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PITFALLS IN THE PREPARATION OF MONOTYPIC AGGLUTINATING ANTISERA FOR *BORDETELLA* *PERTUSSIS*

L. B. HOLT*

*Department of Applied Immunology, Wright-Fleming Institute,
St Mary's Hospital Medical School, London*

CULTURES of *Bordetella pertussis* have one or more of a series of heat-labile surface antigens that are detectable by slide-agglutination (Andersen, 1953; Eldering, Hornbeck and Baker, 1957). The antigenic factor 1 is present in all strains, and most strains also have one or more of the factors 2, 3 and 4. In recent years, Preston (1963, 1965) has claimed that these type antigens play a part in immunity to whooping-cough, and that deficiencies in the antigenic structure of strains of *Bord. pertussis* used for the production of vaccines may have led to incomplete protection. For example, if the infecting strain carries the antigen 3 on its surface in addition to antigen 1, a child will be more effectively protected by a vaccine strain possessing antigens 1 and 3 than by one possessing antigens 1 and 2.

A further point made by Preston (1965) is that the challenge strain of *Bord. pertussis* injected intracerebrally into mice in tests of the immunising potency of vaccines should have the antigen 3, because organisms with this antigen are at present commonly found in whooping-cough. The strain used for this purpose in Britain is no. 18/323, which is usually regarded as possessing only antigen 1 (Preston and Te Punga, 1959). Strain no. 18/323 was isolated in 1923 and has been maintained in a number of different laboratories for many years. It seemed possible, therefore, that the several cultures of it now in use might not be serologically identical. Cameron (1967) found that the culture of no. 18/323 used at the Wellcome laboratories was a mixture of different serotypes.

The starting point of the present investigation was a quantitative serological analysis, carried out by Dr F. Sheffield in the Division of Immunological Products Control, Medical Research Council, of the challenge strains used in different laboratories in this country. The results obtained stimulated an

* Present address: Medical Research Council Laboratories, Holly Hill, Hampstead, London, N.W.3.

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examination of the agglutinins in supposedly pure factor-1 antisera and an investigation of the agglutinins produced by rabbits after injection of cultures that appeared to possess only factor 1.

MATERIALS AND METHODS

Cultures

In the preliminary experiments, Dr Sheffield examined 5 cultures, all stated to be strain no. 18/323, that are used in the mouse intracerebral test in the following laboratories: Burroughs Wellcome Ltd, Beckenham (Wellcome); Glaxo Laboratories Ltd, Greenford (Glaxo); the Lister Institute, Elstree (Lister); Division of Immunological Products Control, Medical Research Council Laboratories, Hampstead, London (MRC) and the Wright-Fleming Institute (WFI).

The origin and serological type of the cultures of *Bord. pertussis* used in the main part of the work are as follows: no. G1353Z, C7061, CN127 (all serological type 1.0.0.), G1260E, 2/Atox, W1791 (all type 1.2.0.) and B179 (type 1.0.3.), all from Dr A. F. B. Standfast, Lister Institute, Elstree; Pillemer 134 (type 1.0.3.) from Dr I. W. Sutherland, Bacteriology Dept, University of Edinburgh; 18/323/1.0.0. (type 1.0.0.), 18/323/1.2.0. (type 1.2.0.), and 18/323/1.2.3. (type 1.2.3.) from Dr J. Cameron, Wellcome Laboratories, Beckenham; and B124 (type 1.2.0.) and B2288 (type 1.2.3.) from Dr Vera Spasojević, of the Institute for Sera and Vaccines, Belgrade, Yugoslavia.

The serological type is expressed in terms of factors 1, 2 and 3, as determined by the senders of the cultures from slide-agglutination tests.

A culture of *Bord. parapertussis* was also obtained from Dr Spasojević.

Stock cultures were stored in the freeze-dried state and recovered on Bordet-Gengou medium as required. They were subcultured 2 or 3 times on this medium before use.

Preparation of bacterial suspensions

Organisms were subcultured from Bordet-Gengou medium on to a liquid-on-solid Casamino Acid medium (Holt, 1962), harvested in 0.01M phosphate-buffered saline, pH 6.8, and killed by the addition of Thiomersal to a final concentration of 1 in 5000. The bacterial content of the suspension was standardised by opacity, 10 units being considered equivalent to 1×10^{10} *Bord. pertussis* per ml.

Antisera

"Monospecific" typing sera for factors 1, 2 and 3 made by (1) Dr N. W. Preston, Department of Bacteriology, University of Manchester, (2) the Wellcome laboratories, (3) the MRC laboratories, and (4) the Glaxo laboratories, were used. In addition, a factor-1 antiserum from the Lister Institute, and two batches (no. 15 and 16) of unabsorbed serum made from strain no. G1353Z at the MRC laboratories were also available.

Antisera were made in the Wright-Fleming Institute as follows. Normal cross-bred rabbits, weight c. 2 kg, were used, two rabbits per vaccine. Each rabbit was bled 10 ml before inoculation. The first injection was made subcutaneously, followed at weekly intervals by three intravenous injections. Each injection comprised 2×10^{10} organisms. A second bleeding was made 12 days after the fourth injection; the sera were separated and stored in the deep-freeze (-22°C) without preservative.

Before carrying out absorptions, the agglutination titre of the serum for the absorbing suspension was determined. Each ml of serum was first absorbed with a dose of bacteria that was one-tenth of the product of the number of organisms used in one tube in the agglutination test and the reciprocal of the agglutination titre of the serum for the organism. The calculated number of bacteria were deposited from a suspension by centrifugation for $\frac{1}{2}$ hr at 5000g, and the serum was added to the deposit. The bacteria were well dispersed in the serum and immediately re-deposited by centrifugation for $\frac{1}{2}$ hr at 5000g. When

necessary, the absorption was repeated, so that the absorbed serum finally agglutinated the suspension at a dilution not exceeding 1 in 10.

Agglutination tests

Serological typing of cultures was carried out by the slide-agglutination method recommended by Preston (1963), and the results were expressed semiquantitatively as an "agglutination rating" in which the strength of agglutination by the "monotypic" sera for factors 1, 2 and 3 was recorded on the scale + + + +, + + +, + +, + and $\pm$ (see Holt and Spasojević, 1968).

The agglutinin content of sera was titrated by two different but comparable methods, in which doubling dilutions of serum were used. At the MRC laboratories, Dr Sheffield made serial dilutions of sera in plastic trays; after the addition of the bacterial suspension, the trays were incubated for 15 min. at 37°C with oscillation, and then left in the refrigerator for a further 15–20 min. before reading. Agglutination was recorded on an arbitrary scale (+ +, +, $\pm$); in the analysis of these results, we determined the end-point of agglutination by a system of interpolation described under *Results*.

At the Wright-Fleming Institute, an agglutination machine was used similar to the one described by Miller and Silverberg (1939). Mixtures were rotated intermittently for 1 hr at room temperature; each comprised 1 vol. diluted serum, and 1 vol. bacterial suspension adjusted to contain 5×10^9 organisms per ml. The end-point was taken as agglutination just visible with a $\times 5$ lens.

The titre was recorded as the reciprocal of the initial dilution of serum at the end-point, no allowance being made for the dilution caused by the addition of the bacterial suspension.

RESULTS

Serological examination of the challenge strains of Bordetella pertussis used in Britain

The challenge strains used in 5 British laboratories in tests of the potency of whooping-cough vaccine—all cultures of strain no. 18/323—were examined serologically by Dr Sheffield. He determined the titre of their agglutination by two sets of "monotypic" typing antisera for factors 1, 2 and 3, prepared respectively at the MRC and Glaxo laboratories (table I). This experiment was carried out to detect differences in antigenic constitution between the challenge strains, and these differences are clearly shown. For example, the culture used in laboratory 1 appears to have more factor 3 on its surface than the other cultures.

The results of this experiment may also be used to compare the specificity of the typing antisera. If each pair of factor sera were truly "monotypic", that is to say, if they differed only in their content of the same agglutinin, the ratios of the titres of the pairs of sera when tested with the same suspension should be fairly constant even if the cultures of *Bord. pertussis* had different amounts of each surface agglutinin.

Titres of agglutination were obtained by interpolation from Dr Sheffield's results in the following manner: when the last tube to show agglutination gave a + + reaction, the end-point was half-way between this and the next higher tube; when the last tube gave a + reaction, it was at one-quarter of the interval between this and the next higher tube; when the last tube gave a $\pm$ reaction,

TABLE I
Agglutination of 5 cultures of Bordetella pertussis, strain no. 18/323, by two sets of "monotypic" factor sera*

Culture from laboratory no.	Degree of agglutination of culture by														
	MRC factor-1 serum diluted 1 in					MRC factor-2 serum diluted 1 in					MRC factor-3 serum diluted 1 in				
	20	40	80	160	320	640	1280	2560	512	1024	2048	4096	8192	40	80
1	++	++	++	++	++	++	—	—	++	++	+	±	—	++	++
2	++	++	++	++	++	++	—	—	++	++	+	±	—	++	++
3	++	++	++	++	++	++	—	—	++	++	+	±	—	++	++
4	++	++	++	++	++	++	—	—	++	++	+	±	—	++	++
5	++	++	++	++	++	++	+	+	++	++	+	—	—	++	++

Culture from laboratory no.	Degree of agglutination of culture by														
	Glaxo factor-1 serum diluted 1 in					Glaxo factor-2 serum diluted 1 in					Glaxo factor-3 serum diluted 1 in				
	20	40	80	160	320	640	1280	2560	20	40	80	160	320	640	1280
1	++	++	++	++	++	++	++	++	++	++	++	++	++	±	—
2	++	++	++	++	++	++	++	++	++	++	++	++	++	—	—
3	++	++	++	++	++	++	++	++	++	++	++	++	++	—	—
4	++	++	++	++	++	++	++	++	++	++	++	++	++	—	—
5	++	++	++	++	++	++	++	++	++	++	++	++	++	—	—

* Cultures used as challenge strains in 5 different laboratories in tests for potency of whooping-cough vaccines. All these tests were performed by Dr F. Sheffield. The amount of agglutination was recorded on an arbitrary scale (see text).

this dilution was the end-point. Table II shows the ratio $\frac{\text{titre with Glaxo serum}}{\text{titre with MRC serum}}$ for each culture with each pair of factor sera.

TABLE II
*Comparison of agglutination titres obtained with two sets of
"monotypic" factor sera (see table I)*

Culture from laboratory no.*	Serum			Ratio: $\frac{\text{titre of Glaxo serum}}{\text{titre of MRC serum}}$
	Nature	Origin	Titre against culture †	
1	anti-factor 1	MRC	960	> 2·7
		Glaxo	> 2560	
2		MRC	800	
		Glaxo	1600	
3		MRC	1600	
		Glaxo	> 2560	> 1·6
4		MRC	800	1·0
		Glaxo	800	
5		MRC	> 2560	...
		Glaxo	> 2560	
1	anti-factor 2	MRC	4096	> 0·6
		Glaxo	> 2560	
2		MRC	512	
		Glaxo	20	
3		MRC	> 8192	
		Glaxo	> 2560	...
4		MRC	1024	0·2
		Glaxo	200	
5		MRC	2560	< 1·0
		Glaxo	> 2560	
1	anti-factor 3	MRC	640	1·0
		Glaxo	640	
2		MRC	160	
		Glaxo	20	
3		MRC	320	
		Glaxo	20	0·06
4		MRC	1600	< 0·01
		Glaxo	< 20	
5		MRC	160	0·1
		Glaxo	20	

* Five cultures of *Bordetella pertussis*, all of strain no. 18/323.

† See text for method of determining the end-points.

... = Ratio could not be determined.

The ratios between the titres obtained with the same pair of sera and different cultures often differed widely. This was so with the factor-1 sera and cultures no. 1 and no. 4, with the factor-2 sera and cultures no. 1, no. 2 and no. 4, and similarly with the factor-3 sera. It must therefore be concluded that the sera were not "monotypic", but contained variable amounts of other agglutinins.

Examination of supposedly "monotypic" antisera

A more detailed examination of a number of factor-1 antisera was next undertaken in an attempt to identify and measure the different agglutinins in them.

For the preparation of "monotypic" sera, cultures previously shown to be clearly different in terms of recognised surface antigens are injected into rabbits. Absorptions are carried out to remove cross-reacting antibodies as far as possible, and the sera are used for slide agglutination at a dilution at which they react only with cultures believed to possess the homologous factor. For the preparation of factor-2 serum, animals are given injections of a strain with the antigenic constitution 1.2.0. and the serum is absorbed with a 1.0.3. strain; factor-3 sera are prepared in an analogous manner. This absorption serves also to remove the minor antigen 7 if it is present.

All strains of *Bord. pertussis* have antigen 1; to make factor-1 serum it is therefore necessary to choose a vaccine strain as far as possible devoid of antigens 2 and 3. Strain no. G1353Z is generally used for this purpose (Preston and Te Punga), and the antiserum is absorbed with an autoclaved suspension of the homologous organism (Preston, 1963) to remove heat-stable O-antibodies. This absorption does not remove factor 7 (Andersen; Eldering *et al.*, 1957).

In all cases the assumption is made that a vaccine strain will not evoke antibodies to agglutinogens that are not detectable on the surface of the organism used. If this were generally true it would follow that absorption of an antiserum with the suspension used to make it would remove all relevant agglutinins from the serum. When an anti-G1353Z serum was absorbed with no. G1353Z this was found not to be so; the agglutination titre of the absorbed serum for some other strains of *Bord. pertussis* was not detectably changed. This observation was investigated further.

Ten strains of *Bord. pertussis* were used for the tests: no. B124, B2288, G1353Z, 2/Atox, C7061, CN127, W1971, G1260E, Pillemer 134 and B179. They were typed by slide agglutination with "monotypic" antisera made by Dr Preston, and with Wellcome antisera; their "agglutination rating" is shown in table III, together with the result of typing of some of them by Dr A. F. B. Standfast with Lister antisera.

The 10 cultures were examined in tube-agglutination tests with a number of antisera made with strain no. G1353Z; these included "monotypic" factor-1 sera prepared by Dr Preston and by Dr J. Cameron at the Wellcome laboratories, and unabsorbed sera from the MRC laboratories (batches no. 15 and 16) and from the Lister Institute. At the WFI, two batches of serum were prepared as described in *Materials and methods*; since their agglutination titres for no. G1353Z were similar they were pooled to become batch E5329. Separate

TABLE III
Agglutination titres of antisera made with strain no. Gl353Z against 10 cultures of *Bordetella pertussis*

Culture no.	Serological typing of culture*	Titre against culture of							
		factor-1 ("mono-typic") sera of		unabsorbed sera				WFI serum absorbed with no. G1353Z	WFI serum absorbed with <i>Bordetella parapertussis</i>
		Wellcome	Preston	MRC no. 15	MRC no. 16	Lister	WFI		
G1353Z (Lister)	P. + + + - - W. + + +	80	<20	160	30	160	<5	300	
2/Atox	P. + + + + + - W. + + + + + 2. L. 1. 0.	500	800	<10	25	500	2500	2500	
B124	P. + + + + + + W. + + + + + -	640	...	<10	30	640	2000	3000	
Pillemer 134	P. + + + + + + W. + + + + + +	250	...	30	30	500	640	500	
B2288	P. + + + + + + W. + + + + + +	500	...	<10	15	320	3000	3000	
C7061	P. + + + - - - W. + + + - - - L. 1. 0.	60	...	10	120	100	<5	15	
CN127	P. + + + + + - W. + + + + + 0. L. 1. 0.	60	...	160	10	100	<5	15	
W1971	P. + + + + + + W. + + + + + 2. L. 1. 0.(4)	500	...	<10	20	600	3000	3000	
G1260E	P. + + + + + + W. + + + + + 2. L. 1. 0.(4)	500	...	<10	20	320	2500	2500	
B179	P. + + + + + + W. + + + - - -	60	...	30	10	320	200	320	

* P: serotype rating with Preston's sera; W: serotype rating with Wellcome sera; L: typing at Lister Institute. For serotype rating see Holt and Spasojević (1968).

portions of this serum were absorbed with no. Gl353Z and with *Bord. parapertussis*. All the sera, with the exception of Dr Preston's factor-1 serum, were tested with all the strains.

The results of the agglutination tests are shown in table III. The two "monotypic" sera, as well as the unabsorbed Lister and WFI sera, had higher titres of agglutination for strains with the surface antigens 2 and 3 than for strains with antigen 1 only, with the exception that the Wellcome "monotypic" serum agglutinated no. B179 (factors 1.0.3.) to low titre only. For all strains with antigen 2, the titre was markedly higher than for no. Gl353Z, C7061 and CN127. The two MRC sera differed from the other sera and from each other. Both reacted to significant titres with 1.0.0. strains only; serum no. 15 agglutinated strains no. Gl353Z and CN127 to a titre of 160 and no. C7061 to a titre of 10 only, but the highest titre with serum no. 16 was with strain no. C7061. These results are difficult to explain unless the assumption is made that the sera contained two different agglutinins in different proportions.

More information was obtained by examining the results for WFI serum E5329, unabsorbed and absorbed with *Bord. pertussis* no. Gl353Z and with *Bord. parapertussis*. The latter absorption was to remove antibodies for factors 7 (and 13) if they were present (Eldering *et al.*, 1957). Absorption with the homologous strain led to a great reduction of the titre for no. Gl353Z, C7061 and CN127, all strains with the antigenic constitution 1.0.0.; presumably therefore these strains all have similar agglutinating antigens. However, this absorption produced no reduction in titre for strains that carried factor 2 on the surface (no. 2/Atox, B124, B2288, W1971 and Gl260E). This suggested that the anti-Gl353Z serum contained a considerable amount of antibody for factor 2. In respect of factor-3 antibody, the results with strain no. B179 are the most satisfactory, and show that anti-Gl353Z serum does contain this agglutinin, because absorption reduced the titre for this strain from 500 only to 200.

Absorption with *Bord. parapertussis* made no significant difference to the titres that appeared to be attributable to factors 2 and 3, but did reduce the titres for no. C7061 and CN127. It may be concluded, therefore, that much of the titre of the unabsorbed sera for these two strains was due to antibodies for factor 7.

Agglutinin content of unabsorbed WFI Gl353Z antiserum. The titre of the serum for factor-1 agglutinin was estimated to be about 100, by comparison of the titre of the unabsorbed serum and that of the serum absorbed with *Bord. parapertussis* in tests with strains no. CN127 and C7061. From the same data, the titre of antibody for factor 7 was 80. The titre of factor-2 agglutinin was shown to be about 2500 (no. 2/Atox, B2288, W1971 and Gl260E after absorption with no. Gl353Z and with *Bord. parapertussis*). The factor-3 agglutinin had a titre of about 200 (no. B179 after absorption with no. Gl353Z, on the assumption that no. Gl353Z absorbs both factors 1 and 7).

In order to determine the titre of the serum for O-agglutinins, it was tested with an autoclaved suspension of no. Gl353Z. The titre was 40. Nevertheless, if we accept the contention of Eldering *et al.* (1957) that O-antibody does not

act on undamaged organisms, the presence of this agglutinin does not complicate the results shown in table III.

In summary, therefore, the WFI serum E5329 contained the following agglutinins:

O agglutinin:	titre about 40;
Factor-1 agglutinin:	titre about 100;
Factor-2 agglutinin:	titre about 2500;
Factor-3 agglutinin:	titre about 200;
Factor-7 agglutinin:	titre about 80.

*Antisera made with strains of Bordetella pertussis
apparently lacking factors 2 and 3*

Since antisera made with strain no. G1353Z contained a variety of agglutinating antibodies, it seemed important to find out whether other strains caused the production of antibodies to antigens not detected by agglutination-typing. Five strains of *Bord. pertussis* were used; 3 of them (no. C7061, CN127 and 18/323/1.0.0.) appeared to possess only factor 1 when tested by slide agglutination. The two "mutants" of 18/323/1.0.0., with the antigenic constitution 1.2.0. and 1.2.3. respectively (Cameron), were included as controls. Two rabbits were vaccinated with each strain (see *Materials and methods*). Portions of each serum collected after vaccination were absorbed with *Bord. paraptussis*. All the antisera, both absorbed and unabsorbed, were examined in tube-agglutination tests with each of the 5 vaccine strains. The WFI serum (E5329) made with no. G1353Z and the corresponding suspension were included in the tests. Sera collected from the rabbits before vaccination were tested with suspensions of no. G1353Z and of *Bord. paraptussis*.

The results (table IV) showed that the rabbits frequently responded to "concealed" agglutinogens by producing antibody in high titre to factors not detected in slide-agglutination tests. Strains that typed as 1.0.0. frequently produced large amounts of factor-2 agglutinin; one of the two sera made with each of no. C7061, CN127 and 18/323/1.0.0. gave very much higher titres of agglutination of 18/323/1.2.0. than of the homologous strain, both before and after absorption. In each case, however, a second rabbit responded much less well to the "concealed" antigen.

It will be noted that titres were in many cases lowered considerably by absorption with *Bord. paraptussis*. This indicated that antibody to factor 7 was frequently produced by the inoculation of *Bord. pertussis* into rabbits. According to Andersen, and to Eldering *et al.* (1957), this is the only agglutinin shared by *Bord. pertussis* and *paraptussis*.

A probable objection to the conclusions drawn from table IV is that the results obtained would be explicable if strains no. 18/323/1.2.0. and 18/323/1.2.3. were more easily agglutinated by factor-1 antisera than were strains no. C7061, CN127 and 18/323/1.0.0. This possibility was investigated by absorbing the antisera made with strains no. C7061 and CN127 with suspensions of no. CN127, and measuring the titres of the absorbed and unabsorbed sera for no. CN127

TABLE IV
Agglutination titres of Bordetella pertussis anisera, before and after absorption with Bordetella parapertussis

Culture no.	Serotype	Titre, against culture, of serum made against strain no.											
		C7061 (serotype 1.0.0.) in		CN127 (serotype 1.0.0.) in		18/323/1.0.0. (serotype 1.0.0.) in		18/323/1.2.0. (serotype 1.2.0.) in		18/323/1.2.3. (serotype 1.2.3.) in		GI353Z (serotype 1.0.0.) in	
		rabbit 1	rabbit 2	rabbit 1	rabbit 2	rabbit 1	rabbit 2	rabbit 1	rabbit 2	rabbit 1	rabbit 2	rabbit 1	rabbit 2
		Un.* Abs.†	Un. Abs.	Un. Abs.	Un. Abs.	Un. Abs.	Un. Abs.	Un. Abs.	Un. Abs.	Un. Abs.	Un. Abs.	Un. Abs.	Un. Abs.
C7061 CN127 18/323/1.0.0. 18/323/1.2.0. 18/323/1.2.3. GI353Z Bord. parapertussis	1.0.0.	640	500	640	1250	60	30	120	40	250	160	260	300
	1.0.0.	800	500	500	1000	80	30	120	60	320	160	300	300
	1.0.0.	1250	1000	1000	1000	120	120	250	320	250	500	300	300
	1.2.0.	4000	1500	1000	1500	250	120	4000	2000	640	500	600	200
	1.2.3.	4000	320	3500	900	250	120	4000	2000	5000	4000	4000	2000
	1.0.0.	2500	2500	320	1000	160	150	4500	3500	5000	5000	3500	2000
GI353Z Bord. parapertussis	1.0.0.	2500	1000	1250	1600	200	60	320	320	160	160	100	<10
	1.0.0.	2500	<10	5	<10	20	<10	120	<10	160	<10	100	<10
	1.0.0.	640	500	640	1250	60	30	120	40	250	160	260	300
	1.0.0.	800	500	500	1000	80	30	120	60	320	160	300	300
	1.0.0.	1250	1000	1000	1000	120	120	250	320	250	500	600	200
	1.0.0.	4000	1500	1000	1500	250	120	4000	2000	640	500	600	200
GI353Z Bord. parapertussis	1.0.0.	640	500	640	1250	60	30	120	40	250	160	260	300
	1.0.0.	800	500	500	1000	80	30	120	60	320	160	300	300
	1.0.0.	1250	1000	1000	1000	120	120	250	320	250	500	600	200
	1.0.0.	4000	1500	1000	1500	250	120	4000	2000	640	500	600	200
	1.0.0.	4000	320	3500	900	250	120	4000	2000	5000	4000	4000	2000
	1.0.0.	2500	2500	320	1000	160	150	4500	3500	5000	5000	3500	2000

* Un. = Unabsorbed serum.

† Abs. = Serum after absorption.

and 18/323/1.2.0. The results are shown in table V; they confirm that antibodies evoked by strains with the surface-antigenic constitution 1.0.0., and detectable in high titre by the agglutination of 1.2.0. strains, are not removed from the serum by absorption with 1.0.0. strains. They show further that the sera made in different rabbits contain antibodies for factors 1 and 2 in different proportions.

TABLE V

Agglutination titres of antisera prepared against strains no. C7061 and CN127, before and after absorption with no. CN127, against strains CN127 and 18/323/1.2.0.

Culture no.	Serotype	Titre, against culture, of serum prepared against strain no.							
		C7061 (serotype 1.0.0.) in				CN127 (serotype 1.0.0.) in			
		rabbit 1		rabbit 2		rabbit 1		rabbit 2	
		Un.*	Abs.†	Un.	Abs.	Un.	Abs.	Un.	Abs.
CN127	1.0.0.	640	< 10	640	< 10	640	10	900	10
18/323/1.2.0.	1.2.0.	3500	2500	640	80	3500	3500	1250	80

* Un. = Serum unabsorbed.

† Abs. = Serum after absorption.

DISCUSSION

Agglutination tests with *Bordetella pertussis* give information only about antigens present on the cell surface. It is now clear that agglutinogens may often be present in the organism that are revealed only by the antibody response in animals.

Evidence for the importance of the type-specific antigens in immunity to whooping-cough is based partly on experiments with animals (Preston and Evans, 1963) and partly on epidemiological observations (Preston, 1963, 1965). In fact, there is no solid evidence that any recognised agglutinin is of importance in immunity to experimental infection with *Bord. pertussis* (Horton and Standfast, 1953; Eldering, Holwerda and Baker, 1967; Holt and Spasojević, 1968), and the available epidemiological information is probably insufficient to form a basis for definite conclusions. Since about 1955, there is said to have been a change in the predominant serotype of *Bord. pertussis* in Britain. Before this date, the infecting strains are stated generally to have had antigens 1 and 2 but not 3, but the number of cultures examined was very small. Since 1960, most of the strains isolated here had factor 3, and this is attributed to the fact that strains of *Bord. pertussis* used for vaccine production in the 1950's usually possessed factor 2 but not factor 3.

The evidence now is that strains that appear on typing by slide-agglutination to be deficient in factors 2 and 3 provoke the formation of antibodies to these

factors. Indeed, strain no. G1353Z, which appears to have only factor 1 on its surface, gives exceptionally good results as a vaccine strain in the mouse-protection test, and evokes antibody for factors 1, 2 and 3 when injected into rabbits.

It follows, therefore, that any argument based on the assumption that such a vaccine would not give rise to antibody to factors 2 and 3 is untenable, and that no constant relation can be established between the serotype of an organism and its protective value in a whooping-cough vaccine.

SUMMARY

Agglutination tests showed that 5 cultures of *Bordetella pertussis*, strain no. 18/323, which are used in Britain to assess the protective value of whooping-cough vaccine by mouse-protection tests, are not serologically identical.

"Monotypic" antisera used for the typing of *Bord. pertussis* by slide-agglutination were found not to be serologically "pure". A number of sera supposed to contain antibodies for factor 1 only proved to contain antibodies for a variety of other antigens, including factors 2 and 3.

Several cultures in which only factor 1 was detected by slide agglutination were used as vaccines in rabbits. The resulting antisera contained other antibodies, and in some cases large amounts of antibody for factor 2 were detected. Individual rabbits varied considerably in the relative amounts of agglutinin for factors 1, 2 and 3 produced as a result of vaccination with the same bacterial suspension.

The implications of these findings were examined in relation to the claim that immunity to whooping-cough is influenced by the serological type of the vaccine strain of *Bord. pertussis*.

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