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# Mouse or man? Which are pertussis vaccines to protect?

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## SUMMARY

Type 1 strains of *Bordetella pertussis* can infect mouse brain and have been recovered as type 1 organisms after death. When introduced into the naso-pharynx of the marmoset, they immediately acquired agglutinogen 2 or 3, and the resulting type 1,2 or 1,3 infection persisted for many weeks.

As in the child, agglutinogens 2 and/or 3 appear to be essential for infection of the marmoset, whereas they are quite unnecessary in mouse brain. A vaccine (extract or whole cell) containing agglutinogen 1 may be sufficient to pass the mouse protection test but it may fail to immunize children. The mouse test is inadequate even for the screening of such extracts.

## INTRODUCTION

The type-specific pertussis agglutinogens (2 and/or 3) appear to be essential to infection of the child: type 1 organisms are rarely recovered from cases of whooping-cough, and never predominate (Preston & Stanbridge, 1972). Similarly in the marmoset (Stanbridge & Preston, 1974*a*) type 1 mutants, from an initial experimental infection with type 1,2,3 or type 1,2 or type 1,3, appear only at a late stage of the infection. They seem to be a degraded form of the bacterium, heralding its final elimination from the host.

In contrast, type 1 organisms are among the most virulent for infection of mouse brain (Preston, 1966). We therefore decided to study their fate when deliberately inoculated into the marmoset naso-pharynx and into mouse brain.

## MATERIALS AND METHODS

### *Strains of* Bordetella pertussis

Three strains of type 1 were used, each of them being preserved in the lyophilized state. One was the type 1 component (Stanbridge & Preston, 1974*b*) of a mixed culture, 41633, isolated in Coventry during the whooping-cough survey of the Public Health Laboratory Service (1969). The second was the standard challenge strain for mice, W.18-323, which in our laboratory is a type 1 strain

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(Preston, 1966). The third, 353/Z, was another mouse-virulent strain (Preston & Evans, 1963) which was confirmed by Eldering, Holwerda & Baker (1966) as a culture possessing only factor 1.

The serotyping of single colonies in pertussis cultures has been described in detail previously (Stanbridge & Preston, 1974*b*).

*Naso-pharyngeal infection of marmosets*

The experiments were performed on a number of common marmosets bred in our laboratory by Mr E. H. Hutton. Details of their inoculation and swabbing have been given previously (Stanbridge & Preston, 1974*a*).

*Intracerebral infection of mice*

Mice of the Manchester strain, inbred from the Schofield strain, were inoculated (Kendrick, Eldering, Dixon & Misner, 1947) with a dose of *ca.* 200 LD 50 of *Bord. pertussis* (strain W.18-323 or 353/Z).

## RESULTS

### *Type* 1 *strains of* Bordetella pertussis *in the marmoset naso-pharynx*

Seven marmosets were inoculated into the naso-pharynx with a type 1 culture of *Bord. pertussis*. Three of them (Table 1) received the type 1 variant of a strain (41633) isolated from a child. Within two days, type 1,2 organisms were detected in every animal and, by four days, this had become the predominant serotype. A further change to type 1,3 occurred in one marmoset before the infections were finally eliminated after 7–8 weeks.

Table 2 shows that, when marmosets were inoculated with the standard challenge strain for mice (W.18-323), an even more rapid change of serotype occurred. By the next day, a mixture of type 1,2 and type 1,3 had emerged in one marmoset, though type 1 still predominated in the other. By the second day, type 1,3 organisms predominated in both animals, and these persisted for 5 weeks in one and 19 weeks in the other.

With the other mouse-virulent strain (353/Z), only type 1 organisms were found in specimens taken from the marmosets within a few hours of inoculation (Table 3). But by two days type 1,2 had appeared in both animals, and by four days it had become the predominant serotype. Subsequently, type 1,3 was recovered from both animals before the final elimination of *Bord. pertussis* after 6–7 weeks.

Thus, all three strains of type 1 behaved in a similar way in the marmoset. Within 1–2 days of inoculation, strains possessing agglutinogen 2 or 3 had emerged; and, within 1–4 days of inoculation, these new serotypes predominated – as in natural pertussis infection in the child.

### *Type* 1 *strains of* Bordetella pertussis *in mouse brain*

Table 4 shows that the fate of strains 353/Z and W.18-323 is very different in the mouse. In every one of 17 mice that died within the two-week period of observation adopted in the potency testing of pertussis vaccine, *only* type 1

Table 1. *Detailed serotyping of* Bordetella pertussis *recovered with pernasal swabs from three marmosets inoculated with a type* 1 *variant of a strain* (41633) *freshly isolated from a child*

(No. of colonies of each serotype at period 'T' after inoculation. After the first week, each marmoset was usually swabbed two or three times per week. About 8 colonies were tested from each primary culture.)

| Marmoset ... ... | M 16 | | | | M 25 | | | | M 51 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serotypes of colonies tested ... | 1,2,3 | 1,2 | 1,3 | 1 | 1,2,3 | 1,2 | 1,3 | 1 | 1,2,3 | 1,2 | 1,3 | 1 |
| 1 day | 0 | 1 | 0 | 7 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 8 |
| 2 days | 0 | 1 | 0 | 7 | 0 | 2 | 0 | 6 | 0 | 1 | 0 | 7 |
| 3 days | 0 | 3 | 0 | 5 | 0 | 2 | 0 | 6 | 0 | 6 | 0 | 2 |
| 4 days | 0 | 5 | 0 | 3 | 0 | 6 | 0 | 2 | 0 | 6 | 0 | 2 |
| 7 days | 0 | 8 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 8 | 0 | 0 |
| 2nd wk | 1 | 23 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 22 | 1 | 1 |
| 3rd wk | 0 | 11 | 0 | 1 | 0 | 12 | 0 | 0 | Nil recovered | | | |
| 4th wk | 0 | 6 | 9 | 1 | 0 | 8 | 0 | 8 | 0 | 16 | 0 | 0 |
| 5th wk | 0 | 1 | 15 | 0 | 0 | 11 | 0 | 4 | 0 | 16 | 0 | 0 |
| 6th wk | 1 | 0 | 15 | 0 | Nil recovered | | | | 0 | 16 | 0 | 0 |
| 7th wk | 0 | 0 | 15 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 3 |
| 8th wk | Nil recovered | | | | 0 | 0 | 0 | 2 | Nil recovered | | | |

Table 2. *Detailed serotyping of* Bordetella pertussis *recovered with pernasal swabs from two marmosets inoculated with the type* 1 *strain W*.18-323 *used for intracerebral challenge in mice*

(No. of colonies of each serotype at period 'T' after inoculation. See note to Table 1.)

| Marmoset ... ... | M 55 | | | | M 56 | | | |
|---|---|---|---|---|---|---|---|---|
| Serotypes of colonies tested ... | 1,2,3 | 1,2 | 1,3 | 1 | 1,2,3 | 1,2 | 1,3 | 1 |
| 1 day | 0 | 0 | 1 | 7 | 0 | 3 | 4 | 0 |
| 2 days | 0 | 0 | 6 | 2 | 0 | 0 | 7 | 1 |
| 3 days | 0 | 2 | 6 | 0 | 0 | 0 | 7 | 1 |
| 6 days | 0 | 3 | 5 | 0 | 0 | 0 | 8 | 0 |
| 7 days | 0 | 3 | 5 | 0 | 0 | 1 | 6 | 1 |
| 2nd wk | 1 | 4 | 11 | 0 | 0 | 0 | 11 | 0 |
| 3rd wk | Nil recovered | | | | 0 | 0 | 1 | 0 |
| 4th wk | 0 | 16 | 0 | 0 | 0 | 0 | 13 | 1 |
| 5th wk | 0 | 0 | 1 | 0 | 0 | 0 | 8 | 0 |
| 6th wk | Nil recovered | | | | Nil recovered | | | |
| 7th wk | Nil recovered | | | | Nil recovered | | | |
| 8th wk | 0 | 0 | 14 | 0 | Nil recovered | | | |
| 9th wk | 0 | 1 | 10 | 0 | Nil recovered | | | |
| 10th wk | 0 | 0 | 5 | 0 | | | | |
| 11th wk | 0 | 3 | 21 | 0 | | | | |
| 12th wk | 0 | 6 | 9 | 0 | | | | |
| 13th wk | 0 | 3 | 13 | 0 | | | | |
| 4th month | 0 | 1 | 31 | 0 | | | | |
| 5th month* | 0 | 0 | 5 | 0 | | | | |

* *Bord. pertussis* last recovered during 19th week after inoculation.

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Table 3. *Detailed serotyping of* Bordetella pertussis *recovered with pernasal swabs from two marmosets inoculated with the mouse-virulent type* 1 *strain* 353/Z

(No. of colonies of each serotype at period 'T' after inoculation. See note to Table 1.)

| Marmoset ... ... | M 53 | | | | M 54 | | | |
|---|---|---|---|---|---|---|---|---|
| Serotypes of colonies tested ... | 1,2,3 | 1,2 | 1,3 | 1 | 1,2,3 | 1,2 | 1,3 | 1 |
| 4 hours | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 8 |
| 1 day | 0 | 0 | 0 | 8 | 0 | 3 | 0 | 5 |
| 2 days | 0 | 2 | 0 | 6 | 0 | 1 | 0 | 3 |
| 4 days | 0 | 7 | 0 | 1 | 0 | 6 | 0 | 2 |
| 7 days | 0 | 7 | 0 | 1 | 0 | 8 | 0 | 0 |
| 2nd wk | 0 | 19 | 1 | 0 | 0 | 18 | 0 | 2 |
| 3rd wk | 0 | 6 | 3 | 7 | 0 | 13 | 2 | 1 |
| 4th wk | 0 | 1 | 5 | 15 | 0 | 8 | 11 | 5 |
| 5th wk | 0 | 5 | 0 | 2 | 0 | 0 | 7 | 0 |
| 6th wk | | Nil recovered | | | 0 | 0 | 8 | 0 |
| 7th wk | 0 | 0 | 0 | 8 | | Nil recovered | | |

Table 4. *Detailed serotyping of* Bordetella pertussis *recovered from the brains of mice which died after intracerebral inoculation with the type* 1 *strains* 353/Z (10 *mice*) *and W*.18-323 (8 *mice*)

(No. of colonies of each serotype in mouse dying on *N*th day after inoculation. Each row of four figures indicates the serotyping results for one mouse.)

| Inoculum ... ... | 353/Z | | | | W.18-323 | | | |
|---|---|---|---|---|---|---|---|---|
| Serotypes of colonies tested ... | 1,2,3 | 1,2 | 1,3 | 1 | 1,2,3 | 1,2 | 1,3 | 1 |
| 6th day | (None died) | | | | 0 | 0 | 0 | 1* |
| | | | | | 0 | 0 | 0 | 1* |
| 7th day | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 1* |
| | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 1* |
| | 0 | 0 | 0 | 5 | | | | |
| 8th day | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 1* |
| | 0 | 0 | 0 | 4 | | | | |
| 9th day | 0 | 0 | 0 | 1* | 0 | 0 | 0 | 1* |
| | 0 | 0 | 0 | 1* | | | | |
| 10th day | 0 | 0 | 0 | 6 | (None died) | | | |
| 11th day | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 8 |
| | 0 | 0 | 0 | 8 | | | | |
| 19th day | (None died) | | | | 0 | 0 | 4 | 9 |

* Test performed on confluent growth from brain, not on subcultures of individual colonies.

organisms were detected. Even in the mouse which died during the third week after inoculation with W.18-323, there was still a predominance of type 1, though some type 1,3 had emerged.

Thus, whilst marmosets had converted to predominantly type 1,2 or type 1,3 infection within 1–4 days of inoculation, mice died with a persistent type 1

infection after 6–11 days. Agglutinogens 2 and/or 3 appeared to be necessary for infection of the marmoset naso-pharynx but unnecessary in mouse brain.

## DISCUSSION

### *Differences between pertussis infection in primate and mouse*

Strains of *Bordetella pertussis* freshly isolated from the child possess one or both of the agglutinogens 2 and 3 (types 1,2,3; 1,2; 1,3), and all can colonize the marmoset naso-pharynx (Stanbridge & Preston, 1974*a*). Type 1 (devoid of factors 2 and 3) never predominates in the child. In our present study, we have inoculated marmosets with type 1 organisms – as nearly pure as possible for a species with such a potentially high mutation rate (Stanbridge & Preston, 1974*b*). These have failed to colonize the marmoset as type 1; but variants, of type 1,2 or type 1,3, rapidly appeared and established prolonged colonization for 5–19 weeks.

Conversely, fresh isolates of all serotypes are usually of very low virulence in mouse brain (Andersen, 1952; Standfast, 1958; Preston & Evans, 1963). Only occasional, atypical, laboratory strains are mouse-virulent, and these may be of type 1. Cameron (1967) has already noted the stability of serotypes during the course of an infection in mouse brain. Our present findings agree with his: we inoculated mice with type 1 mouse-virulent strains and recovered only type 1 organisms from their brains after death. Factors 2 and 3 seemed to be unnecessary for mouse-virulence.

In contrast, on the few occasions when cultures recovered *post mortem* from a child have been examined in this laboratory, they have all been of type 1,2,3 or 1,3 (Preston, unpublished).

### *The importance of agglutinogen* 1 *in mouse brain*

It is convenient to consider the major agglutinogen of type 1 strains as if it were a single entity, though this is probably an over-simplification. Nakase, Takatsu & Kasuga (1969) showed, in their table 3, serological evidence of two components. Ross & Munoz (1971) achieved a similar separation by electrophoresis, and their non-migratory component was associated with mouse protection (see their Figure 3). Moreover, contrary to their conclusions, their table 1 shows a good correlation between mouse protection and content of agglutinogen 1. It was histamine sensitization, not mouse protective activity, which their results showed to be poorly related to agglutinogen content.

Virulence for mouse brain may perhaps depend on one of these components of agglutinogen 1 or, more probably, on some other factor. Lack of mouse-virulence, in strains freshly isolated from the child, does not appear to result merely from a masking of the effect of factor 1 by factor 2 or 3: we found that the type 1 variant of strain 41633 was avirulent for mouse brain even in a dose of 5 million organisms. Nevertheless, whatever the property on which mouse-virulence depends, vaccines of avirulent strains will *protect* mice against intracerebral challenge. Moreover, only agglutinogen 1 is necessary for such protection (Eldering *et al.* 1966; Preston, 1966; Dolby & Bronne-Shanbury, 1975).

Cameron (1967) argued against the prime importance of agglutinogen 1 in the mouse. He claimed that strains of type 1 had 'little immunizing power' whereas vaccines of type 1,2,3 or 1,2 or 1,3 'protected highly'. Actually, his table I showed that it was only types 1,2,3 and 1,2 that gave potent vaccines; but type 1 and *also* type 1,3 vaccines provided poor protection. This is not surprising, for type 1 strains and most type 1,3 strains react more feebly with antibody 1 than do type 1,2,3 and 1,2 strains (Preston, 1965, 1966, 1975*a*). If, then, type 1 and 1,3 strains have less agglutinogen 1 than strains of types 1,2,3 and 1,2, Cameron's evidence is not only consistent with but even confirms the important role of agglutinogen 1 in mouse brain.

### *Agglutinins and immunity – pertussis folklore*

The stromata protective antigen (SPA) of Pillemer, Blum & Lepow (1954) was reported by the Medical Research Council (1959) to give good protection to both mouse and child but 'only a relatively poor agglutinin response'. This response could have been expected if the differences between the SPA strain (type 1,3) and those used for agglutination tests and vaccine production (mostly type 1,2) had been appreciated at the time (see Preston & Te Punga, 1959; Preston, 1966). Preston & Te Punga showed that, against a type 1,2 suspension, anti-SPA serum had only one-quarter of the titre possessed by serum prepared against type 1,2 vaccine, when both sera were diluted to have the same content of antibody 1.

Nowadays, the word 'relatively' in the Medical Research Council (MRC) report needs more emphasis, because agglutinin *was* produced by SPA. Indeed, the titres in SPA-vaccinated children were more than a quarter of the *highest* titres given by any vaccine in the trial (MRC, 1959 – table VII). Moreover, SPA (V17) produced *better* agglutinin titres in *children* than the whole bacterial vaccine (V16) with which it was compared in Liverpool children; and it was found to give them better protection. This relatively *good* (!) agglutinin response to SPA would be antibody 1 – antibody detected by agglutination of a type 1,2 strain with sera of children who had received type 1,3 SPA. The SPA would also have stimulated a good response to factor 3, thus enabling these children to cope with the emergence of type 1,3 variants better than those who received type 1,2 vaccine.

Another misleading statement was made by Dolby & Stephens (1973), claiming that they were 'unable to relate immunity of the child to the titres...of agglutinins'. Three comments are necessary: first, only titres of antibodies 2 and 3 (not 1) were recorded; secondly, they gave no evidence of the serotype to which *each* child was exposed, but merely a suggestion that type 1,3 was prevalent; thirdly, there was no evidence that *any* child who developed clinical disease had antibody 3 when exposed, and this would be the most relevant agglutinin for countering a type 1,3 infection.

### *The mouse protection test*

What then of the mouse brain test, with its undue dependence on the factor 1 content of the vaccines that it is testing? A good correlation between potency for mouse and man can be expected if human infection is of type 1,2,3 or 1,2 – strains

that are rich in factor 1. Indeed, vaccine which has passed the mouse test *does* seem to be effective against such infection. This was so, even in the gloomy 1960s (Preston & Stanbridge, 1972), and *before* the re-introduction of adjuvant or the raising of the mouse-potency from 2 to 4 units (Perkins, 1969). Those vaccinated children were rarely infected with type 1,2,3 or 1,2 but they had been inadequately protected against type 1,3 – strains with a low content of factor 1. For protection against this serotype, antibody 3 is needed too; and the mouse test will give little indication of the content of this antigen in a vaccine, since mouse infection is largely independent of antigens 2 and 3.

In the MRC trials, Pillemer's SPA was used in very large doses, causing a disturbing number of adverse reactions (MRC, 1959). Presumably because of this dosage, it *did* produce quite respectable levels of antibody 1 in the child, even though it was made from a type 1,3 strain; and this antibody 1 would protect a child from type 1,2,3 or 1,2 infection. The SPA would have produced even higher levels of antibody 3 (Preston & Te Punga, 1959) and would therefore be effective against type 1,3 mutants also. That may explain why it was even more effective in children than many of the type 1,2 vaccines in the trial. Even in mice also, it *did* produce agglutinin – more than was produced by the poor vaccines which protected fewer than 50 % of vaccinated children – and this would explain its good performance in the mouse test. Moreover, the higher titres of agglutinin produced by some of the other good vaccines were probably of antibody 2 – not related to mouse protection.

### *Potency testing of pertussis vaccine*

Our present study confirms and extends our previous discovery (Stanbridge & Preston, 1974*a*) of strong similarities between marmoset and child infection, in which the three agglutinogens play an important role. Prevention of such infection requires immunity against the three serotypes, not merely against factor 1. It is probable that either antibody 1 or 2 will protect against types 1,2,3 and 1,2 (strains rich in both 1 and 2 antigens). But against type 1,3 strains, with their low content of antigen 1, antibody 3 is required as well; and the mouse test cannot assure us of a vaccine's ability to provide this. Vaccines must therefore be shown to have an adequate content of the necessary agglutinogens (Preston, 1975*a*, *b*).

### *The search for a good extract vaccine*

At a time when a search is being made for a protective extract of *Bord. pertussis* that will minimize the incidence of adverse reactions, it cannot be too strongly emphasized that the mouse test is an unreliable indicator of success. An agglutinogen extract, capable of protecting children, would *probably* protect the mouse also. But the converse may not be true: if it contained only antigen 1 (or 1 and 2), it may well protect the mouse but not the child. There is even a theoretical possibility, also, that an extract may contain antigens 2 and 3, but not 1; and this may be excellent in the child but fail to pass the sacred mouse test!

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