

## Variation of serotype in strains of *Bordetella pertussis*

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### SUMMARY

The four main serotypes of *Bordetella pertussis* (1, 2, 3; 1, 2; 1, 3; 1) undergo spontaneous variation involving loss or gain of antigen 2 or antigen 3. By serial subculture from single colonies on charcoal-blood-agar medium, we have detected loss-mutations from type 1, 2, 3 to 1, 2 or 1, 3, and from type 1, 2 to type 1. Likewise we have found gain-mutations from type 1 to 1, 2 or 1, 3, and from 1, 2 to 1, 2, 3.

These mutations apparently occur with a high frequency in some strains. Other strains have a lower mutation-rate and are more stable antigenically. We have not detected, by this method, either gain- or loss-mutations from the type 1, 3 strains that we have tested.

These findings offer an explanation for the changes in serotype that occur during the course of a pertussis infection in the child and in the marmoset. They also constitute a warning on the possible antigenic instability of laboratory strains, especially relevant in the production, absorption and testing of diagnostic antisera and in the preparation of pertussis vaccine.

### INTRODUCTION

It was suggested by Cameron (1967) that *Bordetella pertussis* may undergo a step-wise loss of heat-labile antigens, converting a parent of type 1, 2, 3 to a degraded strain of type 1. This suggestion is supported by our previous findings in the child (Preston & Stanbridge, 1972) and in the marmoset (Stanbridge & Preston, 1974), in which we showed that the parent (1, 2, 3) and the intermediates (1, 2; 1, 3) could each establish infection and could change to a different serotype during the course of the infection, whereas type 1 organisms were found only at a late stage of the infection and, even then, did not constitute the predominant serotype.

The object of our investigation was to obtain some indication of the frequency with which such changes in serotype may occur spontaneously *in vitro*, as this may be of importance for the antigenic stability of laboratory strains used in the preparation of diagnostic antisera and of pertussis vaccine.

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## MATERIALS AND METHODS

*Strains of Bordetella pertussis*

Four of our strains had been isolated from children in England: three in 1967 (M/S23 in Manchester, SF/S8 in Sheffield, 41633 in Coventry) and one in 1970 (M21356 in Manchester). All were initially mixed cultures of more than one serotype (Tables 1 and 2). The fifth strain was the Kendrick challenge strain, W.18-323, which in our laboratory is a type 1 strain (Preston, 1966).

*Serotyping of pertussis strains*

Full details have been described previously (Preston, 1970).

*Typing and serial subculture of single colonies*

Each strain was cultured on a plate of charcoal-blood-agar (Oxoid, 1965) so as to obtain, after 3-4 days at 35-36° C., confluent growth suitable for serotyping, and also well-separated colonies for subculture. Individual colonies of *Bord. pertussis*, of this age, are too small for accurate serotyping, whilst older colonies tend to give auto-agglutinable suspensions. Serotyping of single colonies was, therefore, determined retrospectively by testing the confluent growth of the next subculture.

On each occasion, about eight single colonies were subcultured, each on a separate plate, and single colonies from one such plate were used for the next subcultures. Whenever a change of serotype was detected on one of the eight plates, several single colonies were subcultured from this plate as well as from a plate with the original serotype.

## RESULTS

Initially, four of the five strains recorded in Tables 1 and 2 were mixed cultures of two or three different serotypes: not all the colonies of the first plate culture were of the same serotype. But, with all five strains, single-colony subculture usually yielded a pure growth of the same serotype as the parent colony. Occasionally, whereas the majority of colonies tested were of the same serotype as the parent, a single colony of a different serotype appeared, and this in turn yielded pure subcultures of the new serotype. The emergence of these occasional mutant colonies, in an otherwise pure population, is strong evidence to suggest that each colony was usually derived from a single cell, for mixed populations were rarely encountered after the first two subcultures.

Ignoring, then, any changes of serotype detected in less than three serial single-colony subcultures, there is evidence of both loss- and gain-mutation. In Table 1, all three strains show loss of antigen 2 from type 1, 2, 3. Strain M/S23 shows also loss of antigen 3, changing 1, 2, 3 to 1, 2, and subsequent loss of antigen 2, changing 1, 2 to type 1. The only loss-mutation, concerning antigens 2 and 3, that was not detected was the change from 1, 3 to type 1. On two occasions, with strain SF/S8, type 1 growth was found in subculture from a type 1, 3 parent, but this was only a phenotypic suppression of antigen 3, the cause of which is not known (Preston, 1970). On both occasions, further subculture reverted to a pure growth of type 1, 3.

Table 1. Variations of serotype involving loss of antigen 2 or antigen 3

Serial n	subculture*... Serotype of original culture	Serotypes of colonies tested	No. of colonies tested on each subculture (tabulated according to s pe)																				
Strain			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
M/S23	, 2, (3)	$\left\{ \begin{array}{l} 1, 3, \dots \\ 1, 2, 3, \dots \\ 1, 3, \dots \\ 1, 2, \dots \\ 1, \dots \end{array} \right.$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M21356	, (2), 3	$\left\{ \begin{array}{l} 1, 2, \dots \\ 1, 3, \dots \\ 1, 2, 3, \dots \\ 1, 3, \dots \end{array} \right.$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
SF/S8	, 2, 3	$\left\{ \begin{array}{l} 1, 3, \dots \\ 1, 2, 3, \dots \\ 1, 3, \dots \\ 1, \dots \end{array} \right.$	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

( ) = We action, with antibody 2 [type 1, (2), 3] or antibody 3 [type 1, 2, (3)]. Usually indicative of mixed culture of  
 \* Each : Iture derived from a single colony of the previous one (see Materials and Methods).  
 † Type : out phenotypic, not genotypic: subculture yielded only type 1, 2, 3.  
 ‡ Type : ; phenotypic, not genotypic: subculture yielded only type 1, 3.

Table 2. Variations of serotype involving gain of antigen 2 or antigen 3

Serial no. of subculture*...	Serotypes of original culture	Serotypes of colonies tested	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	7	18			
41633	1, (2), 3	1, 3, ... 1, (2), 3, ... 1, 2, ... 1, 2, 3, ... 1, 2, ... 1, ... 1, 2, ... 1, ...	12	9																		
			1	2	1	9	3	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
			1	8	1	8																
			1	8	3	8	7	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
			1	8	1	8	1	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
W. 18-323	1	1, 3, ... 1, ... 1, 3, ... 1, 2, ...	3	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8		
			1	8	1	8	6	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
			1	8	1	8	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
			1	8	1	8	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

( ) = Weak reaction, with antibody 2 [type 1, (2), 3]. Usually indicative of mixed culture of different types.  
 \* Each subculture derived from a single colony of the previous one (see Materials and Methods)

Another example of phenotypic suppression is seen in strain M21356, in which type 1, 2 growth was found in subculture from a type 1, 2, 3 parent, but reverted to a pure growth of type 1, 2, 3 on the next subculture.

In Table 2, both strains show gain of antigen 2 by type 1. They also show gain of antigen 3, changing 1, 2 to 1, 2, 3 with strain 41633, and changing type 1 to 1, 3 with strain W.18-323. The only gain-mutation that was not detected was the change from 1, 3 to 1, 2, 3.

The relative stability of type 1, 3 strains, indicated by these results, was a feature of other 1, 3 strains studied less extensively than the ones recorded here.

It is also noteworthy that on no occasion did we detect a mutant having antigen 2 and/or 3 but devoid of antigen 1.

#### DISCUSSION

As we were deliberately looking for evidence of mutation in the serotypes of *Bord. pertussis*, we chose strains from children which were found to be mixed cultures of different serotypes when first tested. This may have been evidence, we felt, that they had already undergone mutation in the child and they may therefore be more fruitful objects of study than the possibly more stable strains which appeared to be pure cultures each of a single serotype. We are not able even to guess at the possible mutation rates of the more stable strains, and we thought that it would be perhaps unnecessary to attempt to determine them, and certainly too laborious and too expensive to do so with the only method available to us.

The mutations involving the loss or gain of antigen 2 or 3 by some of our type 1, 2, 3, type 1, 2, and type 1 strains, were of a high frequency, perhaps similar to the phase variation in the H antigens of salmonellas (Stocker, 1949). Such a mutation rate, of about  $10^{-3}$  or  $10^{-4}$ , would be consistent with our detection of one mutant colony out of eight, after an average of about nine or ten serial single-colony subcultures (Tables 1 and 2). The mutation rate of our type 1, 3 strains was lower than that of the other serotypes: indeed, we did not detect such mutation *in vitro*. But its occurrence *in vivo* is suggested by our studies in marmosets (Stanbridge & Preston, 1974) in which type 1, 2, 3 and type 1 organisms were occasionally isolated some weeks after inoculation of the nasopharynx with a pure culture of type 1, 3.

We also failed to detect a mutant having only antigen 2 or only antigen 3. This was unfortunate, for such mutants would be most useful in the preparation of monospecific pertussis antisera, and in the titration of antibodies 2 and 3 in polyvalent sera. It seems, however, that loss of antigen 1 rarely occurs or is a lethal mutation.

The mutations that we have detected provide an explanation for a number of previously unexpected observations. These include changes of serotype during the course of infection in a child (Preston & Stanbridge, 1972) or marmoset (Stanbridge & Preston, 1974), and the not uncommon isolation of different serotypes in the same household (Public Health Laboratory Service, 1973). They also provide a further explanation, in addition to the difficulties in the technique of typing strains of *Bord. pertussis* (Preston, 1970), for the differing serotype results reported by two or more laboratories on strains isolated in that Public Health Laboratory Service

survey, and for the high proportion of mixed serotypes that were discovered when those strains were re-typed later.

There is a danger of confusion in terminology between 'serotypes' and 'phases'. We would note that our studies refer to the heat-labile agglutinogens of Andersen (1953); and we agree with Cameron (1967) that a parent 'serotype' (1, 2, 3) may pass through intermediates (1, 2 or 1, 3) to the degraded form (type 1). It seems likely that such loss of antigens was responsible for the earlier division of laboratory strains of *Bord. pertussis* into four 'phases' by Leslie & Gardner (1931), freshly isolated 'smooth' strains of phase I degenerating to 'rough' variants of phase IV. However, we have not been able to obtain cultures of phase II, III or IV to test this hypothesis. It seems likely that such cultures no longer exist, and it would perhaps be best that the 'phases' of Leslie & Gardner be forgotten.

Another kind of variation in *Bord. pertussis* was described by Lacey (1960) but was quite distinct from the mutations which we record here. Lacey's 'modes' were phenotypic, the organisms being reversibly changed from X-mode to C-mode by alterations in the conditions of growth. All of our mutants were incubated at a temperature and on a medium that would produce growth in the X-mode.

Lastly, it must be accepted that not all strains of *Bord. pertussis* may be subject to such rapid mutation as we have detected, and that relatively stable strains of each serotype have been maintained in laboratories over many years. Nevertheless, their potential instability has serious consequences. In the preparation of mono-specific typing sera, for example, it is essential to test the serotypes of the actual suspensions used for the immunization of animals and those used for the absorption and titration of their sera. Similarly, in the production of pertussis vaccine, it is not sufficient to start with strains having the appropriate range of antigens: the final product must be tested for the presence of all three antigens, in order to guard against both loss-mutation and phenotypic suppression.

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