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A comparison between the intranasal and intracerebral infection of mice with *Bordetella pertussis*

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INTRODUCTION

It has already been shown (Standfast, 1958) that potent pertussis vaccines fall into three groups: (i) those protecting mice against infections both by the intranasal and intracerebral routes, (ii) those protecting mice much better against infections by the intranasal route than the intracerebral route, and (iii) those protecting mice better against infections by the intracerebral route than the intranasal. The best results in the prophylaxis of children against pertussis are given by those vaccines which protect mice well against an intracerebral challenge (M.R.C. report, 1956).

Dolby & Standfast (1958) showed that protective antisera prepared against numerous fractions of *Bordetella pertussis* also fell into three similar groups. In later papers (Dolby & Standfast, 1961; Dolby, Thow & Standfast, 1961) we have investigated the modes of action of antibodies operating specifically against each type of challenge.

In this paper, we present the results obtained when following the course of infection by both intranasal and intracerebral routes, using homologous and heterologous antibodies and vaccines and compare the two routes. Since it has been suggested by Andersen (1958) that the lethal intracerebral challenge and the sublethal intranasal challenge are both measuring the same antibody, results obtained with the latter method of assay have also been included.

METHODS

Infecting suspensions

Bordetella pertussis, strain 18–323, was used for infections by the intracerebral route and strain G 353 for infections by the intranasal route both lethal and sublethal. (For further details, see Standfast, 1958.)

Active immunization tests

Dilutions of vaccines were given by the intraperitoneal route into groups of mice given a constant challenge (see Standfast, 1958).

Passive protection tests

Dilutions of rabbit antisera given by the intraperitoneal route at times stated before or after intracerebral challenge or dilutions of antisera mixed with intranasal challenge and given as a combined instillation (see Dolby & Standfast, 1958).

Viable counts

Counts of brain and lungs were made on Bordet–Gengou media by the Miles & Misra (1938) method (see Dolby & Standfast, 1961; Dolby *et al.* 1961).

Definitions and abbreviations

Throughout the paper 'intracerebral' antigen means the antigen which protects mice against a challenge by the intracerebral route and 'intracerebral' antibody means the antibody which protects mice against challenge by the intracerebral route, similarly with 'intranasal' antigen and 'intranasal' antibody. The route of administration of antigen or serum is always referred to as the intracerebral route, etc. *IC*, *IN* and *IP* are used when it is necessary to abbreviate.

RESULTS

A comparison of intracerebral and intranasal infections

The usual model used for the multiplication of effective organisms in particular systems is shown in Fig. 1A. The usual form of this model postulates that organisms increase *in vivo* at a constant rate so that their number rises exponentially and that the response (in this case the death of the mouse) occurs when the total number of organisms reaches a critical figure.

The growth of *B. pertussis* in the lungs or brains—the only two sites in which this organism will grow in the mouse—fits this model with certain modifications.

Infection of the lung is shown diagrammatically in Fig. 1B, based on the results of Proom (1947), Andersen (1953), and Dolby *et al.* (1961). Three factors additional to the usual model affect this scheme: few, if any, mice die before the 5th day irrespective of the dose; the critical value rises with time from *c.* 10^8 on the 5th day to $10^{9\cdot3}$ on the 10th day; infecting doses smaller than 10^3 (depending on strain of *B. pertussis* and strain of mice) result in a sublethal infection. This sublethal infection results from a race between the organism reaching the critical level of *c.* 10^8 and the development of an immunity in the host, elicited by the infection itself, reaching a level adequate to deal with the infection.

The lung of the mouse can only support a relatively small number of infecting bacterial parasites, the number for *B. pertussis*, strain G 353, being *c.* 10^8 living bacteria. When initial doses larger than this were given, the viable count in the lungs fell until the mice died about the 5th day. When doses approaching the toxic level were given and the mice died on the 1st or 2nd days, the viable count still fell.

The infection in the brain is shown in Fig. 1C based on Dolby & Standfast (1961), Blyth (1955), Andersen (1957) and Brown (1958). Rather surprisingly the critical level in the brain was the same as in the lung, *c.* 10^8. Deaths did not as a rule appear before the 4th or 5th day after infection, but in the brain the critical level fell with time from *c.* 10^8 on the 4th day to *c.* 10^7 on the 13th day. The logarithm of the viable count plotted against time did not give a straight line in intracerebral infections, as the rate of increase was constantly diminishing until the critical level was reached. The reason for this is unknown.

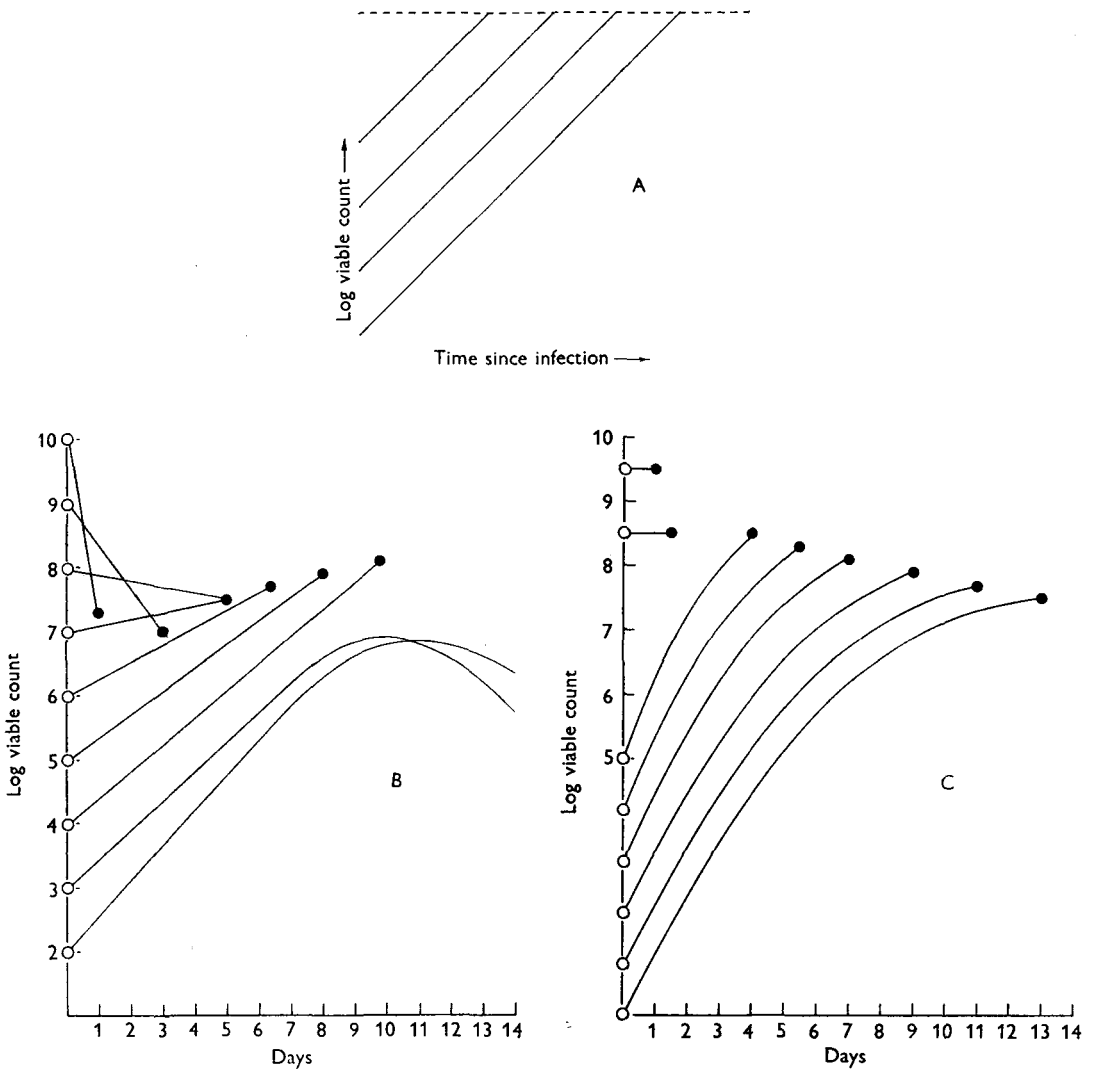

Fig. 1. The growth of *B. pertussis* in mice. A is the model for the hypothesis that effective organisms increase *in vivo* exponentially and that the response occurs when their number reaches a constant critical level. B. Hypothetical growth curves for the intranasal infection of mice. Growth is exponential for 6–7 days, then the lower infecting doses reach a maximum and then decrease to give the typical sublethal curve. No deaths before the 5th day with the true infections and a steadily *rising* critical level. Infecting doses just above the critical level show a fall in count. Doses much above this level are toxic and death takes place in 1–3 days with toxic symptoms and a rapid drop in the viable count; in these mice there is no true infection. C. Hypothetical growth curves derived from our experiments and from the published results of Blyth (1955) and Brown (1958) for the growth of intracerebral infections of *B. pertussis*. Growth is not strictly exponential, there is a steady diminution in rate and a steadily *falling* critical level. Doses much above the critical level are toxic; with such doses death takes place in hours. The viable count remains steady. No sublethal curves.

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Until the blood-brain barrier becomes permeable, the brain is free from circulating antibody and provides all the necessary growth conditions so that very small inocula, probably down to a single cell if it becomes lodged in the brain, can and will grow up to the critical level (Dolby & Standfast, 1961). It is therefore not surprising that with a virulent strain sublethal infections do not occur.

There is some reaction in the brain as Blyth (1955), Andersen (1957) and Brown (1958) all reported sublethal infections with strains of *low mouse virulence*. In Blyth's (1955) example an inoculum of $10^{2.5}$ had grown to $10^{4.5}$ by the 5th day, the numbers then declined until the 10 mice examined on the 21st day had sterile brains. Andersen (1957, fig. 1*a*), working with strain no. 1528, found similar results; an inoculum of *c.* $10^2/0{\cdot}1$ g. mouse brain grew to *c.* $10^5/0{\cdot}1$ g. by the 5th day and then declined to *c.* $10^3/0{\cdot}1$ g. on the 12th day.

Table 1. *Toxic levels of* Bordetella pertussis *suspensions and toxin preparations*

		Route		
		Intranasal	Intraperitoneal	Intracerebral
		TD 50 (ml.) and bacterial count equivalent		
Preparation				
Toxin*	No. 5353/1	0·018 ml. = $1{\cdot}8 \times 10$	0·0017 ml. = $1{\cdot}7 \times 10$	0·00012 ml. = $1{\cdot}2 \times 10$
Toxin	No. 5353/2	> 0·2 ml.	0·015 ml.	0·0017 ml.
Toxin	No. 171258/1	0·1025 ml.	0·0032 ml.	0·000125 ml.
		Viable count		
Terminal count		$1 \times 10^8 - 1 \times 10^9$	—	$1 \times 10^7 - 1 \times 10^9$

* Toxin was prepared by crushing bacteria in a Hughes press (Hughes, 1951) and extracting with sterile saline.

Even if we assume that 90 % of the inoculum spilled out of the brain at inoculation, and Andersen reported that she found the count at 5 hr. usually about one-tenth of the expected amount, this number of cells is hardly enough to act as an effective primary antigen. Experiments with vaccination by the intracerebral route suggested that at least 10^6 bacteria were necessary for this purpose. Until we have further precise information we can only assume that this sublethal curve is due to the temporary flooding and later recovery of the normal clearing mechanism of the brain against which strains of low virulence have no defence, and so differs from the sublethal infection in the lung.

The fact that the terminal count of *B. pertussis* (*c.* 10^8) was the same in the brain as in the lung was unexpected since the susceptibilities of brain and lung to pertussis toxin were quite different and death in intracerebral infection is probably due to toxin. Table 1 shows that the ratio lung:peritoneum:brain is about 1:10:100 in susceptibility to toxin.

The correlation in numbers between the toxin equivalent and the terminal count by the intracerebral route was remarkably close and suggests very strongly that toxin is the cause of death in infection by this route, and that the toxin produced by 10^7 to 10^8 organisms is lethal. The correlation in the lung is not so good and the relative insensitivity of the lung to toxin suggests that if toxin is the

cause of death in lung infections the toxin is absorbed by the lung but acts elsewhere, perhaps on the nervous system. Alternatively, lung infections of *B. pertussis* in mice result in an interstitial pneumonia and death in the mouse may be due to this pneumonia, which may be caused by the action on the lung of toxic products of the bacteria. If this is the case the amount of pneumonia brought about by an infection rising to 10^7 to 10^8 organisms is sufficient to incapacitate the lung, the susceptibility of the lung to acute toxicity (Table 1) has no bearing on the death of the mouse.

Relationship between infecting dose and time of death

The relationship between the geometric mean death time ($\bar{T}$) and the dose, plotted as a number of LD50's contained in the infecting dose, is shown in Fig. 2A and B. With doses of < 1 LD50 the number of mice which died was very small and the estimate of $\bar{T}$ became less precise. The mice with intracerebral infections were discarded after 14 days and the mice with intranasal infections after 28 days as experience has taught us that very few mice in these groups die after this time.

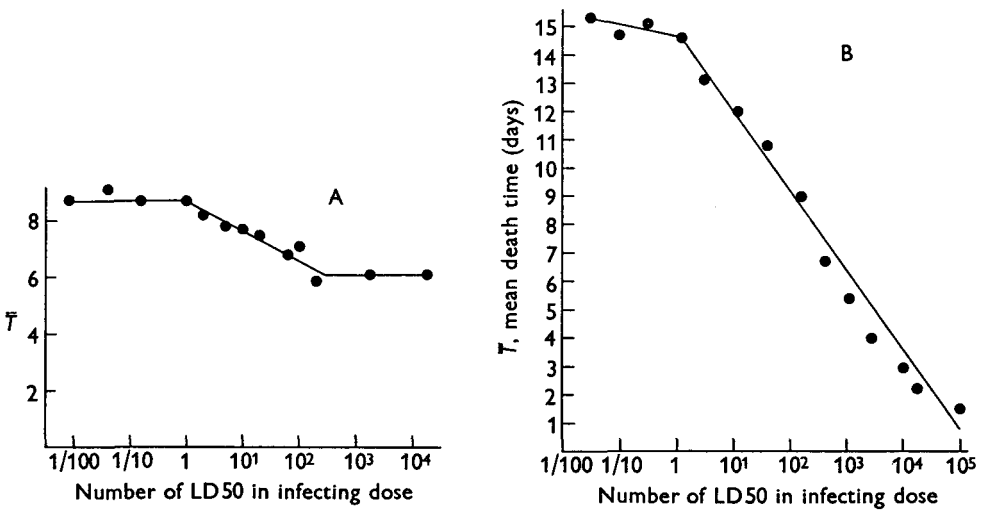

Fig. 2. Geometric mean death time $\bar{T}$ plotted against logarithm of number of LD50 in infecting dose. Straight lines have been fitted by eye. A. Intracerebral infections. B. Intranasal infections.

In intracerebral infections (Fig. 2A) $\bar{T}$ is more or less constant for doses < 1 LD50 and for doses of > 200 LD50. About 10^5 LD50 the dose becomes toxic and death results in 1–2 days with toxic symptoms quite different from the infection. In intranasal infection (Fig. 2B) there was a tendency for $\bar{T}$ to flatten at *c.* 15 days with doses of < 1 LD50. With the largest doses the infective results merged into the toxic results. Doses of 10^5 LD50 which were about 2×10^{10} organisms were toxic by the intranasal route and killed all mice in 1–2 days. Doses of *c.* 10^4 LD50 result in what were probably mixed infective and toxic results, many of the mice dying

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in 1–2 days from toxin, a few dying later from true infections. The usual intranasal challenge containing 10^3 LD50 in 200×10^6 organisms resulted in a pure infection. Few of the mice died before the 4th day with this dose.

Concurrent infections in mice

Intranasal and intracerebral infections in mice were able to run concurrently in the same mouse without any demonstrable interference. There was no synergistic effect from the combination of the two infections. Neither the viable count in the lung or in the brain was increased by the dual infection, nor was the time to death accelerated in any way. The geometric mean time to death was 5·03 days with the intracerebral infection only and 5·18 days with the dual infection. The mice given a lethal intranasal infection died on the 7th and 8th days (Table 2).

Mice given a lethal intranasal infection with a lethal intracerebral infection superimposed on the 3rd day, did not die more quickly than mice with a lung infection only. The two infections appear to be completely independent.

Table 2. *Concurrent infections in mice*

(A lethal intracerebral (*IC*) infection was given to two groups of mice; 3 days later a lethal intranasal (*IN*) infection was given to one of the groups and to a 3rd group. Mice were sacrificed and counts made in the 3rd–6th days of the experiment as below.)

		Group 2 (*IC* and *IN*)		
Day of expt.	Group 1 (*IC*) Intracerebral count (log)	Intracerebral count (log)	Intranasal count (log)	Group 3 (*IN*) Intranasal count (log)
0	3·65	3·65	—	—
3	6·93	6·95	6·74	6·96
4	7·59	7·83	7·75	7·38
5	7·26	7·76	8·25	7·87
6	8·77	8·15	7·59	8·27

Day of death in control cages of similarly treated mice	Group 1 *IC*	3 3 5 5 5 6 6 6 6 $6\bar{T}$ = 5·03 days
	Group 2 *IC* and *IN*	3 5 5 5 5 5 6 6 6 $6\bar{T}$ = 5·18 days
	Group 3 *IN*	No death by 6th day of experiment

A comparison of passive protection tests

In the preceding papers (see p. 200 and p. 212) the results were given for infections in passively protected mice in which the homologous serum was used. Figs. 3 and 4 show the results of giving heterologous sera as well.

Fig. 3 shows in a typical experiment the course of an intracerebral infection in mice given potent pertussis antisera by the intraperitoneal route 4 hr. before the challenge; two types of antisera were used: (i) containing 'intracerebral' antibody, which protects against an intracerebral challenge (curve *c*), and (ii) containing 'intranasal' antibody, which protects against an intranasal challenge (curve *b*). It will be seen that the 'intranasal' antisera had no marked effect on the intracerebral infection.

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In intracerebral infections the crisis occurs at about the 4th day when the blood-brain barrier breaks down and circulating antibody can diffuse easily into the brain; the 'intracerebral' serum exerts its effective action, whilst the 'intranasal' serum is ineffective. The brain counts of the serum treated mice are lower than the brain counts of the control mice, even before the 4th day. Although the differences are small they were a constant finding in all these experiments; presumably some antibody seeps through into the brain all the time. We cannot explain the small

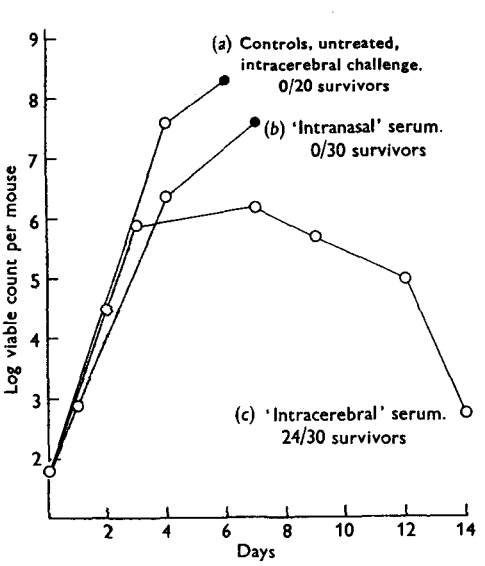

Fig. 3

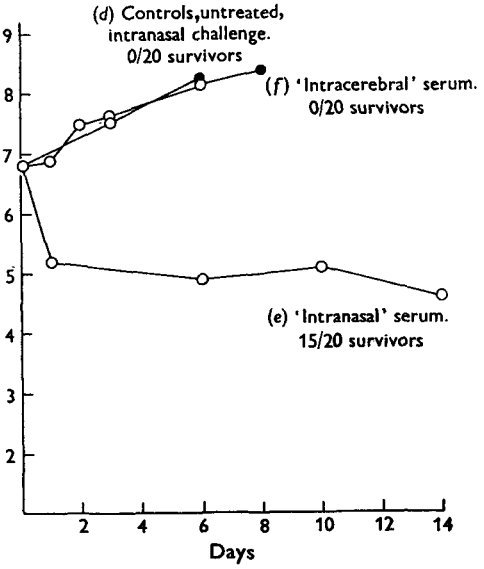

Fig. 4

Fig. 3. Growth of *B. pertussis*, strain 18–323, in treated and untreated mice. Logarithm of the geometric mean of the viable count from the brains of groups of 10 mice plotted against time in days from infection. *a*, Untreated mice given 100 × LD 50, strain 18-323, deaths 6th, 7th, 8th and 9th days; no survivors after 9th day. *b*, Treated mice, 'intranasal' serum D 683 0·2 ml. given undiluted by intraperitoneal route, deaths 7th and 8th, 9th and 14th days; one mouse survived which died on 14th day. *c*, Treated mice, 'intracerebral' serum 2095 given as above, deaths on 5th and 9th days; 80 % mice survived 14th day.

Fig. 4. Growth of *B. pertussis*, strain G 353, in treated and untreated mice. Logarithm of geometric mean of viable count from the lungs of groups of 10 mice plotted against time in days from infection. *d*, Untreated mice given *c*. 1000 × LD 50, strain G 353. Sixteen mice died 5th day, 3 mice died 6th day and the last of the control mice died on the 10th day. *e*, Treated mice were given *c*. 1000 × LD 50, strain G 353 mixed with a potent 'intranasal' serum diluted to 4 × PD 50 per mouse dose. Deaths occurred on the 4th, 6th and 10th days but 75 % mice survived 14th day. *f*, Treated mice were given *c*. 1000 × LD 50, strain G 353, with undiluted 'intracerebral' serum which contained per mouse dose at least 50 × PD 50 assayed by the intracerebral route against strain 18–323.

action of the 'intranasal' serum. Either 'intranasal' serum has a small action against an intracerebral infection or our 'intranasal' serum is contaminated with a small amount of 'intracerebral' antibody.

'Intracerebral' serum has little or no effect on an intranasal infection while the

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'intranasal' serum converts the lethal challenge dose of organisms to a sublethal one (Fig. 4, curves *f* and *e*).

It will be seen that mice given 'intracerebral' serum lived *c.* 2 days longer than the control mice, in this and similar experiments. This may be due to the fact that our 'intracerebral' serum was not pure.

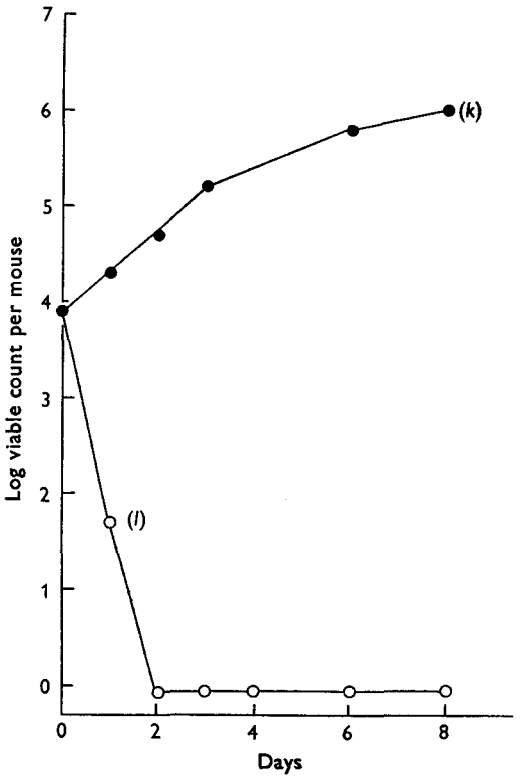

Fig. 5. Action of potent intranasal serum on sublethal intranasal infection. Growth of *B. pertussis* in untreated mice given a sublethal intranasal infection and treated mice given serum known to be potent against a lethal intranasal infection. Logarithm of geometric mean viable count from lungs of groups of mice plotted against time in days after infection. *k*, Sublethal dose G 353 given to control mice. *l*, Treated mice received the same challenge with serum C 876/2 which has a PD 50 of 0·006 ml. against 1000 LD 50 challenge intranasally. All lungs in treated mice sterile after 2nd day.

The sublethal intranasal test

Several attempts have been made in the past to assay vaccines by counting the reduction in numbers in a sublethal lung infection in vaccinated and unvaccinated mice at various times after challenge.

Our experience with the sublethal intranasal test has been that in some respects it resembles the intracerebral test more than the lethal intranasal test, and that vaccines which passed the intracerebral test would pass the sublethal intranasal test while they might or might not pass the lethal intranasal test.

Passive protection tests

'Intranasal' serum by definition must be effective against a lethal intranasal challenge and its action is to reduce this immediately to a sublethal level (Dolby *et al.* 1961, figs. 7, 8). It might therefore be expected to be even more effective against a smaller or sublethal intranasal challenge and this is so (Fig. 5, curve *k*).

The effect of an 'intracerebral' serum against intranasal challenges is shown in Fig. 6A–D. Such an 'intracerebral' serum had some action against a sublethal challenge (Fig. 6A), but as the challenge increased the action became less and less until against a lethal challenge of *c.* 100 × LD50 it was useless (Fig. 6D).

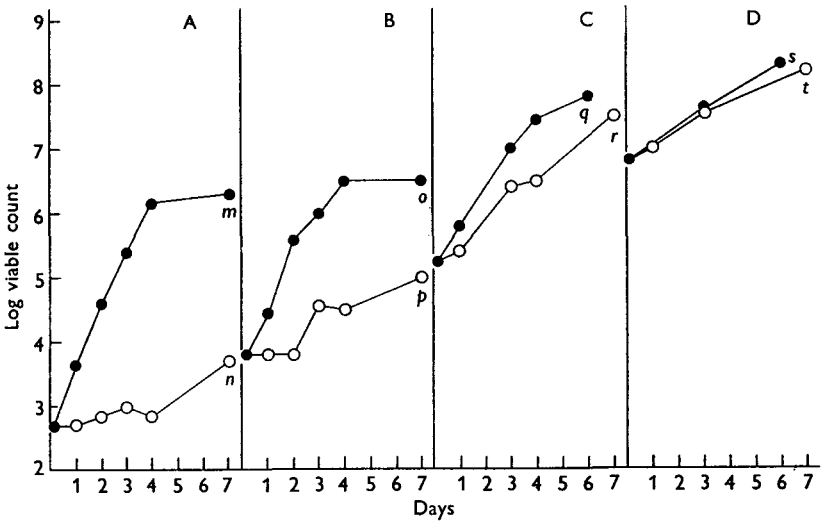

Fig. 6. Action of potent intracerebral serum on sublethal intranasal infection. Growth curves of *B. pertussis* in untreated mice given increasing intranasal infections and treated mice given known potent intracerebral serum E014 with the same infecting doses. A: *m*, infecting dose, $10^{2\cdot8}$ = < 1 LD50; *n*, infecting dose, serum treated. B: *o*, infecting dose, $10^{3\cdot8}$ = 1 LD50; *p*, infecting dose, serum treated. C: *q*, infecting dose, $10^{5\cdot25}$ = *c.* 10 LD50; *r*, infecting dose, serum treated. *s*, infecting dose, $10^{6\cdot8}$ = *c.* 100 LD50; *t*, infecting dose, serum treated.

Thus both 'intracerebral' and 'intranasal' sera were active against sublethal lung infections. From figs. 5 and 6A it will be seen that the action of 'intracerebral' serum against a sublethal intranasal infection was different from 'intranasal' serum. 'Intracerebral' serum did not reduce the numbers of organisms but was able to keep the count stationary either by stopping growth or killing off as many organisms as grew. The larger the infecting dose the shorter was this period, from 4 days with an infecting dose of $10^{2\cdot7}$ to 1 day with $10^{5\cdot25}$ and zero with $10^{6\cdot8}$ organisms (Fig. 6).

These experiments showed that as potent 'intracerebral' sera had a marked action against a sublethal intranasal challenge as, of course, had 'intranasal' sera, the sublethal intranasal test cannot differentiate between 'intracerebral' and 'intranasal' sera and hence between 'intracerebral' and 'intranasal' antigens.

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Active immunization tests

Active immunization tests were carried out with sublethal challenges on vaccines K 205, K 205 B, V 18 and F 68. Previous experience with vaccine K 205 (Fisher, 1955; Standfast, 1958) had shown that this vaccine when assayed by the intracerebral test and the lethal intranasal test was potent in both tests, having an ImD 50 of 440 million and 205 million. After heating at 100° for 1 hr. (vaccine K 205 B) the ImD 50 was α and 450 million, showing the destruction of the 'intracerebral' antigen as

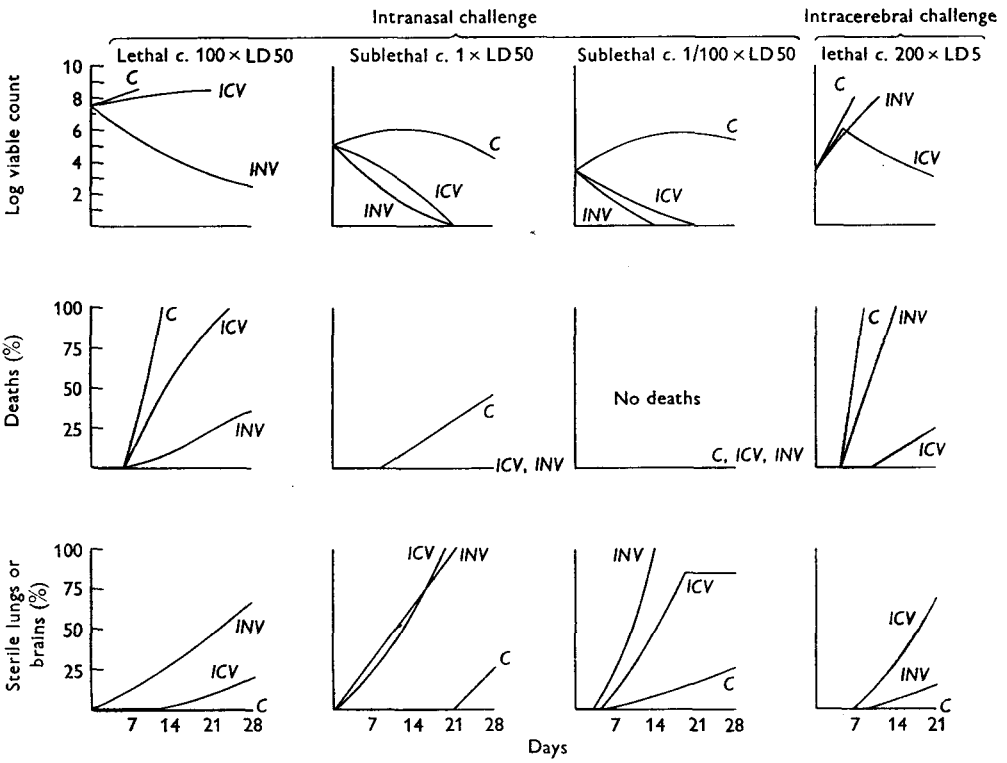

Fig. 7. Active immunization tests with lethal and sublethal intranasal challenges and lethal intracerebral challenge. Mice immunized with 'intracerebral' vaccines (*ICV*) or with 'intranasal' vaccines (*INV*) were given increasing challenges of *c.* 1/100 × LD 50, 1 × LD 50, and 100 × LD 50 of *B. pertussis* by the intranasal route and *c.* 200 × LD 50 by the intracerebral route. The degree of immunity was measured by plotting the rise and fall of the viable count in the lungs or brains of the mice, by the accumulated deaths and by the number of sterile lungs or brains. *C*, controls, unvaccinated mice.

measured by the routine mouse assay and the relative stability of the 'intranasal' antigen. An unheated vaccine V 18 was found to be useless against an intracerebral infection but very potent against an intranasal one.

Vaccine F 68 was a chemically-treated fraction of *B. pertussis*; it was found by test to protect mice against an intracerebral challenge but not against an intranasal challenge (Dolby & Standfast, 1958).

These vaccines were then tested in mice against a lethal intranasal challenge

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c. 100 LD50, sublethal intranasal challenges of $1 \times$ LD50 and $1/100 \times$ LD50, and a lethal intracerebral challenge of *c.* $200 \times$ LD50 (Fig. 7).

The routine methods of mouse assay are based on the proportion of mice dying or surviving as a lethal challenge is normally used, but other criteria can be used—percentage of sterile lungs or brains, or the growth of *B. pertussis* in vaccinated or unvaccinated mice.

As the proportion of mice dying or surviving cannot be used with sublethal challenges we used all three criteria mentioned above in the tests. In Fig. 7 they are shown for unvaccinated control mice (*C*), for mice given a potent 'intracerebral' vaccine (*ICV*) and for mice given a potent 'intranasal' vaccine (*INV*).

It will be seen (Fig. 7) that against a high challenge the mice vaccinated with the 'intracerebral' vaccine (*ICV*) behaved like the control mice in intranasal tests, and the mice vaccinated with the 'intranasal' vaccine (*INV*) behaved like the control mice in the intracerebral test. There was some action shown by the 'intracerebral' vaccine (*ICV*) against an intranasal challenge when this was of a low order, e.g. with a challenge of $1/100 \times$ LD50 or even $1 \times$ LD50 the *ICV* curves resemble the *INV* rather than the controls, confirming the results obtained in the passive protection experiments.

CONCLUSIONS

The main difference between intracerebral and intranasal infections lies in the differing responses to small infecting doses. In the mouse lung small inocula of virulent bacteria (10^4 to 10^6 depending on strain) grow steadily until the 10th to 14th day after infection and then slowly decline until after some weeks or even months the lung becomes sterile—the sublethal infection or curve. In the brain, with virulent organisms, there is no sublethal infection as probably even single organisms if they become lodged in the brain grow up to the critical level and kill the mouse. The only sublethal brain infections reported in the literature have been with strains of lowered virulence (Andersen, 1957; Blyth, 1955).

The relative insensitivity of the lung to toxin means that much larger doses of organisms can be tolerated. Vaccination by the intranasal route is possible, though not as efficient as the intraperitoneal or subcutaneous route, which means that organisms can get from the lung to the site of antibody formation. Living organisms are at least 10 times more efficient than killed ones as a vaccine, and an intranasal infection could therefore supply the antigen necessary to immunize the mouse. Attempts to measure antibody during lung infection by agglutination or protective titre failed, and it is unfortunate that the behaviour of the viable count is easily the most sensitive way of detecting antibody. The only other known cases of progressively declining viable counts are due to the presence of antibody. The most probable explanation of the sublethal curve in the lung is that it is the result of a race between the growing bacteria and the developing immunity of the host, won by the developing immunity.

In the brain much smaller numbers are involved. 10^2, 10^1 or fewer will initiate an infection although up to 90 % of the inoculum is lost at once (Mims, 1960). This material which is lost into the circulation is not, however, enough to act as a

primary stimulus for antibody production; at least 10 living *B. pertussis* given intraperitoneally or intravenously were required for this. This leakage only occurs initially, after which the brain is sealed off until the growth of the organism in the brain reaches a stage at which it causes sufficient local reaction to render the blood-brain barrier permeable. This is the first time after the original inoculation at which organisms can leak out of the brain and by this time it is too late for them to act as an efficient antigenic stimulus. Mice can be vaccinated by the intracerebral route only if the immediate leak contains enough antigen, at least 10^6, cells. Since there is no sublethal curve in the brain we conclude that there is no local production of antibody in the brain.

We do not know why an 'intranasal' serum is ineffective against an intracerebral infection or an 'intracerebral' serum against an intranasal infection. That there is a difference in the mechanism of action of the two sera is suggested by the fact that while the action of an 'intranasal' serum against an intranasal infection is to reduce the viable count immediately (Figs. 4, 5), the action of an 'intracerebral' serum against an intranasal infection when the infecting numbers are very small, is to keep the count steady for 3 or 4 days (Fig. 6A). It cannot apparently reduce it and so sterilize the lung (Fig. 5).

An animal can be protected from an intranasal infection if antibody can reduce the infecting dose to a sublethal level, whereas protection against an intracerebral infection necessitates the sterilization of the brain.

Another important difference between intracerebral and intranasal infections is the part played antigenically by the organism. In the first place there is a considerable difference in numbers between the two infecting doses although the terminal count is the same, *c.* 10^8. The LD 50 is the number of organisms that will kill 50 % of the mice on the 5th to 14th days and what is happening in the first 5 days is important: in the brain probably not much, as the infection is sealed off and the numbers of bacteria involved are very small. In the lung, the numbers are much greater, and since we can vaccinate mice by the intranasal route there is always a way for bacteria to get from the lung to the antibody forming cells. In the intranasal route immunization of the host by the infection is a major factor.

SUMMARY

1. The main differences between intracerebral and intranasal infections in mice with virulent strains of *Bordetella pertussis* are in: (1) the responses to small infecting doses (< 1 LD 50); (2) the action of antisera in controlling infection; (3) the action of toxin on brain and lung; and (4) the rates of increase of the viable count. The two infections can run concurrently in the same mouse without any demonstrable interference.

2. The terminal viable count in the lung and brain is *c.* 10^8 organisms.

3. In the brain there is no sublethal infection with virulent strains; probably even single organisms can grow up to the critical level and kill the mouse. In the lung sublethal infections are found in which the count rises to a figure below the critical level and then declines.

4. The action of 'intranasal' antiserum is to reduce a lethal infection to a sublethal one in the lung but there is no effect in the brain. 'Intracerebral' antisera cannot act until the blood-brain barrier becomes leaky, when they are able to reduce the viable count and eventually sterilize the brain. In the lung 'intracerebral' sera have no action against lethal infections but can control small infections ($< I$ LD50). The sublethal intranasal test measures this effect but it also measures the action of 'intranasal' sera and so cannot be used to distinguish the two types of sera and hence the two antigens.

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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. (1957). Demonstration of promunity in the early immunity of Pertussis vaccinated mice. *Acta path. microbiol. scand.* **40**, 227.

ANDERSEN, E. K. (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.

BLYTH, W. A. (1955). The effects of immunity on the intracerebral infection of mice with *Haemophilus pertussis*. Thesis, Victoria University of Manchester.

BROWN, A. M. (1958). Intracerebral infection of mice with *Haemophilus pertussis* and passive protection by hyperimmune rabbit sera. *J. gen. Microbiol.* **18**, 48.

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.

DOLBY, J. M. & STANDFAST, A. F. B. (1961). The intracerebral infection of mice with *Bordetella pertussis*. *J. Hyg., Camb.* **59**, 205.

DOLBY, J. M., THOW, D. & STANDFAST, A. F. B. (1961). The intranasal infection of mice with *Bordetella pertussis*. *J. Hyg., Camb.* **59**, 191.

FISHER, S. (1955). Multiplication of *H. 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.

MIMS, C. A. (1960). Intracerebral injections and the growth of viruses in the mouse brain. *Brit. J. exp. Path.* **41**, 52.

M.R.C. REPORT (1956). Vaccination against whooping-cough. *Brit. med. J.* ii, 454.

PROOM, H. (1947). The immunological aspects of experimental *Haemophilus pertussis* infection. *J. Path. Bact.* **59**, 165.

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.