Responses to *Bordetella pertussis* mutant strains and to vaccination in the coughing rat model of pertussis

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Summary. Phase I strains 18-323, Tohama and L-84 of *Bordetella pertussis* produced paroxysmal coughing when encased in agarose beads and administered intrabronchially to adult Sprague–Dawley rats. In contrast, the Phase IV variant of strain L-84 was inactive in cough induction, as was strain BP 357, a transposon-insertion mutant which is deficient only in pertussis toxin (PT). Strain BPM 1809, which lacks only the heat-labile toxin, was similar to the unmodified Phase I strains for cough induction, indicating that this toxin is not needed to induce coughing. *B. parapertussis* also was inactive as a cough inducer. These results indicate that PT, present in Phase I strains of *B. pertussis*, and absent from Phase IV strains, strain BP 357 and *B. parapertussis*, is essential for the induction of paroxysmal coughing in this rat model of whooping cough. Prior injection of DTP (whole-cell) vaccine greatly reduced the incidence of coughing in rats challenged subsequently with Phase I *B. pertussis*. Serological responses were monitored after intrabronchial infection with the various bacterial strains and after vaccination and challenge. The PT-positive or -negative status of the strains *in vivo* was confirmed by the appropriate presence or absence of anti-PT IgG in the convalescent sera.

Introduction

Despite recent advances in knowledge of the individual virulence factors of *Bordetella pertussis*,^{1–3} and their regulation by the *bvg* gene,⁴ much remains to be learned about the basic mechanisms of pathogenesis and immunity in whooping cough. For example, the bacterial components responsible for the paroxysmal cough have not yet been identified. Nor is it decided how many and which antigens, additional to pertussis toxoid (PTd), should be included in the new pertussis vaccines currently being investigated as replacements for the traditional whole-cell preparations.^{2,3,5,6} Study of the cough and the factors responsible is important because of its central role in the pathogenesis of the disease. Most complications and deaths occur during the paroxysmal stage. Respiratory complications and central nervous system disturbances are relatively frequent and due mainly to pressure effects and anoxia, both resulting from the severe coughing.^{7–9}

A major obstacle to progress in these areas has been the lack of an accessible animal model in which whooping cough caused by *B. pertussis* can be reproduced. Paroxysmal coughing has been reported in some but not other species of primate,^{10,11} but the mouse and the rabbit, although able to sustain a sublethal respiratory tract infection with *B. pertussis*,^{12,13} do not respond in this fashion. However, the mouse does show the lymphocytosis and hyper-

insulinaemia which occur in the human disease.^{12,14} The intranasally-infected mouse has also been valuable for defining the contribution of individual virulence factors to colonisation and lethal infection;¹⁵ but mice do not cough.

Therefore, it was of considerable interest when Woods *et al.*,¹⁶ in 1989, reported that adult Sprague–Dawley rats, after infection with *B. pertussis* strain Tohama embedded in agar beads, produced two of the cardinal signs of pertussis, namely intermittent paroxysmal coughing and leukocytosis. Fifty years before this, Hornibrook and Ashburn¹⁷ had noted that rats do indeed cough when given *B. pertussis* intranasally, but their paper was overlooked by subsequent investigators.

We have confirmed and extended^{18,19} the observations of Woods *et al.*¹⁶ and Hornibrook and Ashburn¹⁷ on the induction of paroxysmal coughing in rats infected with *B. pertussis*. However, in our work the agar for the beads was replaced by low-gelling temperature agarose; the *B. pertussis* strain Tohama was replaced by strain 18-323, the standard challenge organism for the intracerebral mouse protection test;²⁰ and cough production was quantified by keeping the infected rats in sound-insulated booths equipped with sensitive sound-activated tape recorders.

In the present study, the production of coughing by Phase I strains other than 18-323, by a Phase IV variant,²¹ by transposon-insertion mutants of *B. pertussis* lacking pertussis toxin (PT) and heat-labile toxin (HLT), and by *B. parapertussis* were explored. The

protective effect, against coughing, of prior injection of whole-cell pertussis vaccine was also investigated and the antibody responses in convalescent sera from the variously infected animals were measured.

Materials and methods

Bacterial strains

The following strains of *B. pertussis* were used: 18-323, Phase I (NCTC 10739); Tohama Phase I (kindly provided by Dr C. R. Manclark, Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892, USA); L-84 Phase I (NCTC 11089); L-84 Phase IV (NCTC 10902); BP 357 (*ptx::Tn5*)²² and BPM 1809 (*dtn::Tn5lac*)²³ (kindly provided by Dr A. Weiss, Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA 23298, USA). A single strain of *B. parapertussis* (NCTC 10520) was also employed. All the strains were grown and maintained as described previously.¹⁹

Infection and monitoring of rats

The methods for encasing bordetellae in fine beads (c. 100 µm diameter) of low-gelling temperature agarose, or agarose plus carrageenan, for administering the beads intrabronchially to anaesthetised adult male Sprague-Dawley rats, and also for housing the animals in sound-insulated booths and recording the occurrence of paroxysmal coughing with sound-activated tape recorders were described previously.¹⁹ In all experiments, the infecting dose was c. 10⁸ cfu of bordetellae/rat. The viscous suspension (0.1 ml) of beads containing the bacteria was delivered deep into the left bronchus *via* a bent, bead-tipped needle inserted through a tracheotomy while the rat was anaesthetised with a mixture of Hypnorm and Hypnovel. On completion of the operation, which took c. 6 min/animal, the rats were subjected to a 2-min period of inhalation of ether to promote subsequent cough production.¹⁹ Control animals were either given sterile beads or were left completely untreated. In the present study, sound recordings were made only during the evenings of days 9–14 after infection, as this was the period in which most coughing was expected to occur.

Vaccination

Rats (6 weeks old; c. 200 g body weight) were each given a single human dose (0.5 ml) of adsorbed diphtheria-tetanus-pertussis (DTP)-vaccine containing killed whole *B. pertussis* (Wellcome Trivax-AD) by intraperitoneal injection and challenged 3 weeks later with the usual infecting dose of *B. pertussis* strain 18-323. The animals were then observed for coughing as described above.

Serology

The methods for making the coating antigens for ELISA tests, i.e., whole-cell sonicates of *B. pertussis* strain 18-323 and of *B. bronchiseptica* NCTC 5376, and purified preparations of pertussis toxin (PT) and filamentous haemagglutinin (FHA) from *B. pertussis*, were described in detail previously.¹⁹ Similarly, the methods for obtaining and evaluating the IgG ELISA results have already been documented.¹⁹

Results

Cough production by various *Bordetella* strains

As a continuation of previous studies,^{18,19} the present experiments examined the cough-producing potential of *B. pertussis* strains other than 18-323. However, this strain was also included as a known positive control. A single strain of *B. parapertussis* was also tested. Thus, seven *Bordetella* strains were tested in a total of 110 rats, together with 24 control animals which were either untreated or given sterile beads. The observations of subsequent coughing in the infected animals, in groups of eight/recording booth, were made on the evenings of days 9–14 (the period of peak coughing) after infection, with audio tapes of 45-min capacity. The results (table I) are expressed as the total number of coughing paroxysms/rat during the 6 days of observation.

Table I shows that the *Bordetella* strains fell into two distinct categories: those that induced paroxysmal coughing and those that did not. Like strain 18-323, the two other Phase I strains, Tohama and L-84, induced coughing as did strain BPM 1809, the HLT-deficient transposon-insertion mutant. The strains that induced much less, or no, coughing were the L-84 Phase IV mutant, the PT-deficient strain and *B. parapertussis*. These observations indicated that paroxysmal cough production was not simply the result of

Table I. Comparison of the effect of Phase I *B. pertussis*, *B. pertussis* mutant strains and *B. parapertussis* on cough production in rats

Rat treatment*	Number of rats	Sum of coughing paroxysms/rat (days 9–14 post-infection)
<i>B. pertussis</i>		
18-323 (Phase I)	32	10.59
Tohama (Phase I)	15	6.86
L84 (Phase I)	8	6.9
L84 (Phase IV)	16	0
BP 357 (PT ⁻)	15	0.96
BPM 1809 (HLT ⁻)	16	10.3
<i>B. parapertussis</i>		
10520	8	1.25
Sterile beads	8	0
Untreated	16	0.06

* Intrabronchial inoculation of organisms in beads containing agarose and carrageenan.

Table II. Effect of vaccination on cough production by Phase I *B. pertussis* strain 18-323 in rats

Rat treatment	Number of rats	Sum of coughing paroxysms/rat (days 9-14 post-infection)
Infected*	23	6.14
Infected†	8	4.0
Vaccinated then infected*	18	1.68
Vaccinated then infected†	7	0.14
Sterile beads	8	0.5
Untreated	8	0

* Intrabronchial inoculation of strain 18-323 in beads containing agarose and carrageenan or
 † agarose alone. Sterile beads contained agarose and carrageenan.

having live bordetellae in the lungs but required strains of *B. pertussis* that were able to produce PT. The rats which were given sterile beads or left completely untreated showed no or only minimal incidence of coughing (or sounds that were interpreted as coughs).

Effect of vaccination

Vaccination and challenge experiments with a total of 72 rats were made with adsorbed DTP (whole-cell) vaccine. A single intraperitoneal dose of 0.5 ml in 6-week-old rats of *c.* 200 g, was followed 3 weeks later by challenge with 10^8 cfu of *B. pertussis* strain 18-323. This experiment included a separate investigation into whether including carrageenan 1% w/v in the agarose 1% w/v beads had any promotional effect on subsequent cough production. The results (table II) show that vaccination had a pronounced protective effect against cough production after intrabronchial challenge; the sum of paroxysms fell from 6.14 to 1.68 in the carrageenan experiments and from 4 to 0.14 in the series without this additive. Carrageenan appeared to increase the incidence of paroxysmal coughing in infected rats. Control animals given sterile beads or left untreated again showed no, or only minimal, coughing.

Serological responses to infection

The serological responses of the rats to infection were measured by ELISA of individual sera taken at the end of each experiment, on day 28 after challenge. IgG antibodies were assayed over a dilution range from 1 in 10 to 1 in 100 000 and with four different antigens: PT, FHA and whole-cell sonicates of *B. pertussis* and *B. bronchiseptica*. The latter was included to explore possible exposure of the rats to *B. bronchiseptica* either before or during the experiments. The antibody responses for each group of sera with each of the four ELISA antigens are summarised in table III as: (a) the number of sera positive/number tested; (b) the median titre of the group; (c) the range of titres within the group; (d) the 95% confidence limits (CL) of the median for each group; and (e) the significance

(probability value) of the difference between this median and that of Group 1 (18-323 challenge), as determined by the Mann-Whitney U-test.

Table III shows that a strong anti-PT response was produced after infection with each of the four strains of *B. pertussis* known to produce PT, i.e., strains 18-323, Tohama, L-84 Phase I and BPM 1809. With all these strains, the proportion of IgG responders to PT (titre ≥ 10) was not less than 87% and the median titres varied between 800 and 3850. Within these groups, the individual rat titres ranged widely, from < 10 in a few animals up to $> 100\,000$. In contrast, the sera from rats infected with the PT-deficient strains, i.e., L-84 Phase IV, BP 357 and *B. paraptussis*, contained none, or at most one responder out of 15 (7%). This was not significantly different from the titres of sera from the control animals.

With FHA as the ELISA coating antigen, strong serological responses were observed in sera from rats infected with two of the three Phase I strains of *B. pertussis*, the two transposon mutants and *B. paraptussis*. However, with strain Tohama, the median titre (15) and proportion of responders (8 of 15) were much lower than with any of the other Phase I strains, although not as low as with strain L-84 Phase IV with which the median titre was < 10 and the proportion of responders was one of 15.

When *B. pertussis* whole-cell sonicate was used as the ELISA coating antigen, there was a high proportion of positive sera in all groups which had received beads containing any of the *B. pertussis* strains. The proportion of responders ranged from 73% (11 of 15) with strain Tohama up to 100% with strains 18-323, L-84 Phases I and IV and BPM 1809. With *B. paraptussis* three of eight rats were responders and the median titre was < 10 , which was much lower than that with any of the *B. pertussis* strains and not significantly above the background level of titres of the control animals.

With *B. bronchiseptica* sonicate as the ELISA coating antigen, the strongest immune responses were observed in sera from rats which had been infected with *B. paraptussis*. In this group of eight rats,

Table III. Summary of IgG ELISA titres of sera from rats infected 28 days previously with various strains of *B. pertussis* and *B. paraptussis* and tested with coating antigens consisting of PT and FHA from *B. pertussis* or sonicates of *B. pertussis* (BP) and *B. bronchiseptica* (BB)

Group no.	Rat treatment†	ELISA titre with coating antigen							
		PT		FHA		BP		BB	
		sera positive	median‡ (range)	sera positive	median‡ (range)	sera positive	median‡ (range)	sera positive	median‡ (range)
		sera tested	[95 % CL]	sera tested	[95 % CL]	sera tested	[95 % CL]	sera tested	[95 % CL]
1	<i>B. pertussis</i> 18-323 (Phase I)	<u>31</u> 32	800 (< 10 , $> 100\,000$) [600, 2600]	<u>32</u> 32	9200 (290, $> 100\,000$) [1999, 23002]	<u>32</u> 32	850 (200, $> 100\,000$) [460, 1700]	<u>21</u> 32	125 (< 10 , 25000) [< 10 , 320]
2	<i>B. pertussis</i> Tohama (Phase I)	<u>14</u> 15	3000 (< 10 , $> 100\,000$) [705, 27263]	<u>8</u> 15	15** (< 10 , 1200) [< 10 , 259]	<u>11</u> 15	300** (< 10 , 1500) [60, 424]	<u>3</u> 15	< 10 ** (< 10 , 170) [< 10 , < 10]
3	<i>B. pertussis</i> L84 (Phase I)	<u>7</u> 7	700 (150, 2800) [304, 2213]	<u>7</u> 7	500* (280, 3000) [368, 1387]	<u>7</u> 7	300* (160, 1300) [175, 787]	<u>7</u> 7	90 (60, 400) [60, 349]
4	<i>B. pertussis</i> L84 (Phase IV)	<u>1</u> 15	< 10 ** (< 10 , 200) [< 10 , < 10]	<u>1</u> 15	< 10 ** (< 10 , 13) [< 10 , < 10]	<u>15</u> 15	920 (100, 20000) [242, 5313]	<u>15</u> 15	140 (23, 1100) [67, 2724]
5	<i>B. pertussis</i> BP 357 (PT ⁻)	<u>0</u> 15	< 10 ** (< 10 , < 10) [< 10 , < 10]	<u>15</u> 15	790** (300, 1800) [550, 1163]	<u>14</u> 15	260** (< 10 , 1100) [120, 424]	<u>4</u> 15	< 10 ** (< 10 , 95) [< 10 , 7]
6	<i>B. pertussis</i> BPM 1809 (HLT ⁻)	<u>14</u> 16	3850 (< 10 , $> 100\,000$) [783, 7237]	<u>15</u> 16	1125** (< 10 , 3000) [556, 2200]	<u>16</u> 16	300** (120, 900) [245, 433]	<u>1</u> 16	< 10 ** (< 10 , 40) [< 10 , < 10]
7	<i>B. paraptussis</i> 10520	<u>0</u> 8	< 10 ** (< 10 , < 10) [< 10 , < 10]	<u>8</u> 8	1125** (440, 2200) [590, 1826]	<u>3</u> 8	< 10 ** (< 10 , 140) [< 10 , 131]	<u>8</u> 8	652* (290, 2200) [337, 2013]
8	Sterile beads	<u>0</u> 8	< 10 ** (< 10 , < 10) [< 10 , < 10]	<u>0</u> 8	< 10 ** (< 10 , < 10) [< 10 , < 10]	<u>0</u> 8	< 10 ** (< 10 , < 10) [< 10 , < 10]	<u>0</u> 8	< 10 ** (< 10 , < 10) [< 10 , < 10]
9	Untreated	<u>0</u> 21	< 10 ** (< 10 , < 10) [< 10 , < 10]	<u>1</u> 21	< 10 ** (< 10 , 190) [< 10 , < 10]	<u>0</u> 21	< 10 ** (< 10 , < 10) [< 10 , < 10]	<u>5</u> 21	< 10 ** (< 10 , 1300) [< 10 , < 10]

† Rats were infected by intrabronchial inoculation of organisms in beads containing agarose and carrageenan.

‡ Medians were compared by Mann-Whitney *U*-test with the median of Group 1: * $p \leq 5\%$; ** $p \leq 1\%$.**Table IV.** Effect of vaccination on IgG ELISA titres of sera from rats infected with *B. pertussis* 18-323 and tested with coating antigens consisting of PT and FHA from *B. pertussis* or sonicates of *B. pertussis* (BP) and *B. bronchiseptica* (BB)

Group no.	Rat treatment†	ELISA titre with coating antigen							
		PT		FHA		BP		BB	
		sera positive	median‡ (range)	sera positive	median‡ (range)	sera positive	median‡ (range)	sera positive	median‡ (range)
		sera tested	[95 % CL]	sera tested	[95 % CL]	sera tested	[95 % CL]	sera tested	[95 % CL]
1	Infected†	<u>16</u> 23	260 (< 10 , 220000) [49, 2239]	<u>19</u> 23	58 (< 10 , 5500) [31, 185]	<u>12</u> 23	135 (< 10 , 85000) [< 10 , 655]	<u>8</u> 23	< 10 (< 10 , 3800) [< 10 , 167]
2	Infected§	<u>4</u> 6	535 (< 10 , 2000) [< 10 , 1679]	<u>6</u> 6	850* (340, 3500) [397, 3214]	<u>6</u> 6	605 (480, 2700) [505, 2164]	<u>6</u> 6	380* (280, 700) [287, 621]
3	Vaccinated then infected†	<u>17</u> 18	61500* (< 10 , 520000) [7132, 134820]	<u>18</u> 18	7450* (30, 20000) [2000, 8000]	<u>18</u> 18	23000* (4400, 700000) [10470, 109640]	<u>18</u> 18	3150* (110, 90000) [1607, 6482]
4	Vaccinated then infected§	<u>6</u> 6	13000 (60, 27000) [2539, 23429]	<u>6</u> 6	6150* (300, 11000) [1585, 10464]	<u>6</u> 6	10000* (1300, 20000) [3657, 16786]	<u>6</u> 6	2700* (360, 4400) [1017, 4114]
5	Sterile beads	<u>0</u> 7	< 10 * (< 10 , < 10) [< 10 , 10]	<u>0</u> 7	< 10 * (< 10 , < 10) [< 10 , < 10]	<u>1</u> 7	< 10 (< 10 , 13) [< 10 , 3.5]	<u>1</u> 7	< 10 (< 10 , 10) [< 10 , 3]
6	Untreated	<u>0</u> 8	< 10 * (< 10 , < 10) [< 10 , < 10]	<u>0</u> 8	< 10 * (< 10 , < 10) [< 10 , < 10]	<u>0</u> 8	< 10 * (< 10 , < 10) [< 10 , < 10]	<u>0</u> 8	< 10 * (< 10 , < 10) [< 10 , < 10]

† Rats were infected by intrabronchial inoculation of *B. pertussis* strain 18-323 (Phase I) in beads containing agarose and carrageenan or § agarose alone. Sterile beads contained agarose and carrageenan.‡ Medians were compared by Mann-Whitney *U*-test with the median of Group 1: * $p \leq 1\%$.

100 % responded and the median titre was 652. The same high proportion (100 %) of positive sera was found with strains L-84 Phases I and IV with median

titres of 90 and 140 respectively. With strain 18-323 sera 66 % (21 of 32) gave positive results, with a median titre of 125. The results obtained with sera

from groups infected with the other *B. pertussis* strains were not significantly different from the controls given sterile beads or left untreated, in which the median titres were < 10 and the proportion of positive results were none of 8 and five of 21 respectively.

Serological responses after vaccination and challenge

Table IV summarises the ELISA results of the vaccination experiments; the sera being collected 28 days after challenge with *B. pertussis* strain 18-323. Extremely high anti-PT titres (median 61 000; Group 3) were observed in the rats which had been vaccinated and challenged. These were significantly higher than with the challenge-alone animals in which the median was 260 (Group 1). Similar high titres were found in the vaccinated group with FHA and *B. pertussis* and *B. bronchiseptica* sonicates as coating antigens.

Carrageenan was included routinely in the agarose beads in an attempt to enhance cough production but, as noted previously,¹⁹ it appeared to have an immunosuppressive effect on responses to the various antigens. Rats infected with *B. pertussis* strain 18-323 in beads containing both agarose and carrageenan (Group 1) had lower antibody titres to all four antigens than those infected with beads containing agarose alone (Group 2). This effect was not seen after vaccination (Groups 3 and 4).

Discussion

This study and the previous reports^{18,19} provide good evidence of the usefulness of the Sprague-Dawley rat for studies of pathogenesis and immunity in pertussis. We have shown that paroxysmal coughing occurs after infection with three Phase I strains of *B. pertussis* but not with a Phase IV strain, nor with *B. parapertussis*. The most significant finding was the apparently specific requirement for PT for cough induction. Thus strain BP 357, the transposon-insertion mutant which is deficient in PT but is believed to contain a full complement of the other virulence factors,²² was essentially inactive as a cough inducer compared with the other Phase I strains in two independent experiments. Therefore, these results provide direct support for the concept proposed by Pittman^{24,25} of pertussis as a toxin-mediated disease, with PT having a central role.

In contrast, the transposon mutant BPM 1809, that lacks heat-labile toxin but contains PT and the other virulence factors,²³ was as effective a cough inducer as strain 18-323 and the other Phase I strains. This indicated that HLT, although a potent dermonecrotising toxin, is not essential for cough induction. In an infant mouse model in which wild-type *B. pertussis* had a lethal effect, Weiss and Goodwin¹⁴ found that strain BP 357 and *B. parapertussis* were unable to cause a lethal infection whereas strain BPM 1809 was fully virulent. So far, other transposon mutants of *B.*

pertussis, such as those lacking FHA or adenylate cyclase toxin, have not been tested in rats but this would clearly be of interest. Such tests might reveal whether PT is the only virulence factor needed for the induction of paroxysmal coughing or whether it requires other factors. Such information could have implications for the selection of components additional to PTd for inclusion in acellular pertussis vaccines.

Prior injection of rats with a single dose of adsorbed DTP vaccine containing killed whole *B. pertussis* greatly reduced the incidence of coughing in animals challenged subsequently with *B. pertussis* strain 18-323. It would be of great interest to determine the protective effect of PTd and other monocomponent and multicomponent acellular vaccines on cough induction in rats.

It was essential in the above experiments to have control rats that were either given sterile beads by the usual surgical procedure, or were not subjected to any experimental treatment and simply kept as normal animals. In such rats, the occasional isolated cough was recorded, but rarely were such coughs repeated in a paroxysmal fashion. Occasionally, it was difficult to interpret particular sounds, as most of the tape records were not of coughs but of other noises such as rattling the food containers, fighting and squeaking. Sometimes these sounds were coincident with coughing, as the microphone in each recording booth recorded the sounds from all eight rats in the typical group and not the sounds from individual animals. Certainly, we make no claims to having used other than a fairly primitive system of recording the coughing, and considerable development work is desirable, particularly to separate the coughing from other noises by electronic analysis. Also, the sound records were limited by the 45-min capability of the tapes on each evening of recording and by the time required to analyse the tapes from each experiment.

The serological results were of value in showing that a major antigenic stimulus was delivered to the rats by the intrabronchial insertion of live bordetellae encased in agarose beads. That such bacteria multiply *in vivo* was indicated by the previous study¹⁹ in which *B. pertussis* killed by heating at 56°C was much less potent as a stimulator of antibodies than the same dose of live organisms. In the present work, the serology was useful to confirm the PT-negative status (*in vivo*) of strains L-84 Phase IV, BP 357 and *B. parapertussis*. Thus the 28-day coalescent sera from rats infected with these strains had little or no anti-PT IgG, whereas these antibodies were abundantly evident in sera from rats infected with the PT-positive strains. There were a few inconsistent results that we are unable to explain. Examples include the single rat with an anti-PT titre of 200 in the L-84 Phase IV group, and the single untreated animal with an anti-FHA IgG titre of 190. The finding that some control sera reacted with *B. bronchiseptica* sonicate is attributed to cross-reaction with antigens from unrelated environmental bacteria,

because the Sprague-Dawley rats were of the highest quality commercially available in the UK and were accompanied by test reports of freedom from a wide range of rat pathogens including *B. bronchiseptica*.

As with many other systems for reproducing the features of a human disease in an experimental animal, the coughing rat model of pertussis has some limitations. For effective cough induction the bordetellae have to be encased in agarose beads and then delivered intrabronchially *via* a tracheotomy,¹⁹ a procedure much more cumbersome than the aerosol infection of mice, for example. However, the encasement in beads may not be totally different from the human situation in which the bacteria expelled from an active case of pertussis are presumably surrounded by a layer of viscid mucus.⁷⁻⁹

Our impression was that the Sprague-Dawley rats were considerably less susceptible to respiratory tract infection with *B. pertussis* than are mice. Thus, the

infecting dose of strain 18-323 (10^8 cfu/rat) was considerably larger than that normally used for sub-lethal infection of mice¹² and yet it did not kill any of the animals. As with mice, the infected rats did not appear to transmit *B. pertussis* infection to other rats in the same room. However, the direct experiment of looking for transmission of infection from an infected to a control animal housed in the same cage has not yet been done. In conclusion, although much remains to be developed and defined with the coughing rat model of pertussis, the system appears to have potential utility for further studies on mechanisms of cough production and immunity in whooping cough, including the investigation of virulence factors, acellular pertussis vaccines and antitussive agents.

We thank Mrs Berit Adam for performing the ELISA tests and Mr G. N. Fellah for his assistance. This work was supported by research grant K/MRS/50/C1494 from the Scottish Home and Health Department.

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