

## The intranasal infection of mice with *Bordetella pertussis*

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### INTRODUCTION

Since Proom (1947), Cooper (1952), Andersen (1953) and Fisher (1955) published their work on the course of infection with *Bordetella pertussis* in the lungs of normal and immune mice as indicated by the number of viable bacilli in the lungs, it has become evident that infection in the lungs of mice is quite different from infection in the brain.

Dolby & Standfast (1958) and Dolby (1958) showed that protection against challenge by the intranasal and intracerebral routes is governed by different antibodies operating in different ways. Standfast (1958) also showed that the potency of vaccines assayed by challenge by the lethal intranasal route is different from the potency assayed by the intracerebral route.

Two types of challenge by the intranasal route have been used by different workers, a lethal (Burnet & Timmins, 1937; Standfast, 1958) and a sublethal (Fisher, 1955; Andersen & Bentzon, 1958). Andersen & Bentzon (1958), estimating the potency of pertussis vaccine by making lung smears from mice after sublethal infection by the intranasal route, obtained values similar to those from assays using the intracerebral route.

The course of disease in both normal and protected mice after infection by the intranasal route is reported in this paper. The findings confirm and extend the results of previous workers, indicate that the lethal and sublethal intranasal challenges may be measuring two different antibodies, and suggest a mode of action of the antibody which protects mice against the lethal challenge by the intranasal route.

### METHODS

#### *Strains used for challenge*

*Bordetella pertussis*, strains C2621 and G353, after passage through mouse lungs, were freeze-dried. An ampoule of dried culture was opened each week and suspensions were made by emulsifying the growth from Bordet-Gengou plates after 20 hr. at 37°, and diluting the emulsion to a standard opacity by the N.I.H. ground glass standard for *B. pertussis*. The routine lethal challenge was  $200 \times 10^6$  organisms and the sublethal challenge was usually 10,000 organisms. Between 10 and 20% of the organisms in suspension were viable.

All dilutions were made in 1% (w/v) Difco-Casamino acids.

*Active immunization tests*

Vaccines were injected by the intraperitoneal route in 0.2 ml. volumes 10 days before the intranasal challenge (c. 1000 LD<sub>50</sub>) which was given in a volume of 0.04 ml. under light anaesthesia (chloroform + ether, 1 + 3).

*Passive protection tests*

Rabbit antisera, previously tested for activity by determining the PD<sub>50</sub> dose (protective dose 50%: the dose of serum in ml. which protects 50% of the treated animals), against an intranasal challenge (c. 1000 LD<sub>50</sub>) and an intracerebral challenge (c. 100 LD<sub>50</sub>) (Dolby & Standfast, 1958), were mixed with equal volumes of a double strength challenge suspension and 0.04 ml. of the mixture instilled by the intranasal route under light anaesthesia.

*Viable counts*

At intervals, groups of 5 or 10 mice were killed with coal gas, the lungs from each mouse were removed individually to 10 ml. 1% (w/v) Casamino acids in a McCartney bottle containing 2 ml. of glass beads (4–5 mm. diam.) and shaken in a vertical shaker 2½ in. throw, 325 r.p.m. for 15 min. Mice dying from the infection on the day of the count were included. Serial tenfold dilutions were made in Casamino acids and counts made with a 50-drop pipette on to pre-dried Bordet–Gengou plates according to the method of Miles & Misra (1938). The counts are reported as the geometric mean of the individual counts of the lungs in each group.

*Toxicity tests*

Graded doses of whole bacterial cells or of an extract of bacterial cells crushed in a Hughes Press (Hughes, 1951) were given by either the intracerebral, intravenous, intraperitoneal or intranasal routes to groups of 10 mice at each of four tenfold dose levels. The toxic dose (TD<sub>50</sub>) was calculated by the Reed & Muench (1938) method on the 3rd day.

*Definitions and abbreviations*

Throughout this paper 'intranasal' antigen means the antigen which elicits protection in mice against a challenge by the intranasal route; 'intranasal' antibody means antibody which protects mice against a challenge by the intranasal route; and similarly for 'intracerebral' antigen and 'intracerebral' antibody. The route of administration of antigen, challenge or serum is always referred to as the 'Intracerebral route', etc.; *IC*, *IN* and *IP* are used as abbreviations for intracerebral, intranasal and intraperitoneal.

## RESULTS

*The growth of Bordetella pertussis in the mouse's lung*

*B. pertussis* by the intranasal route multiplied in the lungs of mice without difficulty even from small inocula, although it is not a natural pathogen for mice. The number of bacilli in the lung was critical at a figure of  $10^8$ – $10^9$  viable organisms. When the count reached this level the mouse died. When it failed to reach this level the mouse recovered though it was not immediately freed from the infecting organism; in many cases the mouse's lung harboured small numbers of viable *B. pertussis* for months.

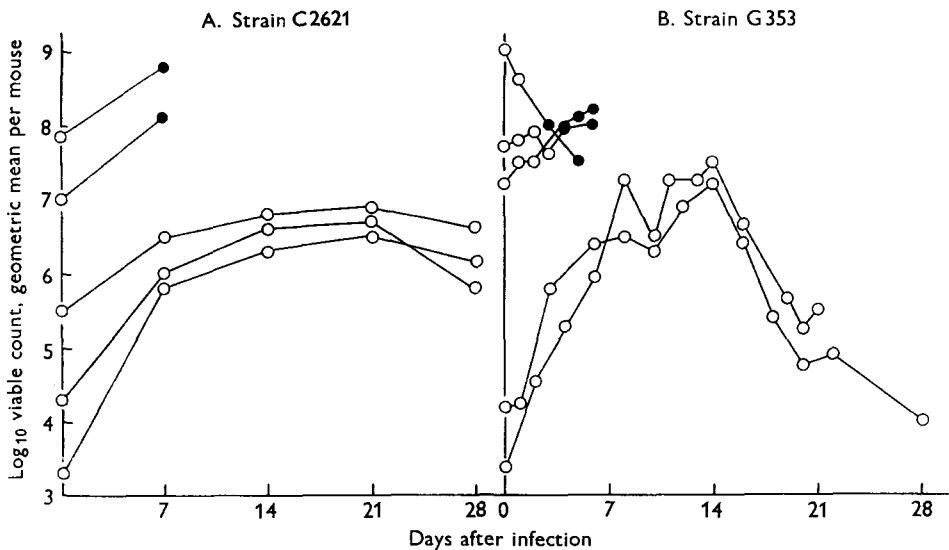


Fig. 1. A. Growth curves for *Bordetella pertussis* strain C2621 instilled into the lungs of normal mice. Each point is the geometric mean count from ten mice infected by the intranasal route with  $\log$  3.3, 4.3, 5.5, 7.0 and 7.85 viable organisms. Groups of mice were sacrificed on the 7, 14, 21 and 28 days. Mice infected with  $\log$  7.0 and 7.85 organisms started to die on the 6th day (●); there were no deaths in the other doses (○). B. Similar growth curves for *B. pertussis*, strain G353. Each point is the geometric mean of the counts in Fig. 2A–E. Counts from living mice are shown with open circles (○); counts including dead mice with closed circles (●).

From the growth curves for a series of increasing doses of *B. pertussis* given by the intranasal route (Figs. 1 A, B; 2 A–E) it will be seen that with strain C2621 an inoculum of  $10^7$  or more was necessary to initiate a lethal infection; an inoculum of  $< 10^7$  resulted in a sublethal infection, the count rising to a maximum in c. 21 days and then falling (Fig. 1 A). The curves for strain G353 have the same characters (Fig. 1 B). When the growth was sufficiently rapid for the number of viable organisms in the lung to reach c.  $10^8$  before 10–14 days, the mice died; when, however, the inoculum was too small to allow this to happen, it appears that multiplication to the critical level was inhibited by the specific immunity and a sublethal infection resulted.

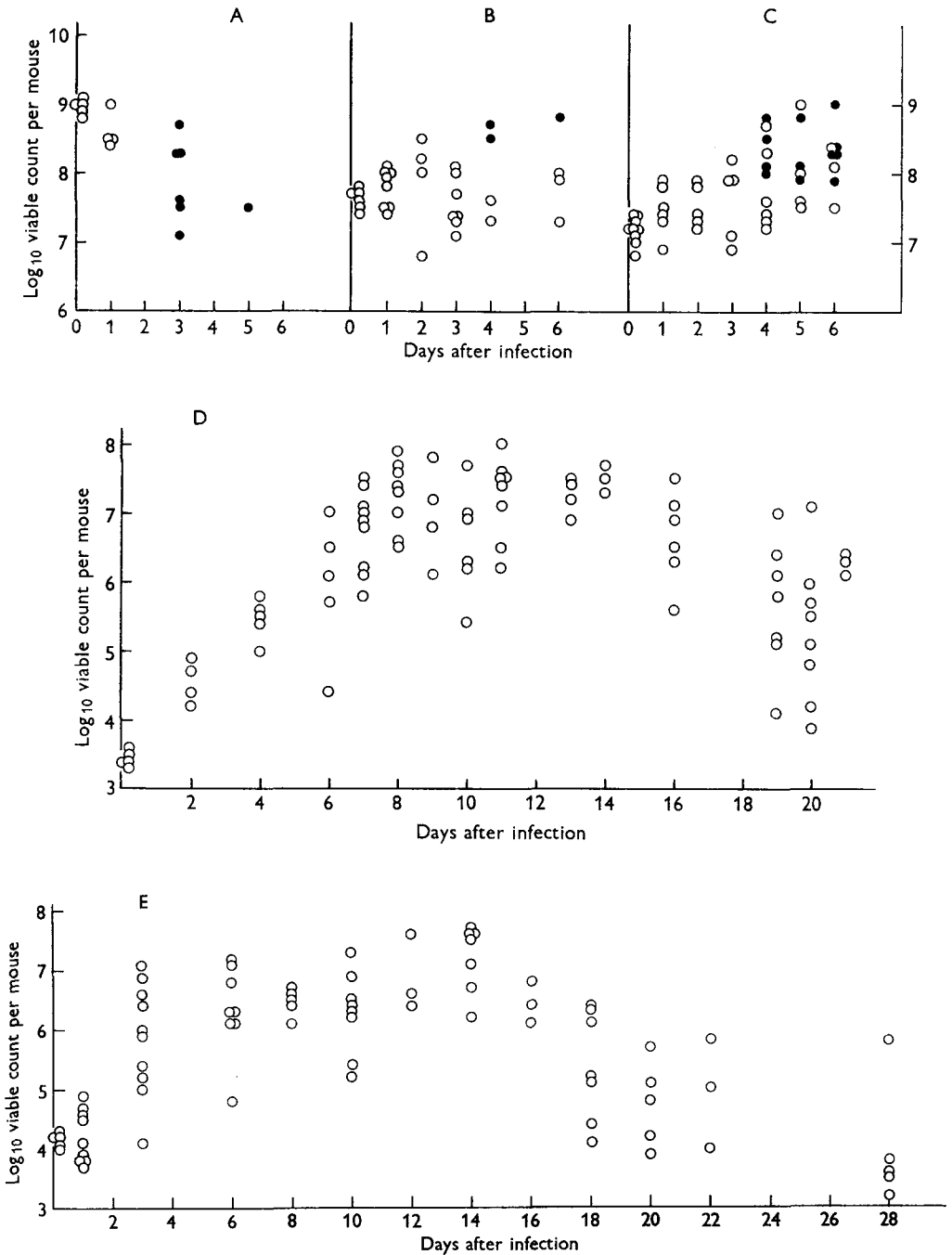


Fig. 2. Detailed growth curves of experiments in Fig. 1B, where five groups of mice were infected with  $10^{3.4}$ ,  $10^{4.2}$ ,  $10^{7.2}$ ,  $10^{7.7}$  and  $10^9$ . Each count gives the viable count on the lungs of one mouse, living mice open circles (○), dead mice closed circles (●). The point on the ordinate corresponds to the inoculum; the first counts shown on 0.1 of the first day are the 2 hr. counts. There were no deaths in the mice given  $10^{3.4}$  and  $10^{4.2}$  organisms.

Mice can be vaccinated by the intranasal route though this was not as efficient as the intraperitoneal route. The IMD 50 (immunizing dose protecting 50 % mice) of vaccine V 3 was  $1650 \times 10^6$  *IN* and  $190 \times 10^6$  *IP* against a challenge of 100 LD50 given by the intracerebral route. Living vaccines were about 10 times as effective as killed ones. IMD 50 of living vaccine 407L was  $24 \times 10^6$  and of killed 407K was  $300 \times 10^6$  against a challenge of 185 LD50. Cooper (1952) immunized mice with  $5 \times 10^6$  by the intranasal route and demonstrated this immunity against challenge by both routes.

Similar growth curves to those shown in Figs. 1 and 2 were found with three other strains. For one of these, the American strain 18-323 (NCTC 9797), not very virulent by the intranasal route though exceptionally so by the intracerebral route, the critical level was  $10^9$ , therefore the relative inability of this strain to kill by this route is not due to its inability to grow.

The shape of the growth curve was characteristic of the strain; that of strain C2621 (Fig. 1 A) was a flat-topped curve with the maximum between the 14th and 25th days; that of strain G 353 (Fig. 1 B) was sharper with a maximum between the 10th and 14th days. The curves for the strains used by Proom (1947) and Cooper (1952) were similar to that of strain G 353, but that for Proom's strain was maximum on the 14th day, whereas that for Cooper's strain (CN 134) was maximum on the 7th day and had dropped to half the maximum by the 14th day.

Table 1. *Terminal count at death and highest sublethal count of groups of mice infected with different strains of Bordetella pertussis by the intranasal route*

(The inoculum varied from  $10^{5.0}$  to  $10^{7.5}$ .)

Strain		Day after infection on which count was made				
		4	5	6	8	10
		$\log_{10}$ viable count				
G 353	Terminal count*	8.0	8.6	—	9.0	9.1
G 353	Sublethal count†	7.5	7.2	—	7.0	6.7
C2621	Terminal count	8.02	—	7.89	8.31	—
C2621	Sublethal count	7.7	—	7.13	7.4	—
18-323	Terminal count	8.52	9.35	9.41	—	—
18-323	Sublethal count	8.49	8.63	8.58	—	—
2 atox	Terminal count	7.4	—	8.16	—	—
2 atox	Sublethal count	7.2	—	7.7	—	—
C7294	Terminal count	—	—	8.02	—	—
C7294	Sublethal count	7.3	7.5	—	—	—

\* Terminal count—geometric mean of all counts made immediately after death on all mice dying.

† Sublethal count—geometric mean of all counts on mice in those groups given the highest infecting dose in which there were no deaths within 28 days.

*The lethal level*

The margin between the recovery of the mouse and death was a narrow one. Table 1 shows the mean terminal count for five strains carried out immediately after death of all mice dying in these experiments, and the mean counts of all mice

in the groups given the highest infecting dose in which there were no deaths, i.e. the highest sublethal dose range.

There is a practical difficulty in dealing with lung counts in groups in which some mice have died or are expected to die, because at the moment of killing it is impossible to know whether the mouse would have died from the infection in the near future or not. The relation of the counts in the various circumstances is shown in Fig. 3, which records experiments in which cages of mice identical with those used for the counts were kept for registering daily deaths, etc. Curves *a* and *b* record counts up to the day on which the first mouse died for mice which had been given a known lethal challenge. Curve *c* is of the terminal counts of mice which died from the infection. Curve *e* is of the counts from mice sacrificed on the day shown after a sublethal infection and curve *d* from survivors from a 'low' lethal

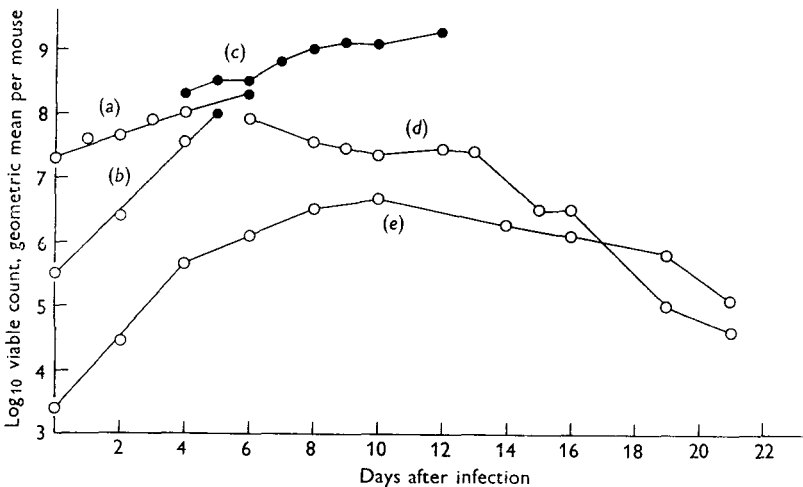


Fig. 3. Composite parts of the general growth curve of *Bordetella pertussis*, strain G 353, in the lungs of mice. Curves *a* and *b* are typical for viable counts in which the 5th or 6th day was the first day on which any mouse died from the infection. Curve *c* is the mean terminal count of all mice which died on each day from the 4th to the 12th day. Curve *d* is the mean viable count for the 6th to the 12th days of survivors from experiments with a low lethal challenge (2–10 LD<sub>50</sub>). Curve *e* is typical of a sublethal infection. Each point on these curves is the geometric mean for counts on 30–50 mice. Counts from living mice open circles (○), counts from dead mice closed circles (●).

challenge (1–5 LD<sub>50</sub>). Curve *d* is typical of many curves of this type which after a rather rapid fall over the first few days take a form similar to that of the sublethal challenge.

There is a gradual rise in the lethal level as shown by the mean terminal count for mice dying on different days, curve *c*, characteristic of all the strains used in different experiments.

The finding of a critical terminal count more or less independent of the size of the initial dose agrees with the findings for many varied infections (see Table 1, Meynell & Meynell, 1958).

*Rate of increase of viable count and virulence*

It is essential in using the intranasal technique of Burnet & Timmins (1937) to ensure that the whole dose reaches the lung. Mice which did not appear to take in the whole dose by deep inspiration were discarded, nevertheless a small immediate drop in the viable count occurred. In nineteen consecutive experiments, in which the inoculum ranged from  $10^{3.23}$  to  $10^{7.92}$  (0.1 LD<sub>50</sub>–1000 LD<sub>50</sub>), the viable count of the lungs of mice killed immediately after instillation was between 1 and 20% lower than the viable count of the inoculum. The drop was evident at all dose levels.

Table 2. *Growth of Bordetella pertussis during first day of lung infections in mice*

			Numbers of bacteria	log base <sub>10</sub>	log increase per hour
Expt. 1	Inoculum*	—	1,000,000	19.932	—
	Mean viable count at†	0 hr.	479,000	18.869	0.034
		2 hr.	502,000	18.936	0.172
		4½ hr.	677,000	19.367	0.133
		7½ hr.	852,000	19.699	0.223
		24 hr.	7,420,000	22.822	0.221
		30 hr.	18,700,000	24.150	
log 22.822 – log 18.869 = 3.95 generations in 24 hr.					
Expt. 2	Inoculum	—	19,960,000	24.250	—
	Mean viable count at	0 hr.	9,780,000	23.221	0.099
		2 hr.	11,123,000	23.419	0.132
		4½ hr.	14,130,000	23.751	0.133
		7½ hr.	18,630,000	24.150	0.118
		24 hr.	57,550,000	25.744	
		log 25.744 – log 23.221 = 2.52 generations in 24 hr.			

\* Inoculum calculated from viable count of bacterial suspension.

† Mean viable count is the geometric mean of counts carried out at each of the times stated on groups of 9 or 10 mice.

There is no very distinct lag phase in the mouse, and it will be seen in Fig. 4 and Fig. 2B, C that the rate of increase of the viable count (slope) for the 1st day in each of the six curves is not less than the 2nd and 3rd days. If there were an appreciable lag over the first few hours, it seems improbable that the rate of increase of viable count over 12th–24th hours would be more rapid than in the later days. An experiment to illustrate this point is shown in Table 2; although growth was slower over the first 2 hr., it was constant over the first 24 or 30 hr. The technical difficulties of doing counts on small numbers of *B. pertussis* in mouse lungs make it impossible to carry out similar experiments with smaller challenges.

In a series of challenges with differing intranasal doses in normal mice there were obvious different rates of growth, and the rate of growth was greater with the smaller than with the larger doses (Fig. 5).

The growth curve can accurately and conveniently be represented by a straight line, at least for the first 4 days (Fig. 4; also Fig. 3, curves *a*, *b* and *e*). Over a period of years, in only three out of thirty-two experiments did the growth curve flatten before the 4th day.

The growth rate can be calculated from the formula

$$i = (\log n_2 - \log n_1) / (t_2 - t_1),$$

and since  $t_2 - t_1$  is the same in all these tests, i.e. 4 days, then  $i$  can be represented by  $\log n_2 - \log n_1$ . In Fig. 5, the growth rates for thirty-two consecutive experiments are plotted from a common source, and the initial dose ( $\log n_1$ ) and the doubling time of the viable count are indicated.

There was a progressive diminution in the growth rate as the initial dose

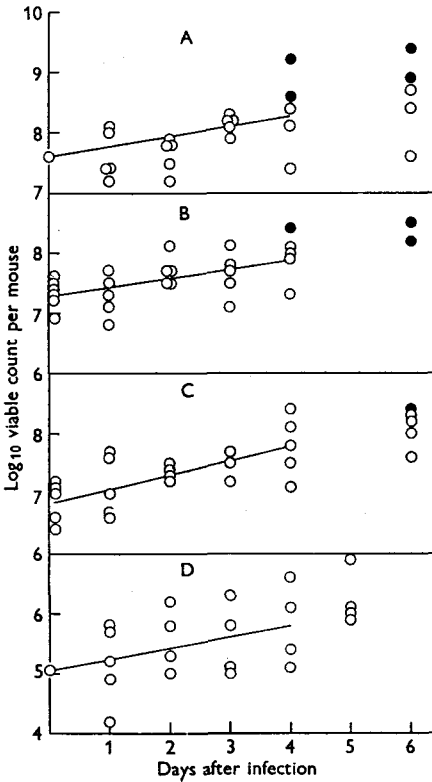


Fig. 4

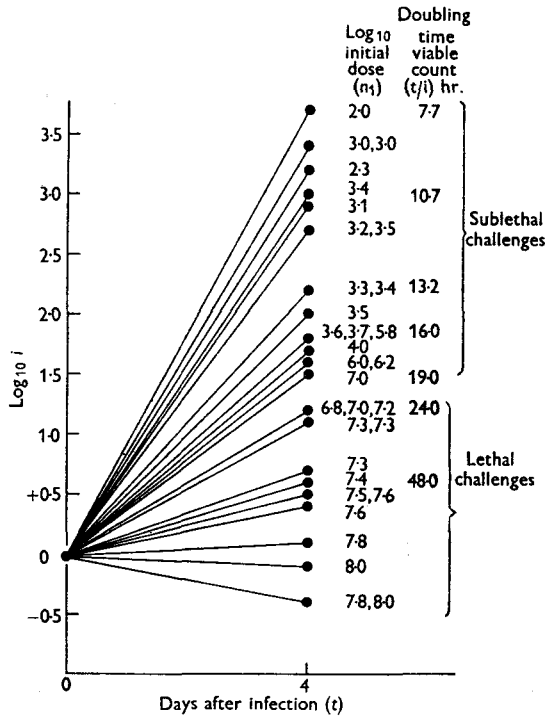


Fig. 5

Fig. 4. Growth curves of four experiments in which groups of mice were infected with  $10^{7.6}$ ,  $10^{7.3}$ ,  $10^{6.85}$  or  $10^{5.05}$ . The dose in A and D was calculated from the viable count of the inoculum and is shown on the ordinate; in B and C an initial count was carried out on mice killed at 2 hr. shown as 0.1 day. Each line joins the geometric mean of the inoculum or the initial count with the geometric mean count on the 4th day. Counts from living mice open circles (○), counts from dead mice closed circles (●).

Fig. 5. Rates of increase of viable counts ( $i$ ), from 32 experiments, in logarithms plotted from a common source showing also the initial dose, and the mean doubling time.



increased to  $10^{7.83}$  or more, when growth in the mouse lung ceased and the number of viable organisms diminished steadily.

The smaller the initial dose the more rapid the growth over the first 4 days, but mice died only when the viable count reached the lethal level. In surviving mice the curve started to flatten off, reaching a maximum lower than the lethal

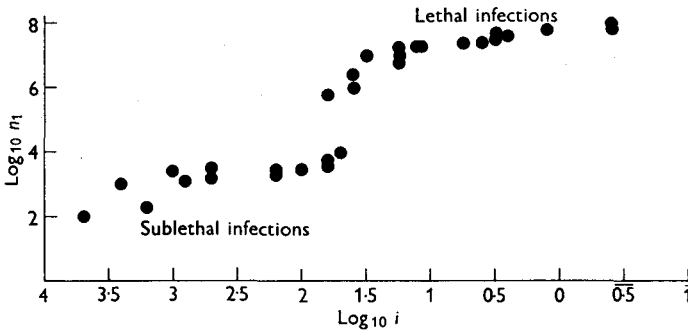


Fig. 6. Logarithms of the initial dose from 32 experiments plotted against the the rate of increase of viable count  $i$  ( $\log n_2 - \log n_1$ ). Negative readings mean that the 4th day count was lower than the initial count. The distinction between lethal and sublethal challenges is obvious.

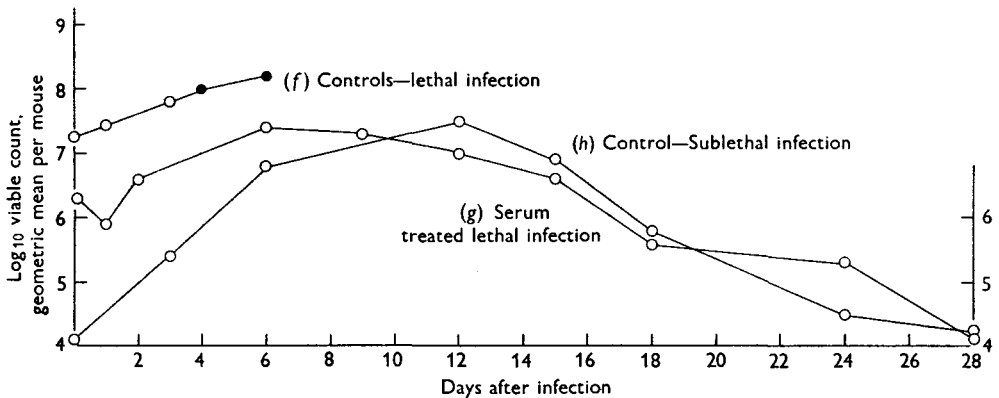


Fig. 7. The effect of 'intranasal' serum on a lethal intranasal infection. Curve *f* untreated mice given a dose of  $10^{7.3}$  viable organisms, first death on 4th day. Curve *g* mice given same dose  $10^{7.3}$  combined with 2 PD 50 'intranasal' serum There were 16 deaths in 60 mice exposed. Mean viable count of survivors killed on days indicated is shown. Curve *h* untreated mice given a sublethal dose of  $10^{4.1}$  viable organisms. In this series two mice died on the 20th day, one on the 21st day and one on the 22nd day. These four mice had counts of  $c. 10^9$ . The curve is plotted from surviving mice killed on the days indicated.

level at about the 10th day or later (Fig. 3, curve *e*). Lethal doses tended to grow exponentially until the death of the mouse (Fig. 4A, B; Fig. 3, curves *a* and *b*). In this series of experiments the lowest dose leading to a lethal infection was  $10^{5.24}$ .

In Fig. 6 the initial dose is plotted against the increase in viable count. It is possible to draw two lines, one through the points for the 'lethal' doses and one

through the 'sublethal' doses which divide fairly sharply at the dose  $10^4$ . Unfortunately we had no initial doses between  $10^4$  and  $10^{5.8}$  in this series.

*Infection with Bordetella pertussis by the intranasal route in passively protected mice*

Fig. 7 shows the effect of a potent 'intranasal' serum on a lethal dose. Compared with the control there was a drop from the initial dose of  $10^7$  viable organisms to  $10^{5.9}$  on the first day, this was followed by a rise to a maximum on the 6th day, and thereafter a decline to  $10^{4.01}$  at the 28th day. Superimposed on this curve is a

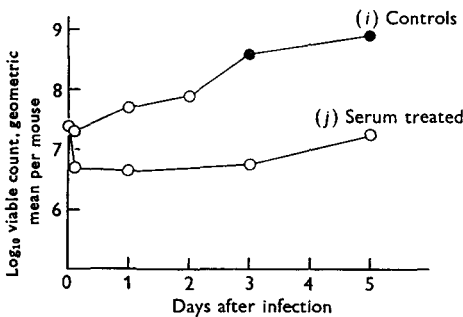


Fig. 8

Fig. 8. The effect of an 'intranasal' serum on a lethal intranasal infection showing the rapid drop in viable count within 2 hr. Curve *i* untreated mice given an inoculum of  $10^{7.4}$  viable organisms which fell to  $10^{6.7}$  in 2 hr. and then rose. Curve *j* mice given the same inoculum mixed with 2 PD 50 'intranasal' serum no. 454. Viable count fell to  $10^{6.7}$  in 2 hr. and to  $10^{6.65}$  in 24 hr.

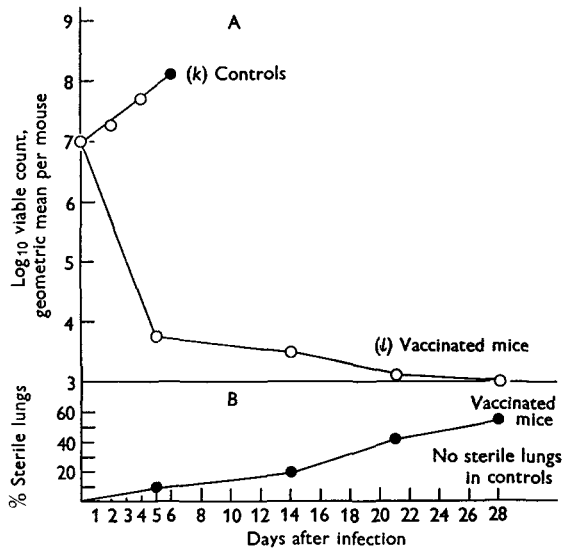


Fig. 9

Fig. 9. The effect of active immunization on a lethal intranasal infection. A. Curve *k* untreated mice given an initial dose of  $10^7$  viable organisms—first deaths on 6th day, all dead by 14th day. Curve *l* mice given the same dose 14 days after vaccination by intraperitoneal route with 400 million killed *B. pertussis*. 55/60 survivors on 28th day. B. Accumulated sterile lungs per hundred in vaccinated mice. There were no sterile lungs amongst the control mice.

curve for a sublethal infection started by a dose of  $10^{4.1}$ , which rose to a maximum on the 12th day and then fell away more or less parallel with the curve for the treated mice. The serum converted a lethal infection to a sublethal one by an immediate reduction in the numbers of viable organisms in the infecting dose.

The rapidity of the fall in viable count with 'intranasal' serum batch no. 454 is shown in Fig. 8. Another batch of 'intranasal' serum 394 in a similar experiment showed a drop of  $10^{7.4}$  to  $10^{6.7}$  in 2 hr., and at 24 hr. the count was  $10^{6.75}$  while the

control count was  $10^{7.3}$  in 2 hr. and  $10^{7.8}$  in 24 hr. Further, the drop depends to some extent on the potency of the actual serum used. Increasing the dose of serum no. 454 from 2 to  $3\frac{1}{2}$  to 7 PD50 resulted in the drop from  $10^{7.8}$  to  $10^{6.7}$ ,  $10^{6.3}$  and  $10^{5.5}$ . No 'intranasal' serum lowered the viable count of a lethal inoculum more than  $2-3 \log_{10}$  units so no serum could sterilize the lung, but 'intranasal' sera always had this effect when given at the same time as, or up to 6 days after, the challenge. They had no effect if given before the challenge.

*Infection by Bordetella pertussis by the intranasal route in actively immunized mice*

The course of infection in mice actively immunized by the intraperitoneal route was similar to that in passively protected mice (Fig. 9). In the sixty control mice 27% died during the first week, 80% by the end of the second week, and 100% by the twenty-fifth day. In the sixty vaccinated mice there were five deaths, on the sixth, seventh, seventh, eleventh and twenty-fifth days, respectively. The lung counts dropped rapidly and the number of sterile lungs increased rapidly.

This steady increase in sterile lungs during the course of the infection was the main difference between the active and passive immunity, and was presumably due to a greater immunity of the actively immunized animals and perhaps the steady elimination of the passively administered rabbit antiserum.

*In vitro tests with Bordetella pertussis antiserum*

As there was an immediate drop in the lung count of infected mice given intranasal antibody, tests were made for *in vitro* activity of antiserum.

Mixtures of antisera, *B. pertussis* suspension and complement were incubated at

Table 3. *A typical experiment showing the antibacterial action in vitro of intranasal sera in the presence of complement*

One ml. of serum under test diluted 1/10, + 1 ml. *Bordetella pertussis* suspension standardized to give c. 50 viable organisms in a drop of 0.02 ml. when diluted 1/100 + 0.1 ml. of a 1/5 dilution of preserved guinea-pig complement. Mixtures were rotated for 1 hr. at 37°. Viable counts were then carried out by the method of Miles & Misra (1938) by plating 0.02 ml. of undiluted, 1/10 and 1/100 dilutions of the mixtures onto Bordet-Gengou plates and incubating for 5 days at 37°. Controls with and without normal serum and with and without complement were put up.

	Intranasal sera								
	Normal serum			B. no. B 394			B. no. B 841		
Dilution of mixture counted	1/1	1/10	1/100	1/1	1/10	1/100	1/1	1/10	1/100
Viable count/drop average of 4 counts	$\alpha$	$\alpha$	65	17*	13	4	8	0	0
Viable count/ml.	325,000			13,250			400		
% decrease in viable organisms	0			94			> 99		

\* The count of the undiluted mixture was often much lower than might be expected from the other dilutions. This was undoubtedly due to the continued action of the complement during the incubation of the count plate. This phenomenon only occurred in the counts of the 'undiluted' mixtures.

37° on a rotating shaker. Viable counts were then made on Bordet–Gengou plates. It was necessary to test each batch of blood used for the Bordet–Gengou medium as some batches were found to inhibit the reaction.

In the presence of complement and sera containing intranasal antibody, the viable count diminished, whereas it was unaffected by normal serum or pertussis antisera not containing intranasal antibody. There was no effect unless complement was present (Table 3). In most of these experiments guinea-pig complement was used as mouse plasma is always said to be lacking in complement. Mouse plasma was found to be active in these tests when special precautions were taken; the mouse blood was kept at +4° until the plasma was collected and citrated when it was kept at –15°. Collected and stored under these conditions it was active.

With six intranasal antisera the average percentage decrease in viable count was 64, 94, 95, 95 and 99. None of six intracerebral sera had any significant effect.

#### DISCUSSION AND CONCLUSIONS

When graded doses of living *B. pertussis* were given to mice by the intranasal route one of two things happened according to the size of the dose. When the dose was large enough, the organisms grew until the numbers reached a critical level at which the mouse died. This critical level—the lethal level—depended to some extent on the strain of mice and the strain of *B. pertussis*. The lethal level with five strains of *B. pertussis* lay between  $10^{7.4}$  and  $10^{8.02}$  on the fourth day after infection. There was a slight rise in the lethal level between the fourth day (on which the first deaths occur) and the tenth day, of not more than one log unit. Mice died when this lethal level was reached irrespective of the time taken by the invading organisms to reach it.

The rate of growth over the first 4 days in the lung depended on the size of the inoculum. The smaller the inoculum the faster the growth rate. With an inoculum of  $10^2$ , the mean generation time was 7.7 hr. for the first 4 days, with an inoculum of  $10^{7.5}$  it was 48 hr. With an inoculum at the lethal level *c.*  $10^8$  there was no growth; the lung count remained stationary until the mice were dead. With inocula of more than the lethal level there was usually a diminution of the lung count until death of the mice (Fig. 2A, etc.).

With strain C2621 the count never reached the lethal level unless the inoculum contained  $10^6$  viable bacteria. The lung count remained more or less constant from the seventh to the eighteenth day and then gradually fell away. Many mice had small numbers of *B. pertussis* in their lungs 3 months after infection. This very characteristic curve was called the sublethal curve (Fig. 1; Fig. 3e).

'Intranasal' antisera, which had a pronounced bactericidal action *in vitro* in the presence of complement, had a striking protective effect *in vivo*, consisting of an immediate reduction in the numbers of bacteria in the lung. In the case of a lethal infection this was reduced to a sublethal level. Antiserum must be in direct contact with the bacterial cells to act and this action was best demonstrated by mixing the serum and challenge before administration or by instilling the antiserum into the lungs after the challenge. Serum given intranasally before the challenge

was ineffective presumably because it was quickly removed from the lung. The action of antiserum required at least two factors, adequate amounts of antibody and complement, for antibody alone (*in vitro*) did not reduce the viable count, and *in vivo* known potent sera were relatively inactive if given intraperitoneally, unless in very large doses, presumably because insufficient quantities of serum reached the lumen of the lungs and came into contact with the bacteria. Most of the effect of the serum was over in 2-3 hr., and all of it in the first 24 hr.

Antiserum had no action *in vitro* without complement, antiserum alone did not seem to affect the bacterial cells in any way and we suggest that the *in vivo* activity was due to complement in the exudate of the lung acting on the sensitized cells.

#### SUMMARY

*Bordetella pertussis* instilled by the intranasal route into the lungs of mice multiply without difficulty even from small inocula, although *B. pertussis* is not a natural pathogen for mice and mouse to mouse infection could not be demonstrated. When the initial dose was large the bacilli multiplied until the number in the lungs reached a critical level at which the mouse died. With smaller doses the critical level was never reached; a maximum count was achieved in 10-14 days, after which the number of viable bacilli declined. These smaller doses were consistently non-lethal and the figures for the viable counts when plotted gave curves of typical shape which were called 'sublethal curves'. The decline corresponded in time with the development of specific immunity. Growth in the lung during the first 4 days of the infection was exponential, the rate of increase in the viable count depending on the size of the inoculum; the smaller the inoculum the faster the increase. With infecting doses at about the critical level, the numbers did not increase during the first 3-4 days, and with larger doses they decreased during the first 2-4 days of infection.

Antiserum given with the inoculum reduced the number of viable organisms in the lung at once. Its effect was short-lived, because after 24 hr. the lung count rose; nevertheless, the initial check on the bacteria had converted a lethal infection into a sublethal infection.

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#### REFERENCES

- ANDERSEN, E. K. (1953). Active pertussis immunity in mice after recovery from pulmonary infection or vaccination against *H. pertussis*. *Acta path. microbiol. scand.* **32**, 125.
- ANDERSEN, E. K. & BENTZON, M. W. (1958). Comparison between pertussis vaccine potency assays in mice challenged by the intracerebral route and mice challenged by the intranasal route (sublethal dose). *Acta path. microbiol. scand.* **42**, 333.
- BURNET, F. M. & TIMMINS, C. (1937). Experimental infection with *Haemophilus pertussis* in the mouse by intranasal inoculation. *Brit. J. exp. Path.* **18**, 83.
- COOPER, G. N. (1952). Active immunity in mice following the intranasal injection of sublethal doses of living *Haemophilus pertussis*. *J. Path. Bact.* **64**, 65.
- DOLBY, J. M. (1958). The separation of the histamine-sensitizing factor from the protective antigens of *Bordetella pertussis*. *Immunology*, **1**, 328.

- DOLBY, J. M. & STANDFAST, A. F. B. (1958). A comparison of passive protection tests against intranasal and intracerebral challenges with *Bordetella pertussis*. *Immunology*, **1**, 144.
- FISHER, S. (1955). Multiplication of *Haemophilus pertussis* in the mouse lung following intranasal infection. *Aust. J. exp. Biol. med. Sci.* **33**, 609.
- HUGHES, D. E. (1951). A press for disrupting bacteria and other micro-organisms. *Brit. J. exp. Path.* **32**, 97.
- MEYNELL, G. G. & MEYNELL, E. W. (1958). The growth of micro-organisms *in vivo* with particular reference to the relation between dose and latent period. *J. Hyg., Camb.* **56**, 323.
- MILES, A. A. & MISRA, S. S. (1938). The estimation of the bactericidal power of the blood. *J. Hyg., Camb.* **38**, 732.
- PROOM, H. (1947). The immunological aspects of experimental *Haemophilus pertussis* infection. *J. Path. Bact.* **59**, 165.
- REED, L. J. & MUENCH, H. (1938). A simple method of estimating fifty per cent endpoints. *Amer. J. Hyg.* **27**, 493.
- STANDFAST, A. F. B. (1958). The comparison between field trials and mouse protection tests against intranasal and intracerebral challenges with *Bordetella pertussis*. *Immunology*, **1**, 135.