INTERFERENCE IMMUNITY PRODUCED BY PERTUSSIS VACCINE TO PERTUSSIS INFECTION IN MICE.

D. G. EVANS AND F. T. PERKINS.

From the Department of Bacteriology, University of Manchester.

Received for publication August 24, 1954.

WE have previously reported (Evans and Perkins, 1954) that a single intraperitoneal injection of pertussis vaccine produces in mice an early immunity to intracerebral infection with *Haemophilus pertussis*. This effect appeared to be specific for pertussis vaccine since it was not produced by a number of other antigenic preparations. The rapidity with which the immunity developed and the fact that it was independent of circulating specific antibodies suggested that the acquired resistance was of a type for which serological mechanisms were not responsible. The possibility was considered that the effect might be analogous to the " interference phenomenon " which has been shown to occur between inactivated and active virus of the same strain (Henle, 1950). This possibility has now been investigated by studying the protective action of pertussis vaccine when given to mice intracerebrally at the site of infection.

MATERIALS AND METHODS.

Vaccines.—Three of the pertussis vaccines, V12, V17 and YPS, which we used in our previous study were tested. Vaccines V12 and YPS were whole bacterial suspensions each containing 20×10^9 organisms/ml. (U.S. National Institutes of Health opacity standard). Vaccine V12 was killed by 1/10,000 thiomersalate and YPS by heating at 55° for ½ hr. Vaccine V17 was a cell-free bacterial extract prepared by Pillemer, Blum and Lepow (1954) by the method they described and which they have called SPA.

Inoculation of mice.—Female white mice with an average weight of 16 g. were used. They were injected intracerebrally with a challenge suspension of virulent *H. pertussis* according to the method described by Kendrick, Eldering, Dixon and Misner (1947) using strain No. 18/323 which they recommended. The challenge suspensions were prepared by suspending, to the desired concentration, the 24 hr. growth from Bordet-Gengou medium in casein hydrolysate. Each challenge suspension was titrated by injecting 5 groups, each of 15 mice, with graded dilutions and the number of LD_{50} contained in the challenge dose was calculated by the method of Reed and Muench (1938) from the number of deaths occurring in each group within 21 days after injection. With the strain of mice used, the LD_{50} of the challenge suspensions lay between 1000 and 2000 organisms (U.S. opacity standard).

Vaccine suitably diluted in casein hydrolysate was also injected intracerebrally into mice, either mixed with, or 3 hr. before the challenge suspension. When vaccine and challenge suspension were given together, the total inoculum was 0·03 ml., and when given separately, each inoculum was 0·03 ml. All intracerebral injections were made into the left cerebral hemisphere. It was found that mice were able to withstand 2 intracerebral injections into the same site and only on rare occasions did an early non-specific death occur. The mice were observed for 21 days after injection and the number of deaths and times to death recorded.

RESULTS.

Protective Effect of Vaccine Mixed with Challenge Dose.

Each of the 3 vaccines was tested for its ability to protect mice against infection when given intracerebrally together with the challenge dose. The results of a test

with vaccine V12 in which the challenge dose contained 40 LD_{50} are given in Fig. 1. In this test, 4 groups, each of 15 mice, were used. Three of the groups were each injected with the challenge dose suspended in vaccine diluted 1/3, 1/30 and 1/300 respectively and the fourth group with the challenge dose only. The doses of vaccine corresponding to the 3 dilutions contained 2×10^8, 2×10^7 and 2×10^6 organisms respectively. All the mice in the control group died by the 11th day after injection. Evidence of protection was shown by the group which received vaccine diluted 1/3 ; 9 of the 15 mice were alive on the 11th day and 7 on the 21st day after injection. There was also evidence of protection, although not so marked, with the groups which received vaccine diluted 1/30 ; 7 of the 15 mice were alive on the 11th day and 4 on the 21st day. Little or no protection, however, was given by vaccine diluted 1/300 ; in the group injected with this dilution, 14 of the mice died at about the same average time as those in the control group and only one mouse survived for 21 days.

The protective effect of vaccine V12, when given mixed with the challenge dose, was also evident from a second test (Fig. 2) in which the challenge dose contained approximately only 1 LD_{50}. Two groups each of 15 mice were used, one group receiving the challenge dose suspended in vaccine diluted 1/100 ; each mouse thus received 6×10^6 organisms of vaccine, and the other group the challenge dose only. Of the 15 mice in the group receiving vaccine, all survived the 21-day period except one which died on the 15th day after injection. Of the 15 mice in the control group, 7 died and all deaths occurred before the 13th day.

Similar tests were made with the other bacterial vaccine YPS and also with the bacterial-extract vaccine V17. The results obtained in one test with each vaccine are shown in Table I together with those obtained with V12. It is evident that each vaccine gave definite protection especially when given in dilutions of 1/3 and 1/30.

The protective property of YPS and V17 was also demonstrated in tests using a range of different challenge doses. Three series of the same 4 graded dilutions of a challenge suspension were made, one in a 1/30 dilution of YPS, a second in a 1/30 dilution of V17 and a third, the control series, in casein hydrolysate. Each dilution was injected intracerebrally into a group of 15 mice. The results are shown in Table II and it is evident that with each of the 4 challenge doses, which ranged from 0·5 to 150 LD_{50}, the two vaccines gave definite protection.

Tests were made which showed that the property of the vaccines to protect mice against infection was not due to an effect of the vaccines on the viability of the organisms *in vitro* before injection. A series of 10-fold dilutions of a challenge suspension was made in each of the three vaccines diluted 1/3 and also a control series in casein hydrolysate. The dilutions were left at room temperature for 1 hr. when plate counts were made in duplicate on Bordet-Gengou medium by the method of Miles and Misra (1938). There was no significant difference in the results obtained with each of the four counts, indicating that there was no effect on the viability of *H. pertussis* by any of the three vaccines.

Protective Effect of Vaccine Given Three Hours Before Challenge Dose.

Two of the vaccines, V12 and V17, were tested for their ability to protect mice against infection when given intracerebrally 3 hr. before the challenge dose. The results of two tests with the whole bacterial vaccine V12 are given in Fig. 3

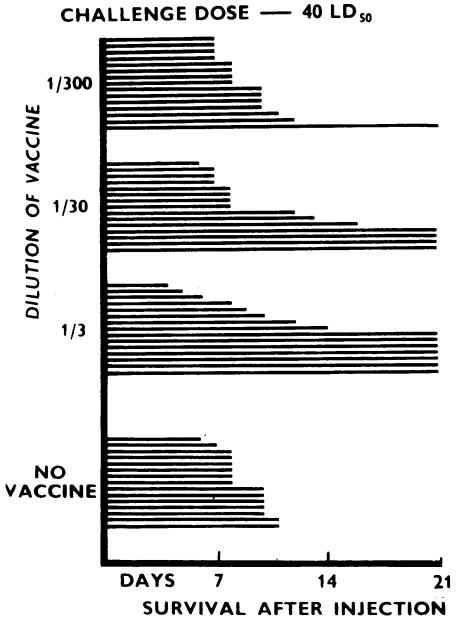

FIG. 1.

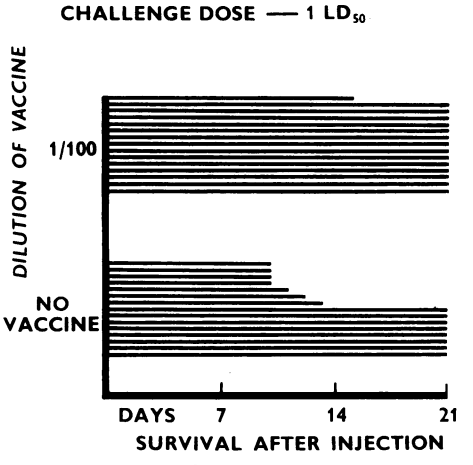

FIG. 2.

FIG. 1 and 2.—The protection of mice with whole bacterial pertussis vaccine V12 injected intracerebrally mixed with challenge dose of *H. pertussis*.
In these Figures and in Fig. 3 and 4, each horizontal line represents the duration of survival of one mouse; mice surviving for 21 days were killed.

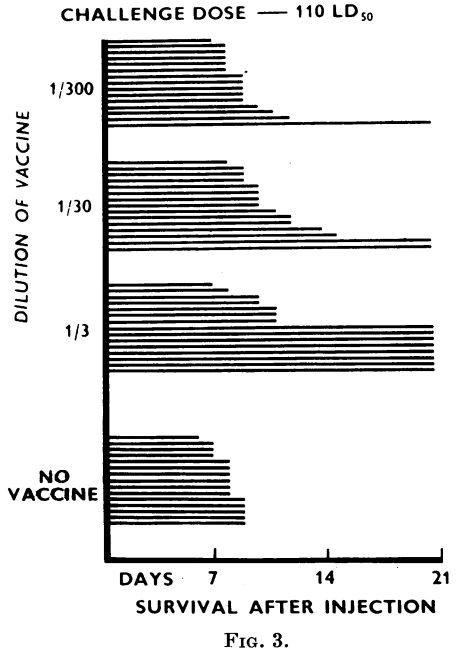

FIG. 3.

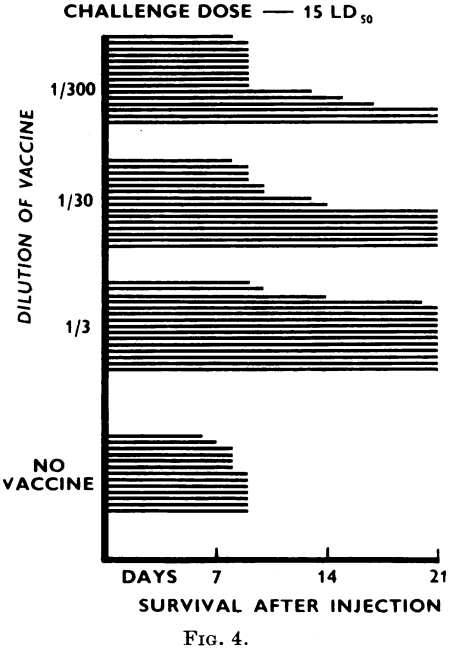

FIG. 4.

FIG. 3 and 4.—The protection of mice with whole bacterial pertussis vaccine V12 injected intracerebrally three hours before challenge dose of *H. pertussis*.

TABLE I.—*Protective Effect of Pertussis Vaccine when given Intracerebrally together with Challenge Dose of* H. pertussis.

Pertussis vaccine.	Dose of vaccine (dilution).	Challenge dose of *H. pertussis* (LD_{50})	No. of mice used.	No. of mice alive at— 10 days.	No. of mice alive at— 21 days.	Percentage of mice alive at 21 days.
V12	1/3	40	15	9	7	47
	1/30		15	7	4	27
	1/300		15	3	1	7
	None		15	2	0	0
V12	1/100	1	15	15	14	93
	None		15	11	8	53
YPS	1/3	150	14	13	10	71
	1/30		15	7	5	33
	1/300		15	5	3	20
	None		15	0	0	0
V17	1/3	150	13	10	5	38
	1/30		14	6	4	29
	1/300		15	3	0	0
	None		15	0	0	0

TABLE II.—*Protective Effect of Pertussis Vaccine when given Intracerebrally together with the Challenge Dose of* H. pertussis.

Vaccine and dose (dilution).	Challenge dose *H. pertussis* (LD_{50}).	No. of mice used.	No. of mice alive at 10 days.	No. of mice alive at 21 days.	Percentage of mice alive at 21 days.
YPS 1/30	0·5	15	15	14	93
	3	15	15	12	80
	15	15	13	8	53
	150	15	11	4	27
V17 1/30	0·5	15	15	15	100
	3	15	14	11	73
	15	15	9	5	33
	150	15	7	5	33
None	0·5	15	12	10	67
	3	15	5	3	20
	15	15	5	0	0
	150	15	0	0	0

and 4. Four groups, each of 15 mice, were used for each test. Three of the groups were each injected with vaccine diluted 1/3, 1/30 and 1/300 respectively and 3 hr. later all four groups were challenged.

In the first of these tests (Fig. 3) the challenge dose contained 110 LD_{50} and killed all the control mice by the 9th day. Vaccine diluted 1/3 showed a protective effect since 13 of the 15 mice which received this dilution were alive on the 9th day and 8 were alive on the 21st day after injection. Vaccine diluted 1/30 also showed a protective effect; 11 of the 15 mice receiving this dilution were alive on the 9th day and 2 on the 21st day. Only a slight effect was evident in the group receiving vaccine diluted 1/300; 4 mice were alive on the 9th day and one on the 21st day.

In the second test with V12 (Fig. 4) the protective effect of vaccine was more definite, which was probably due to the fact that a smaller challenge dose of 15

LD_{50} was used. Of the group of 15 mice which received vaccine diluted 1/3, 14 were alive on the 9th day, when all the mice in the control group were dead, and 11 on the 21st day. Of the group which received a 1/30 dilution of vaccine, 11 of the 15 mice were alive on the 9th day and 7 on the 21st day. Vaccine diluted 1/300 also gave some protection ; 6 of the 15 mice which received this dilution were alive on the 12th day and 3 on the 21st day.

The bacterial-extract vaccine V17 also gave protection when given 3 hr. before the challenge dose. The results of two tests with this vaccine are given in Table III which includes those with V12. In the first test with V17 a high challenge dose containing 350 LD_{50} was used, yet in spite of this vaccine diluted 1/3 gave substantial protection. In the second test a similar result was obtained using vaccine diluted 1/30 followed by a challenge dose containing 35 LD_{50}.

TABLE III.—*Protective Effect of Pertussis Vaccine when given Intracerebrally Three Hours before Challenge Dose of* H. pertussis.

Vaccine	Dose of vaccine (dilution).	Challenge dose of *H. pertussis* (LD_{50}).	No. of mice used.	No. of mice alive at 10 days.	No. of mice alive at 21 days.	Percentage of mice alive at 21 days.
V12	1/3	110	15	11	8	53
	1/30		15	7	2	13
	1/300		15	3	1	7
	None		15	0	0	0
V12	1/3	15	15	13	11	73
	1/30		15	9	7	47
	1/300		15	6	3	20
	None		13	0	0	0
V17	1/3	350	11	7	5	45
	None		15	0	0	0
V17	1/30	35	14	8	5	36
	None		15	3	0	0

DISCUSSION.

These experiments have shown that pertussis vaccine in the form of either a bacterial suspension or a cell-free extract, is able to protect mice against fatal infection produced by an intracerebral challenge injection of virulent *H. pertussis* when given at the site of infection either with or a few hours before the challenge dose. We have not yet followed the fate of the infecting organisms in the brains of mice after injection, but we have shown that in a high proportion of protected mice the brains are free from viable *H. pertussis* when cultivated 28 days after challenge. It is therefore evident that pertussis vaccine, by some mechanism of interference, is able to influence the course of intracerebral *H. pertussis* infection and that this interference effect is not a function of the intact whole bacterial cell.

These results agree with our previous findings (Evans and Perkins, 1954), which showed that an intraperitoneal injection of pertussis vaccine into mice is able to produce within 5 hours a substantial immunity to intracerebral infection with *H. pertussis*, in the absence of detectable circulating specific antibodies. This early immunity is probably due to the same mechanism as that involved when

vaccine is injected locally at the site of infection. Its development, however, would naturally be slower than that after intracerebral injection, since it would depend upon the time taken for the interfering agent to reach the site of infection in sufficient concentration to affect the course of the disease. This hypothesis is supported by the fact that although immunity is evident in mice within 5 hours after intraperitoneal injection, it is not, as we have since shown, evident in mice which have received vaccine only a few minutes before challenge.

What mechanisms are involved in this interference phenomenon are not known, but it is reasonable to suggest that they may be similar to those occurring in the case of viruses where the cellular basis for interference is well established. Support for this suggestion is provided by the work of Dalldorf, Cohen and Coffey (1947) and Cohen (1953) who showed that mice were protected against infection following a small intracerebral challenge dose of *H. pertussis* by a prior intracerebral injection of certain viruses.

The early immunity produced by pertussis vaccine must be distinguished from that which develops later and is especially evident after repeated intraperitoneal injections. We have shown in a few preliminary experiments, using the whole bacterial vaccine V12, that the later immunity is of the antibody type and that serum from immunized mice is able to protect mice passively against intracerebral pertussis infection. It is not known, however, how soon after injection of vaccine the antibody type of immunity manifests itself or at what stage the interference effect begins to wane. It may in fact be difficult to discriminate between these two types of immunity, especially if the antigen responsible for producing the antibody type is also the responsible agent in the production of the early interference phenomenon. These are problems which are at present under investigation.

SUMMARY.

Pertussis vaccine, either in the form of a killed bacterial suspension or a cell-free extract, was shown to interfere with the development of fatal pertussis infection in the mouse. Mice were protected against infection following an intracerebral challenge injection of *H. pertussis* by an intracerebral injection of pertussis vaccine given at the same time or 3 hr. before the infecting challenge dose.

We wish to thank the Medical Research Council for samples of pertussis vaccines received from the Whooping Cough Immunization Committee and for a grant towards the expenses of this work.

REFERENCES.

COHEN, S. M.—(1953) *VI Congr. int. Microbiol.; Riassunti delle Communicazioni*. Vol. II, p. 414.

DALLDORF, G., COHEN, S. M. AND COFFEY, J. M.—(1947) *J. Immunol.*, **56**, 295.

EVANS, D. G. AND PERKINS, F. T.—(1954) *Brit. J. exp. Path.*, **35**, 322.

HENLE, W.—(1950) *J. Immunol.*, **64**, 203.

KENDRICK, P. L., ELDERING, G., DIXON, M. K. AND MISNER, J.—(1947) *Amer. J. publ. Hlth*, **37**, 803.

MILES, A. A. AND MISRA, S. S.—(1938) *J. Hyg., Camb.*, **38**, 732.

PILLEMER, L., BLUM, L. AND LEPOW, I. H.—(1954) *Lancet*, i, 1257.

REED, L. J. AND MUENCH, H.—(1938) *Amer. J. Hyg.*, **27**, 493.