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The Comparison between Field Trials and Mouse Protection Tests against Intranasal and Intracerebral Challenges with *Bordetella pertussis*

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Summary and Conclusions. Fourteen vaccines, ten of which had been tested in the field in the M.R.C. trials, were assayed in the laboratory by the intracerebral (*IC*) and the intranasal (*IN*) routes. The *IC* test arranged the vaccines in quite a different order of potency to the *IN* test.

A satisfactory and reproducible assay can be made by either method, though the dose response curves about the 50 per cent protection point have very different slopes.

The field trails have shown that whereas the mouse potency test using the *IC* challenge corresponds remarkably well with the field results (*M.R.C. Report*), potency tests with the *IN* challenge do not.

Experiments with four different vaccines were consistent in showing that the *IC* antigen is heat labile and will not stand 100° for 1 hr., the *IN* antigen is heat stable and can be so heated for relatively small loss of potency.

Our results with vaccine K205 are similar to those reported by Fisher (1955), except that we were more fortunate in getting a graded dose response with the heated vaccine against the *IN* challenge, probably owing to the larger number of assays we carried out. We would agree with Fisher that individual experiments may give very odd results, and on the whole the dose response curves of individual *IN* assays are not as consistent as *IC* assays; this may be due either to differences in the antigens involved, for two different antigens must be responsible, or to differences in the course of infection in the lung and brain.

INTRODUCTION

THE experiments reported in the previous paper (Standfast, 1958), showing some differences in the factors responsible for virulence by the intranasal and intracerebral routes, suggested a reconsideration of the active protection assays with pertussis vaccines. Earlier assays carried out at the Lister Institute by both methods suggested that there was no difference in the results, at least with the particular vaccines which had been assayed. Both routes have been used for assaying vaccines, in America the intracerebral route (Kendrick *et al.*, 1947; Minimal Requirements, 1948) and in Australia the intranasal route (North, Anderson and Graydon, 1941, etc.).

MATERIALS AND METHODS

Vaccines

A series of vaccines were assayed by both methods, some of which were used in the M.R.C. trials (M.R.C. Report, 1956), others were experimental laboratory batches pre-

pared on solid or liquid media substantially as described in W.H.O. Report (1953), pp. 69 and 82.

Challenge Strains

Bordetella (*Haemophilus*) *pertussis* strain 18–323 received through the kindness of Dr. P. Kendrick, Ann Arbor, Michigan, U.S.A., was used for the intracerebral challenge, and strains 18–323, G353, G863 or M6344 for the intranasal challenge. Challenge strains were kept dried in the frozen state and were passaged through mice at intervals.

Abbreviations

Throughout this paper 'intracerebral' is abbreviated *IC*; *IC* assay means an assay using the intracerebral route of challenge; *IC* antigen means the antigen protecting mice against an intracerebral challenge. Similarly *IN* is used throughout for 'intranasal'; *IN* assay, *IN* antigen, etc.

MOUSE-PROTECTION TEST: INTRACEREBRAL CHALLENGE (*IC*)

Selection of Mice

White mice weighing 12–14 g., all of one sex, from the same Cl stock, were distributed at random into cages of 15 mice. Three cages of 15 mice were used for each antigen and in all assays at least one cage of 15 mice for unvaccinated controls; in most assays a further 3 cages of unvaccinated controls were used for the titration of the challenge dose of *B. pertussis*. Usually 195 mice were distributed into 13 cages, 9 for assay and 4 for controls; 3 antigens were tested at a time, one of which was the reference antigen.

Immunization of Mice

A single dose of vaccine was given intraperitoneally. Usually each antigen was diluted to contain 2000, 400 and 80 million bacilli in 0·2 ml. saline and each dose injected intraperitoneally into a group of 15 mice. The unvaccinated control mice were set aside at the same time and all the cages kept together in the animal house. Period before challenge 10 days.

Challenge of Mice

The suspension of *Bordetella pertussis* strain 18–323 for challenge was prepared from an 18–20 hr. culture on Bordet Gengou medium by emulsifying a little of the growth in a 1 per cent aqueous solution of Difco-Casamino acids (technical grade) so that 0·03 ml. contained 50,000 organisms by opacity, using the N.I.H. ground glass standard opacity tube. For the titration of the challenge dose 3 further dilutions to contain 5000, 500 and 50 organisms in 0·03 ml. were prepared.

The mice were lightly anaesthetized with ether or ether-chloroform mixture and a dose of 0·03 ml. of a suitable dilution injected intracerebrally from a 0·25 ml. syringe with a a small needle such as a $\frac{3}{8}$ in. 27 gauge.

Not more than 3 hr. was allowed to elapse between harvesting the challenge culture and injecting the last mouse.

Test Period and Calculation of Results

The mice were observed for 14 days and a record kept of each death. Deaths occurring in the first 48 hr. after challenge are not included in the calculation. Mice which were

paralysed on the fourteenth day, the last day of the assay, are considered as 'deaths' (recovery by paralysed mice is extremely rare) ImD50s and LD50s were calculated by the method of Reed and Muench (1938).

Comparison of Results

The ImD50 of the unknown vaccine is compared with the ImD50 of the Reference Vaccine to see if the unknown is more or less potent than the reference when tested under identical conditions.

When replicate tests were carried out either the ImD50 were averaged or the tests summed and a pool ImD50 worked out.

MOUSE PROTECTION TEST: INTRANASAL CHALLENGE (*IN*)

In most details the procedure for the *IN* assay was the same as for the *IC* assay; it differed in the following points.

Challenge of Mice

The challenge suspension was prepared from an 18–20 hr. culture of *Bordetella pertussis* on Bordet Gengou medium of a strain known to have an LD50 between 1×10^6. Dilutions were prepared in Difco-Casamino acids (technical grade) so that 0·04 ml. contained 100×10^6, 10×10^6, $1{\cdot}0 \times 10^6$ and $0{\cdot}1 \times 10^6$; the first dilution 100×10^6 is used for the challenge, the others for the titration of the challenge dose.

The mice were lightly anaesthetized with ether-chloroform mixture and two drops from a pipette, calibrated to give 50 drops per ml., are deposited on the external nases of each mouse which is held with the nose pointing vertically upwards.

Test Period

The mice are observed for 28 days, and a record kept of all deaths; those occurring in the first 48 hr. are ignored in the subsequent Reed Muench calculations.

RESULTS

Early assays by the two routes had been carried out on 3 vaccines D231, 087860 and G.61 used in the first series of M.R.C. field trials (*M.R.C. Report*, 1956). It will be seen from Table 1 that there was little difference between the two methods, and from this it was assumed that the two routes were measuring the same type and degree of immunity.

TABLE 1

POOL IMD50 ON 3 ASSAYS BY EACH ROUTE FOR VACCINES G61, D231 AND 087860 AND THE *IC*/*IN* RATIO

Vaccine	*Route of challenge*		*IC : IN ratio*
	Intranasal	*Intracerebral*	
	ImD50 in millions		
G61	2100	600	1 : 3·5
D231	1250	360	1 : 3·5
087860	1480	450	1 : 3·3

The *IC* : *IN* ratio varied from 3·3 to 3·5 and the potency ratio to G61 was about 1·7 for D231 by both routes and 1·3 and 1·4 for 087860.

Later assays by the two routes on M.R.C. field test vaccines V1, V2, V3 showed quite clearly (Table 2) that in certain vaccines there was a real difference in potency between

TABLE 2

ACCUMULATED FIGURES FOR 6 ASSAYS BY INTRACEREBRAL CHALLENGE AND 6 ASSAYS BY INTRANASAL CHALLENGE, ON VACCINES V1, V2, V3

Vaccine		*Intranasal challenge*		*Intracerebral challenge*	
Batch	*Dose millions*	*Survivors/total and % protection*	*ImD50 in millions*	*Survivors/total and % protection*	*ImD50 in millions*
V3	7000	53/88 60%	1300	51/80 64%	2600
	1400	44/81 54%		34/83 41%	
	280	35/72 49%		6/82 7%	
V1	7000	50/88 57%	1650	36/70 51%	4500
	1400	39/83 47%		17/82 21%	
	280	35/88 40%		4/86 5%	
V2	7000	45/86 52%	1670	11/82 13%	∝
	1400	40/86 47%		9/85 10%	
	280	38/89 43%		5/78 6%	

assays by the two routes. Vaccine V2 is of the same order of potency as V1 and V3 by the *IN* test; though no accurated estimate of the ImD50 can be calculated, V2 is clearly far less potent by the *IC* test, at the highest dose level (7000×10^6) only 13 per cent of the mice were protected as compared with 50–60 per cent with the other vaccines. The differences between the dose response curves show in Fig. 1.

A further series of assays by both routes was carried out on the M.R.C. field test vaccines V8, V9, V10, V18. Each vaccine was assayed six times by each route using V8

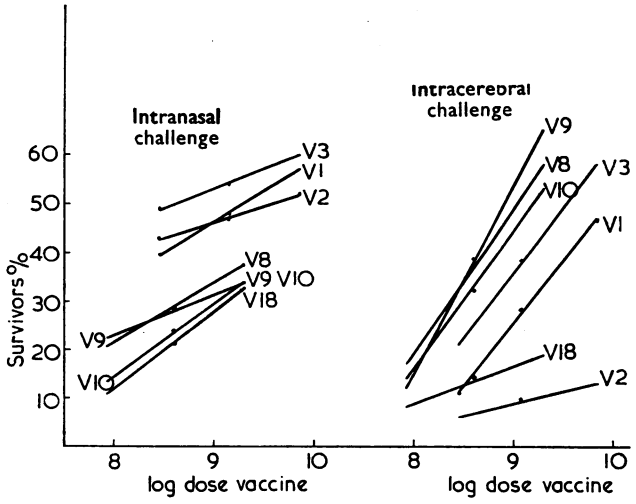

FIG. 1. Dose response curves of vaccines against intranasal (*IN*) and intracerebral (*IC*) challenges based on all available assays for each vaccine.

as the reference vaccine (Table 3 and Fig. 1). In this series V18, although of equal potency to V8 by the *IN* route (ratio 1·07), was much less potent by the *IC*, only about 20 per cent of the mice being protected at the top dose; the calculated ImD50 of 8500 millions is about eight times the ImD50 of V8.

TABLE 3

POOL IMD50 IN MILLIONS OF VACCINES V8, V9, V10 AND V18 ASSAYED BY THE INTRANASAL AND INTRACEREBRAL ROUTES, WITH POTENCY RATIOS IN TERMS OF VACCINE V8, AND *IN*/*IC* RATIOS

Vaccine	*Challenge*				*IN*/*IC ratio*
	Intranasal		*Intracerebral*		
	ImD50 in millions	*Potency ratio*	*ImD50 in millions*	*Potency ratio*	
V18	1200	1·07	*c.* 8500	0·12	0·14
V8	1280	1·00	1050	1·0	1·22
V10	1600	0·80	1900	0·55	0·84
V9	1700	0·75	810	1·3	2·10

RELATIVE POTENCIES OF VACCINES BY BOTH TESTS IN TERMS OF A REFERENCE VACCINE

The estimates of the potency-ratios in terms of the reference vaccine G61, and the log potency ratios, for all vaccines tested by both *IC* and *IN* assay, are shown in Table 4 (see also *M.R.C Report*, 1956, Table IX).

TABLE 4

RELATIVE POTENCIES OF VACCINES BY MOUSE PROTECTION TESTS, IN TERMS OF REFERENCE VACCINE G61

Vaccine	*Intracerebral challenge*		*Intranasal challenge*	
	Potency ratio	*log*	*Potency ratio*	*log*
V9	9·08	0·958	0·81	−0·908
X1	9·08	0·958	4·00	0·602
V8	7·10	0·851	1·07	0·029
V10	3·82	0·582	0·86	−0·934
D231	3·93	0·594	1·37	0·137
087860	2·00	0·300	1·67	0·223
V7	1·03	0·012	0·29	−0·462
G61	1·0	0	1·0	0
V3	0·46	−0·335	0·97	−0·987
V1	0·15	−0·829	0·77	−0·886
X2	0·05	−1·699	1·5	0·176
V18	0·04	−1·602	1·14	0·057
V2	0·03	−1·504	0·75	−0·875
X3	0·02	−1·301	2·06	0·314

The ratios for the 14 vaccines tested by the *IN* challenge vary far less than those for the *IC* challenge. With two exceptions, vaccines XI and V7, the potency ratios of the 14 vaccines do not differ significantly from 1·00. XI is significantly better than G61 and V7 is probably worse than G61.

With the *IC* tests, 5 vaccines, V9–D231 (Table 4), are significantly better than G61; 3 vaccines, 087860, V7 and V3, as good as G61; and 5 vaccines, V1–X3, significantly worse than G61.

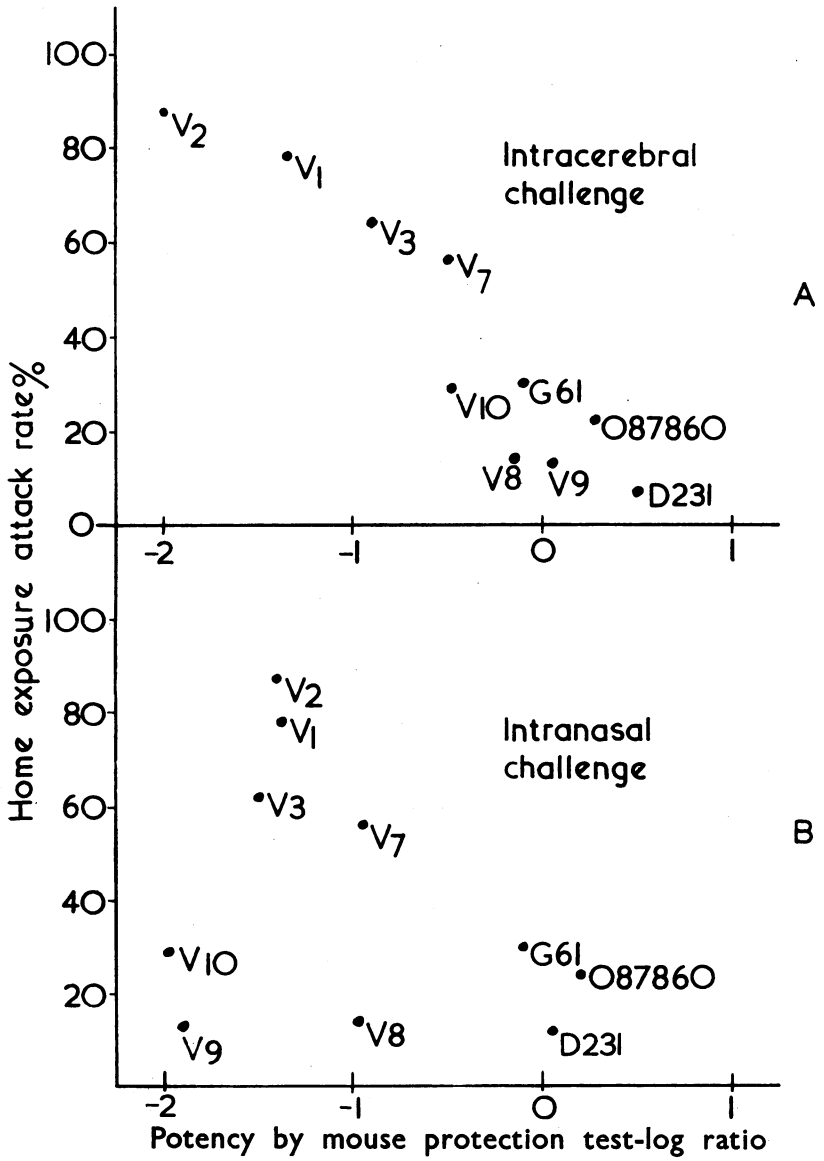

FIG. 2A. Chart showing the relationship in 10 pertussis vaccines between home exposure attack rate in the field and potency as estimated by intracerebral challenge-mouse protection tests, corrected for deterioration of vaccines. Figures taken from *M.R.C. Report*, 1956 (Table XII and chart).

FIG. 2B. Chart showing the relationship in the same 10 vaccines between home exposure attack rate and potency as estimated by intranasal challenge-mouse protection tests, corrected for deterioration of vaccines.

THE COMPARISON OF FIELD TRIALS AND MOUSE PROTECTION TESTS

The relationships between the home exposure attack rates in children and the mouse potency ratios of all the vaccines tested by both protection tests are shown in Fig. 2.

One of the difficulties in assessing the relationship between the results of the field trials and the mouse protection tests is the estimation of the deterioration (if any) of fluid vaccines over the period of time of the trials and the assays (*M.R.C. Report*, 1956; Armitage and Perry, 1957). In Fig. 2A and B the same assumption has been made as in the M.R.C. Report—that all fluid vaccines considered here lost potency at a rate of 0·2 log units per year over the period of test.

Figure 2A shows a significant correlation between the home exposure attack rate in the field and potency as estimated by the intracerebral challenge mouse protection test in the 10 vaccines tested. Figure 2B shows the complete lack of correlation between home exposure and the intranasal challenge mouse protection test, e.g. V9, V8 and D231 with very different log potency ratios show the same home exposure attack rate, while V9, V10, V1 and V2 with about the same log potency ratios have widely differing home exposure rates.

If we assume that fluid vaccines do not deteriorate (Kendrick *et al.*, 1955; Armitage and Perry, 1957) and that the observed fluctuations were due to unavoidable difference in the mice, we obtain the results shown in Fig. 2C and D. Like those in Fig. 2A and B, they support the conclusion of the M.R.C. Report that field results and the *IC* mouse assay are related; but there is no better fit between the field results and the *IN* test. The relative potencies of different vaccines by both methods of assay are of course altered.

The Heat Stability of the IN Antigen

It was suspected that one vaccine which gave good *IN* protection and poor *IC* protection had been overheated during production. Assays were accordingly carried out on 4

TABLE 5

AVERAGE IMD50 IN MILLIONS FOR PAIRED ASSAYS—HEATED AND UNHEATED—ON THREE VACCINES D231, K205 AND K134. VACCINES WERE HEATED AT 100° FOR 1 HR.

Vaccine	*Challenge*		*No. of paired assays*	
	Intranasal	*Intracerebral*	*IN*	*IC*
	ImD50 in millions			
K205	205	440	6	6
K205 heated	450	∝	6	6
D231	1150	410	2	2
D231 heated	*c.* 2500	∝	2	2
V8	245	520	2	2
V8 heated	270	∝	2	2
K134	495	675	2	2
K134 heated	645	∝	2	2

∝ the ImD50 could not be calculated as only a few mice survived scattered at random, e.g. first K205 experiment:—
K205—dose 2000, 800, 40 millions (survivors/total) 11/15, 9/15, 4/15:
K205 heated—dose 10,000, 2000, 800 millions 2/15, 0/15, 3/15.

vaccines, portions of which had been heated for 1 hr. to see whether heating revealed any difference between the antigens responsible for protection against *IN* and *IC* challenge. Boiling for 1 hr. destroyed the *IC* antigen, though this antigen is known to be stable to 56° (Pittman, 1952), but did not affect the *IN* antigen to any great extent (Table 5). In the

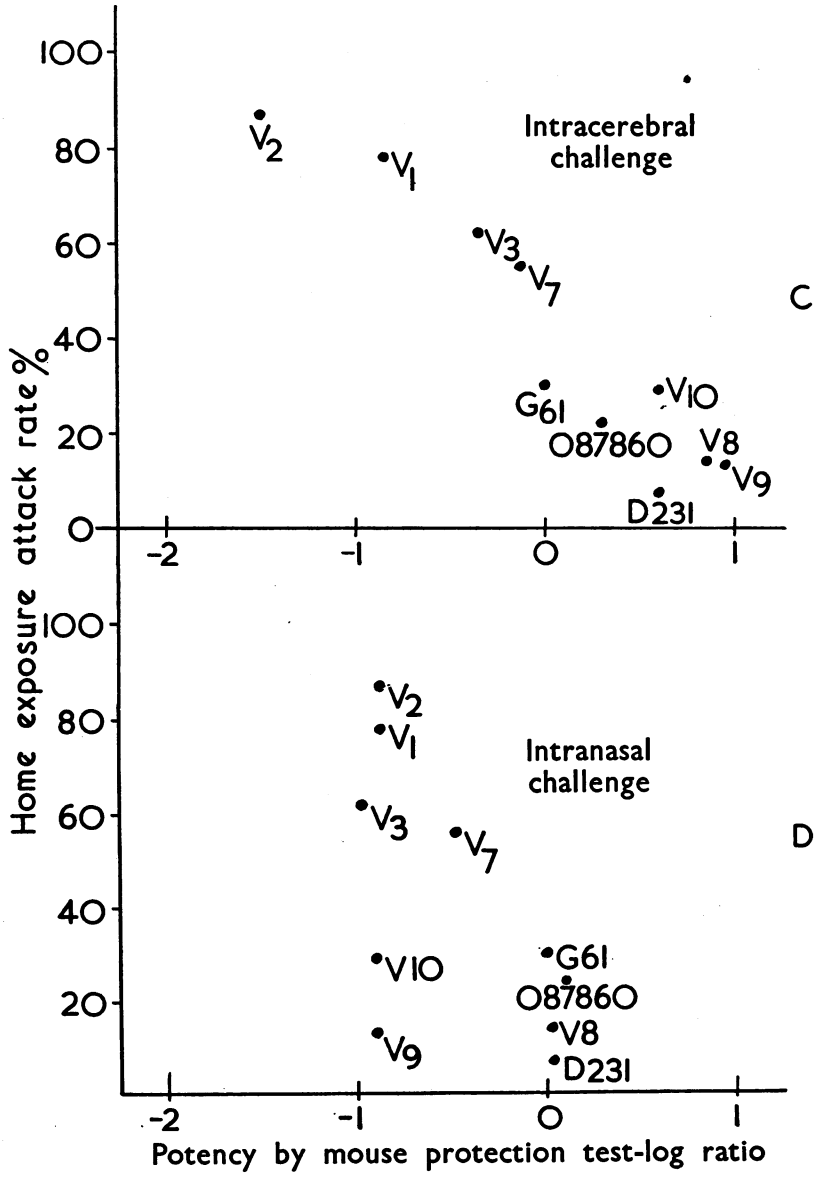

FIG. 2C. Chart showing the relationship in the same 10 vaccines between home exposure attack rate and potency as estimated by intracerebral challenge-mouse protection tests from *M.R.C. Report*, 1956 (Table IX).

FIG. 2D. Chart showing the relationship in the same 10 vaccines between home exposure attack rate and potency as estimated by intranasal challenge-mouse protection tests.

4 vaccines, boiling so affected the *IC* antigen that it can be regarded as totally destroyed even at a dose of 10,000 million, five times the usual maximal dose; only a few mice at random among the three dose groups in the 10 assays survived the challenge. On the other hand, the *IN* antigen lost perhaps half its potency, but not more.

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