STUDIES RELATIVE TO THE APPARENT CLOSE RELATIONSHIP BETWEEN BACT. PERTUSSIS AND B. BRONCHISEPTICUS[1]

I. CULTURAL AGGLUTINATION AND ABSORPTION REACTIONS

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Mallory and his associates in 1912 and 1913, while attempting to prove the relationship of *Bact. pertussis* to whooping cough by animal inoculations, found that the problem was much more complicated than anticipated, their interpretations being clouded by the introduction into the question of the *Bacillus bronchisepticus*, as a result of its presence in some of the animals used for experimental purposes.

During the discussion of the paper by Mallory the observation was made by Dr. J. L. Rhea, that the lesions in pertussis in the human being, due to the bacterium of Bordet are similar to the lesions in the dog, which result from an infection with *B. bronchisepticus*, the cause of distemper, and that this fact suggested an interesting relationship between the two organisms. Later, Mallory states,

> Further experimental work is evidently needed in order to clear up the subject. The two organisms closely resemble each other morphologically and in cultures on potato blood agar, but can be distinguished by their difference in motility and their alkali production in litmus milk.

Soon after the appearance of the work of Mallory, the writers started some experimental work with the two organisms in question, to determine, if possible, just how close was this relationship which apparently existed between them.

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At first the experiments were undertaken with two strains of *Bact. pertussis* which had been cultured since 1911, one having been furnished by the laboratory of Bordet and the other isolated in our own laboratory, and three strains of *B. bronchisepticus* isolated by one of us (N. S. F.), one from a dog in 1908, one from a monkey in 1912 and one from a human subject in 1913. Later on these strains were augmented by ten strains of *Bact. pertussis*, furnished by Dr. Olga R. Povitzky of the New York Board of Health Laboratory, through the courtesy of Dr. Park, and three strains of *Bact. influenzae*, together with one strain of a *Bact. pertussis*-like organism from pertussis sputum, isolated in our own laboratory.

CULTURAL REACTIONS

When first isolated, the *Bact. pertussis* developes slowly and, as a rule, preferably on special media, as reported by Bordet, Woolstein and others. After several months of repeated transplantings, however, its ability to grow on various media gradually increases until it finally presents a growth almost identical to, and nearly as luxuriant as, that of *B. bronchisepticus*, and by that time can be cultured on ordinary media.

It has been found by the writers that the one great difference between the two organisms lies in their power of locomotion; the *B. bronchisepticus* is motile while the *Bact. pertussis* is nonmotile, several months of attempting to develop a strain that would give some evidence of motility resulting in failure.

While the cultural reactions have been found practically identical, even to the alkali production in litmus milk, contrary to the report of Mallory, and the tan color on potato is shared by both, yet, with the *Bact. pertussis*, these reactions are extremely tardy in making their appearance, usually taking about two or three weeks longer than with the *B. bronchisepticus*. At the end of this time, however, it is practically impossible to differentiate between the cultures of the two organisms.

The following outline will show the characteristics of these organisms, in a general way:

	B. BRONCHISEPTICUS	BACT. PERTUSSIS
Morphology	Very small, slender rod, showing bipolar staining	Small, slender rod, showing marked bipolar staining
Gram	Negative	Negative
Motility	Motile	Non-motile
Agar slant	Translucent, filiform growth	Translucent, filiform growth
Bouillon	*Cloudy.* Older growth with heavy, stringy sediment	*Cloudy.* Older growth with heavy, stringy sediment
Potato	*Tan.* Light tan in twenty-four hours to dark tan in three weeks; medium becoming tanned	*Tan.* Light yellow in twenty-four hours to tan in 3–5 weeks; medium tanned.
Litmus milk	*Alkaline.* Slightly blue at the surface in forty-eight hours. This color proceeds downward, becoming very dark greenish blue in about seven days, while the lower part decolorizes	*Alkaline.* In about 6 days the litmus milk begins to decolorize at the dark bottom of the tube, becomes slightly blue in upper portion in four weeks, and in from eight to ten weeks can scarcely be distinguished from *B. bronchisepticus.*
Litmus-lactose agar	Alkaline (forty-eight hours)	Alkaline (four to six days)
Glucose agar	No gas	No gas
Gelatin	Not liquefied	Not liquefied
Indol	Negative	Negative
Nitrites in nitrate broth	Negative	Negative

AGGLUTINATION REACTIONS

The agglutination reactions of these two organisms have presented some very interesting and rather novel phenomena which, to the writers, suggest at least a distant relationship between them.

In the early part of this year Povitzky and Worth reported the results of some agglutination experiments with these organisms, using a *Bact. pertussis* antiserum only, and concluded that,

B. pertussis strains can be specifically identified from hemoglobinophilic bacilli, pertussis-like bacilli and *B. bronchisepticus*. In no instance was there cross agglutination between these organisms—at least not higher than 1 : 40.

Our work has corroborated that of the authors so far as they have gone. Table 1 gives the results of agglutination between anti-

TABLE 1

Agglutination tests between antipertussis serum and heterologous suspensions. Results August 31, 1916. Serum from rabbit 7, treated with Bact. pertussis no. 0363 (Bordet)

DILUTIONS	SUSPENSIONS OF								
	Bact. pertussis		B. bronchisepticus			Pertussislike bacteria	Bact. influenzae		
	No. 0363 (Bordet)	No. 0590 (Ferry)	No. 36 (Dog)	No. 123 (Monkey)	(Human)	No. 932558	T. b. No. 2	"S"	No. 3
1–10	−	−	−	−	−	+	−	−	+
1–20	−	−	−	−	−	−	−	−	−
1–40	−	+	−	−	−	−	−	−	−
1–80	+	++	−	−	−	−	−	−	−
1–200	+++	+++	−	−	−	−	−	−	−
1–400	+++	+++	−	−	−	−	−	−	−
1–800	+++	+++	−	−	−	−	−	−	−
1–1600	+++	+++	−	−	−	−	−	−	−
1–2000	+++	+++							
1–3200	+++	+++							
1–6400	++	+++							
1–10000	+	++							
1–20000	−	+							
1–40000	−	−							
Control	−	−	−	−	−	−	−	−	−

This table and the following one illustrate the presence of pro- agglutinoids in *Bact. pertussis* and *B. bronchisepticus* antiserum making it necessary to test all normal and immune sera in dilutions higher than 1–80.

pertussis serum and suspensions of *Bact. pertussis*, a pertussis-like bacillus, *B. bronchisepticus*, and three hemoglobinophilic bacilli. But on using a *B. bronchisepticus* antiserum, the results are entirely different, as the *Bact. pertussis* suspensions agglutinate

nearly as well as the homologous suspension, the *B. bronchisepticus*. The results of such a test are given in table 2 where agglutination tests were made with suspensions of fourteen strains of *Bact. pertussis* and an homologous suspension of *B. bronchisepticus* against antibronchisepticus serum. And finally, table 3 is a summary of all the homologous and cross agglutination tests.

The results therefor show that the *B. bronchisepticus* antiserum will agglutinate both the *B. bronchisepticus* and *Bact. pertussis* antigens, while the *Bact. pertussis* antiserum will agglutinate only its homologous antigen, the *Bact. pertussis*. This reaction was characteristic of every strain of *Bact. pertussis* and *B. bronchisepticus* under observation.

Whether this shows a true relationship between the two organisms, and can be called a specific reaction, is a question.

Preparation of the antigens for the production of the antisera. *B. bronchisepticus* was grown on plain agar, *Bact. pertussis* no. 0363 (Bordet) and 0590 (Ferry) on ascitic agar, and the New York strains of *Bact. pertussis* and the hemoglobinophilic bacilli on whole-blood (rabbit) agar. Twenty-four hour growths were washed off in 0.2 per cent trikresol in physiologic salt solution—1 cc. to a culture of *Bact. influenzae*, 2.5 cc. to a culture of *Bact. pertussis* and 5 cc. to a culture of *B. bronchisepticus*. The suspensions were thoroughly shaken in a mechanical shaker and, after two days, tested for sterility.

Production of antisera. Before being treated, the serum of each rabbit was tested for agglutinins against all of the organisms under discussion. Any animal showing an agglutination higher than 1–20 against *B. bronchisepticus* antigen or 1–40 against any of the other organisms, was not used.

The rabbits were given three intravenous injections of increasing doses from 0.5 cc. to 2 cc. three days apart and were bled on the fourth day after the last dose; 0.2 per cent trikresol was added to the serum to insure sterility.

Preparation of suspensions for agglutination tests. The suspensions were prepared in general, as follows:

Each culture was transplanted daily for from three days to three weeks—depending upon the organism—on media best suited to it, to insure a good vigorous growth. Then twenty-four hour cultures of

TABLE 2

Agglutination tests showing presence of pertussis agglutinins in antibronchisepticus serum. Results November 7, 1916. Serum from rabbit 1, treated with B. bronchisepticus no. 36 (dog)

DILUTIONS	SUSPENSION OF														
	B. bronchi-septicus	Bact. pertussis													
	No. 36 (Dog)	No. 0363 (Bordet)	No. 55	No. 93	No. 95	No. 98	No. 100	No. 109	No. 110	No. 114	No. 154	No. 163	No. 248	No. 251	No. 253
1–10	++	++	++	−	+	+	+	+	++	+	+	−	+	−	+
1–20	+++	++	+++	+	++	++	+	++	++	+	+	−	+	−	++
1–40	+++	+++	+++	++	+++	+++	++	++	+++	+	++	+	++	+	++
1–80	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
1–200	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1–400	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
1–800	+++	+++	+++	+++	++	+++	++	+++	++	+++	+++	+++	++	++	+++
1–1600	+++	+	++	+	+	++	+	++	+	+++	++	+	+	+	++
1–2000	+++	−	−	+	−	+	+	+	−	+++	+	+	−	−	+
1–3200	+++	−	−	−	−	−	−	−	−	+	−	−	−	−	−
1–6400	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1–10000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1–20000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1–40000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Control	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

Agglutination tests of B. bronchisepticus, Bact. pertussis, and Bact. influenzae suspensions against homologous and heterologous sera

ANTI-SERA	RABBIT NO.	SUSPENSIONS: B. bronchisepticus			Bact. pertussis														Pertussis-like bac.	Bact. influenzæ		
		No. 36 (Dog)	No. 123 (Monkey)	(Human)	No. 0363 (Bordet)	No. 0590 (Ferry)	No. 55	No. 93	No. 95	No. 98	No. 100	No. 109	No. 110	No. 114	No. 154	No. 163	No. 248	No. 251	No. 253	No. 032558	T.b.2	"S"	No. 3
B. bronchisepticus																							
No. 36.....	1	1-6400	1-6400	1-6400	1-1600	1-800	1-1600	1-2000	1-1600	1-2000	1-2000	1-2000	1-1600	1-3200	1-2000	1-2000	1-1600	1-1600	1-1600	1-10	1-40	—	—
	2	1-10000	1-20000	1-10000	1-400	1-400	1-2000	1-800												1-20	1-10	—	1-20
No. 123....	3	1-6400	1-10000	1-20000	1-3200	1-3200	1-20000	1-6400												1-20	1-10	—	—
	4	1-10000	1-10000	1-20000	1-3200	1-6400	1-6400	1-3200	1-2000	1-6400	1-3200	1-6400	1-3200	1-3200	1-6400	1-6400	1-3200	1-3200	1-6400	1-20	—	—	1-80
(Human)..	5	1-2000	1-3200	1-6400	1-800	1-2000	1-800	1-800	1-800	1-2000	1-1600	1-2000	1-2000	1-1600	1-2000	1-2000	1-800	1-800	1-2000	—	—	—	1-10
	6	1-10000	1-10000	1-10000	1-800	1-800	1-800	1-800												1-10	—	—	1-10
Bact. pertussis:																							
No. 0363...	7	—	—	—	1-10000	1-20000	1-10000	1-20000	1-800	1-10000	1-10000	1-20000	1-10000	1-20000	1-20000	1-20000	1-10000	1-10000	1-20000	1-10	—	—	1-10
	8	1-80	1-80	1-80	1-10000	1-20000	1-40000	1-20000												—	—	—	1-20
No. 0590...	9	—	1-10	—	1-6400	1-6400	1-20000	1-10000												1-20	1-10	—	—
	10	1-10	1-10	—	1-6400	1-10000	1-6400	1-10000												1-20	—	—	1-10
No. 55.....	19	—	—	—		1-40000	1-20000																
No. 93.....	20	—	—	—		1-20000		1-20000															
No. 95.....	21	—	1-10	1-10		1-20000			1-3200														
No. 98.....	22	—	—	—	1-20000					1-20000													
No. 100....	23	1-20	1-40	1-20	1-20000						1-20000												
No. 109....	24	—	—	—	1-10000							1-10000											
No. 110....	25	1-10	1-10	1-10		1-10000							1-20000										
No. 114....	26	1-10	—	—		1-20000								1-20000									
No. 154....	27	1-10	1-10	—		1-20000									1-20000								
No. 163....	28	1-10	1-10	1-20		1-20000										1-20000							
No. 248....	29	1-20	1-20	1-20		1-10000											1-20000						
No. 251....	30	1-40	1-80	1-40	1-2000													1-3200					
No. 253....	31	1-20	1-10	1-10	1-6400														1-20000				
Bact. pertussis-like:																							
No. 032558.	11	1-10			—	—														1-20000	—	—	1-10
Bact influenzae:																							
T.b.2.....	13	—	1-20	—	—	—	—	1-10												1-10	1-3200	1-20	1-80
"S".....	15	1-20	1-20	1-10	1-10	1-20		1-20												1-40	1-200	1-6400	1-200
No. 3.....	17	1-20	1-20	1-20	1-20	1-20		1-40												1-20	1-10	1-20	1-1600

these were planted on plain agar in quart whiskey flasks—except in the case of *Bact. influenzae* for which whole-blood agar was used. It has been found advantageous to use agar in whiskey flasks, as this method not only gives a larger amount of suspension for less labor, but it also gives a far heavier and healthier growth than when tubes are used, probably because of the greater supply of media, moisture and air. An abundant growth of *Bact. pertussis* can be obtained on plain agar in flasks when it will grow only slightly or not at all on plain agar in tubes. And a young, vigorous growth is necessary to the production of homogeneous suspensions. Also in using agar without either ascitic fluid or blood, all possibility of clumping from this source is avoided. In the case of the influenza bacillus it is necessary to use blood agar to obtain any growth. Just enough whole blood is added to agar to insure growth and it is used before any hemolysis takes place, in order that the suspension may contain as little blood as possible.

The flasks were incubated for from eighteen to twenty-four hours and the growth washed off with 0.5 per cent formalin in physiologic salt solution. The suspensions were then shaken for a few hours and later, after being tested for sterility, were filtered through paper and standardized to about 2000 million per cubic centimeter.

With this technique, homogeneous suspensions of all the organisms used, were produced.

Agglutination tests. In carrying out the tests, the serum was diluted with physiologic solution and each tube contained 0.5 cc. suspension plus 0.5 cc. diluted serum. The tests were all macroscopic and were incubated at 37°C. for twenty-four hours. (+++) represents complete agglutination with fluid clear; (++) partial agglutination with marked clumping, but fluid not entirely cleared up; (+) slight agglutination, but still with positive clumping; and (−) no clumping, no clearing.

Specially graduated 1 cc. pipettes were used for making the serum dilutions and a different pipette was used for each dilution. All glassware used in connection with the tests was clean and sterile.

AGGLUTINATION REACTIONS WITH SERUM FROM DISTEMPER RABBITS AND DOGS

In testing apparently normal rabbits for agglutinins, before beginning inoculation for the production of antisera, we found that if a serum agglutinated *B. bronchisepticus* in a dilution higher

than 1–20, it also agglutinated *Bact. pertussis* and generally in higher dilutions. In one instance when there was no agglutination against *B. bronchisepticus*, the serum agglutinated *Bact. pertussis* in a dilution of 1–400 (rabbit E) (see table 4).

A few of these rabbits subsequently developed symptoms of distemper; the others may have recovered from an attack.

Sera from only two distemper dogs have been tested, but, with these, similar results were obtained. As in rabbits, agglutinins for *Bact. pertussis* were manifest in higher dilutions than for *B. bronchisepticus*. These dogs exhibited typical symptoms of distemper and were in the later stages of the disease.

Dog 1 agglutinated *B. bronchisepticus* no. 36 (dog) at 1–20; and *Bact. pertussis* no. 0363 (Bordet), 1–400 and no. 93, 1–1000. Dog 2 agglutinated *B. bronchisepticus* no. 36 (dog) at 1–80; and *Bact. pertussis* no. 0363, 1–1000 and no. 93, 1–1000.

On absorption with *B. bronchisepticus* the agglutinins for that organism are removed, while the agglutinins for *Bact. pertussis* are affected little or not at all. On absorption with *Bact. pertussis*, the pertussis agglutinins are removed, while those for *B. bronchisepticus* are unaffected.

ABSORPTION REACTIONS

Upon submitting to absorption tests those antiserums which were produced by the injection of *B. bronchisepticus* antigen into rabbits, and which were found to agglutinate both the *B. bronchisepticus* and *Bact. pertussis* suspensions, the following results were obtained.

Upon absorbing with *B. bronchisepticus* suspension, the agglutinins for *B. bronchisepticus* (the major agglutinins) were absorbed, but the *Bact. pertussis* agglutinins (the minor agglutinins) were still intact (table 5), and an absorption with *Bact. pertussis* was necessary before they were neutralized. In other words, the *B. bronchisepticus* antigen stimulated the formation of both *B. bronchisepticus* and *Bact. pertussis* agglutinins, but contrary to what one would expect, was not able to absorb the *Bact. pertussis* agglutinins, (the minor agglutinins). It was also found, in the

TABLE 4

Agglutinin reaction in rabbits not used for production of anti-sera

Dilutions	Results																			
	Rabbit A		Rabbit B		Rabbit C		Rabbit D		Rabbit E		Rabbit F		Rabbit G		Rabbit H		Rabbit I	
	B. bronchisepticus no. 36	B. pertussis no. 55	B. bronchisepticus no. 36	B. pertussis no. 55	B. bronchisepticus no. 36	B. pertussis no. 109	B. bronchisepticus no. 36	B. pertussis no. 114	B. bronchisepticus no. 36	B. pertussis no. 163	B. bronchisepticus no. 36	B. pertussis no. 163	B. bronchisepticus no. 36	B. pertussis no. 248	B. bronchisepticus no. 36	B. pertussis no. 251	B. bronchisepticus no. 36	B. pertussis no. 253
1–10	+ + +	−	+ + +	+	+ + +	+	+ + +	+ + +	−	−	+ + +	−	+ + +	+ + +	+ + +	−	+ + +	−
1–20	+ + +	+	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	−	−	+ + +	−	+ + +	+ + +	+ + +	−	+ + +	+
1–40	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+	+	+ +	+	+ + +	+ + +	+ +	+	+ + +	+ + +
1–80	+ +	+ + +	+ +	+ + +	+ +	+ + +	+ +	+ + +	+ +	+ + +	+ +	+	+ +	+ +	+	+ + +	+ +	+ + +
1–200	+	+ + +	+	+ + +	+	+ + +	+	+ + +	+	+ +	+	+	+	+ +	+	+ +	+	+ + +
1–400	−	+ + +	−	+ + +	−	+	−	+ +	−	+	−	−	−	+	−	+	−	+ +
1–800	−	+ +	−	+ +	−	+	−	+	−	+	−	−	−	+	−	+	−	+
1–1600	−	+	−	+	−	−	−	−	−	−	−	−	−	+	−	+	−	+
Control	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

case of dogs suffering with distemper, that the serum agglutinated *Bact. pertussis* antigen in higher dilutions than the *B. bronchisepticus* antigen. Absorption with *B. bronchisepticus* antigen took out only the *B. bronchisepticus* agglutinin, while it was necessary to absorb with *Bact. pertussis* antigen before the *Bact. pertussis* agglutinin was neutralized (see table 7). The *Bact. pertussis* agglutinins, therefore, were fixed or stable, as far as the *B. bronchisepticus* was concerned, but absorbable for the *Bact. pertussis*, and this type of an agglutinin, which can be produced

TABLE 5

Serum from rabbit 1, treated with B. bronchisepticus absorbed (1–40) with B. bronchisepticus

DILUTIONS	AGGLUTINATION			
	Before absorption		After absorption	
	B. bronchisepticus No. 36	Bact. pertussis No. 0590	B. bronchisepticus No. 36	Bact. pertussis No. 0590
1–80	+++	+++	++	+++
1–200	+++	+++	−	+++
1–400	+++	+++	−	+++
1–800	+++	++	−	++
1–1600	+++	−	−	−
1–2000	+++	−	−	−
1–3200	++	−	−	−
1–6400	+	−	−	−
1–10000	−	−	−	−
Control	−	−	−	−

by one antigen to be taken up or absorbed more readily by another, has been called by the writers, for the lack of a better term, a transitive agglutinin.

The antisera were identical to those used for the agglutination tests.

The heavy suspensions used for absorption were made from twenty hour growths on plain agar in whiskey flasks, suspended in .85 per cent salt solution to which had been added 0.5 per cent formalin. About 10 cc. salt solution was used to a flask for *B. bronchisepticus* and 4 cc. for *Bact. pertussis*. The suspensions were shaken over night in a mechanical shaker and then strained through mull. Each strain had

been transplanted daily for several days before using so that very heavy growths were obtained.

Absorption tests. Equal parts of heavy suspension and serum were mixed; incubated at 37°C.; then centrifugalized and the supernatant

TABLE 6

Absorption tests with B. bronchisepticus and Bact. pertussis antisera

SERUM	ABSORBED WITH	AGGLUTINATION AFTER ABSORPTION	
		B. bronchisepticus	Bact. pertussis
Antibronchisepticus.			
No. 36 (dog), rabbit 1	*(Original titre)*	*1–6400*	*1–800*
	B. bronchisepticus no. 36	—	—
	Bact. pertussis no. 0590	1–6400	1–80
No. 36 (dog), rabbit 2	*(Original titre)*	*1–10000*	*1–400*
	B. bronchisepticus no. 36	—	—
	Bact. pertussis no. 0590	1–10000	—
No. 123 (monkey), rabbit 3	*(Original titre)*	*1–10000*	*1–3200*
	B. bronchisepticus no. 123	—	1–2000
	Bact. pertussis no. 0590	1–10000	1–80
No. 123 (monkey), rabbit 4	*(Original titre)*	*1–20000*	*1–3200*
	B. bronchisepticus no. 123	—	1–80
	Bact. pertussis no. 0590	1–6400	—
Human, rabbit 5	*(Original titre)*	*1–6400*	*1–800*
	B. bronchisepticus (Human)	—	1–800
	Bact. pertussis no. 0363	1–6400	—
Human, rabbit 6	*(Original titre)*	*1–10000*	*1–800*
	B. bronchisepticus (human)	—	1–800
	Bact. pertussus no. 0363	1–10000	1–80
Antipertussis			
No. 0590, rabbit 10	*(Original titre)*	*1–10*	*1–10000*
	Bact. pertussis No. 0590		—
	B. bronchisepticus No. 36		1–6400

fluid tested for agglutinins. The time of incubation, the number of absorptions and the dilution of the serum were varied in a number of ways. For example, we found that when either antipertussis or antibronchisepticus serum was diluted to 1–40, we obtained a more nearly

complete absorption than when diluted 1–10, which may be due to the fact that complete agglutination does not occur in the lower dilutions with either of the organisms, especially with *Bact. pertussis*. And in the absorption tests, it was noted that clumping and clearing was not so complete at 1–10 or 1–20 as at 1–40.

Again, serum from rabbit 5, treated with *B. bronchisepticus* (Human) was absorbed as many as four times, the dilutions being from 1–5 to 1–40 and the tests being shaken in a mechanical shaker before each incubation, with no effect on the agglutinins for *Bact. pertussis*.

It was found that when antibronchisepticus serum was absorbed with *B. bronchisepticus* sufficiently to remove the agglutinins for *B. bronchi-*

TABLE 7

Absorption tests with serum from distemper dogs

SERUM	ABSORBED WITH	AGGLUTINATION AFTER ABSORPTION		
		B. bronchisepticus no. 36 (dog)	Bact. pertussis	
			No. 0363	No. 93
Dog 1	*(Original titre)*	*1–20*	*1–400*	*1–1000*
	B. bronchisepticus no. 36	—	1–200	1–1000
	Bact. pertussis no. 0363	1–20	—	1–20
Dog 2	*(Original titre)*	*1–80*	*1–1000*	*1–1000*
	B. bronchisepticus no. 36	—	1–800	
	Bact. pertussis no. 0363	1–80	—	

septicus the agglutinins for *Bact. pertussis* were still unaffected. This could be done by an absorption at 1–40, incubated 24 hours. Table 5 gives the results of such an experiment.

But it required repeated absorption with *B. bronchisepticus* before any marked effect was produced in the pertussis agglutinins, and this happened only with the dog strain.

Finally, the following method was used with the six antibronchisepticus sera. The results of these experiments are given in table 6.

Each serum was absorbed three times with its homologous antigen—that is, serum from rabbit treated with *B. bronchisepticus* (dog) was absorbed with the dog strain—and with *pertussis* antigen as follows:

First absorption, serum 1–10 incubated two hours.
Second absorption, serum 1–20 incubated two hours.
Third absorption, serum 1–40 incubated eighteen hours.

SUMMARY AND DISCUSSION

1. After repeated transplantings *Bact. pertussis* has been found to give the same cultural reactions as *B. bronchisepticus*—the tan growth on potato and the alkaline reaction in litmus mik being the most prominent characteristics. The difference in motility and the tardiness in the reactions of *Bact. pertussis* on culture media are differential characteristics.

2. *B. bronchisepticus* antiserum agglutinates not only *B. bronchisepticus* (1–6400 to 1–2,000), but also *Bact. pertussis* (1–400 to 1–6400).

3. *Bact. pertussis* antiserum on the other hand agglutinates only the pertussis bacillus.

4. There was no cross agglutination between *B. bronchisepticus* and a pertussis-like bacillus or three hemoglobinophilic bacilli. Also, there was no agglutination between *Bact. pertussis* and these organisms.

5. *Bact. pertussis* antiserum of high agglutination titre (1–3200 to 1–20000) has been produced, in rabbits, by three intravenous inoculations (three days apart) of sterile, unheated vaccines of fifteen strains of *Bact. pertussis*.

Povitzky and Worth, in the article cited above, conclude that,

> A strongly agglutinating pertussis serum was best obtained in the rabbit by ten to twelve intraperitoneal inoculations of living cultures at seven-day intervals. Agglutinins are also produced by vaccines, but not as abundantly as by living cultures.

After ten to twelve inoculations of live cultures, their rabbits show a titre of from 2400 to 5000 and one rabbit went to 10000.

Over 50 per cent (9 out of 17) of our rabbits show a titre of 20000, and only two were as low as 3200. The one rabbit in the tables of Povitsky and Worth treated with killed vaccine (heated) shows a titre of 1600 after the sixth inoculation and no agglutination after the fourth. They also say,

Two rabbits, more responsive to vaccines, developed, after a few inoculations, a comparatively high agglutination titre—up to 1000.

6. Sera from rabbits treated wtih *Bact. pertussis* grown on blood media developed marked agglutinins for *Bact. pertussis* grown on plain agar.

This is contrary to Bordet's statement that animals inoculated with *Bact. pertussis* grown on Bordet-Gengou medium develop agglutinins for *Bact. pertussis* grown on the same media, not for the organism grown on plain agar; and is in accordance with Povitzky's and Worth's conclusion that,

> From our experience it would seem that the culture medium influences an agglutinable strain in so far as it affects its growth and best development, not in its production of different kinds of agglutinins.

Also, serum from a rabbit treated with *Bact. pertussis* no 0363 (Bordet) grown on *ascitic agar* for the past three years agglutinates all thirteen strains grown on *blood media* (with only one generation, for suspensions, on plain agar); and sera from rabbits treated with thirteen strains grown on blood media agglutinate the ascitic agar strains.

7. When *B. bronchisepticus* antiserum is absorbed with *B. bronchisepticus* sufficiently to remove the agglutinins for *B. bronchisepticus*, the agglutinins for *Bact. pertussis* are still unaffected. These unaffected or fixed agglutinins have been called by the writers, transitive agglutinins.

Upon repeated absorption, agglutinins for *Bact. pertussis* have been removed by the dog strain of *B. bronchisepticus*, slightly reduced by the monkey strain and affected not at all by the human strain.

It may be that, by the absorption tests, grades of differences are brought out between *B. bronchisepticus* from dog, monkey and human which are not shown in the agglutination tests. A similar differentiation is thought to have been brought out by complement fixation tests. (See article in press by Ferry and Klix).

8. When *B. bronchisepticus* antiserum is absorbed with *Bact. pertussis*, agglutinins for *Bact. pertussis* are removed, but agglutinins for *B. bronchisepticus* are unaffected.

9. When *Bact. pertussis* antiserum is absorbed with *Bact. pertussis*, agglutinins for that organism are removed.

10. When *Bact. pertussis* antiserum is absorbed with *B. bronchisepticus*, agglutinins for *Bact. pertussis* are unaffected.

11. The similar morphology, the identical cultural reactions on differential media, the presence of *Bact. pertussis* agglutinins in artificially produced antibronchisepticus serum and in serum from dogs and rabbits suffering or recovered from distemper, all point toward a close relationship between *Bact. pertussis*, the cause of whooping cough and *B. bronchisepticus*, the cause of distemper.

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