THE EFFECT OF HEMOPHILUS INFLUENZAE SUIS VACCINES ON SWINE INFLUENZA

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Early experiments indicated that the bacterium, *Hemophilus influenzae suis*, (1) administered intranasally to swine, conferred no immunity to swine influenza (2). These had not been intended to test the possible value of the organism for use as a prophylactic agent in controlling swine influenza, and no experiments in which the bacterium was administered in other ways than intranasally were conducted. More recent experiments (3), however, have indicated that, when accompanied by human influenza virus, *H. influenzae suis* does play a rôle in immunizing swine to swine influenza. It has been found that while swine recovered from infection with a mixture of human influenza virus and *H. influenzae suis* were usually immune to swine influenza, those recovered from infection with human influenza virus alone were usually not immune. These experiments were believed to indicate that *H. influenzae suis*, in the presence of a concomitant human influenza virus infection, immunized swine to the bacterial component of the swine influenza etiological complex (4).

The apparent discrepancy between the earlier and the more recent experiments could be explained by assuming that when the bacterium alone was administered intranasally to swine it was applied superficially to an intact mucosa that was impermeable to its deep penetration. The inability of the bacterium to penetrate the respiratory tract mucous membranes could account for its failure to induce an immunity response. However, when *H. influenzae suis* was given intranasally to swine in mixture with human influenza virus it was not only afforded a portal of entry into deeper tissues through lesions produced by the virus, but the influence of the virus may have en-

dowed it with invasive properties that it did not possess alone. Under such circumstances the bacterium might be expected to elicit an immunity response. This possible explanation of the observed phenomena raised the question of whether *H. influenzae suis* vaccines given intramuscularly might not immunize swine to the bacterial component of the etiological complex of swine influenza.

EXPERIMENTAL

Preparation of H. influenzae suis Vaccines.—Cultures 18 and 23 *H. influenzae suis*, originally obtained from naturally occurring field cases of swine influenza, were used either singly or pooled in the experiments. The 48 hour growths from chocolate agar slants were scraped off and suspended in a small amount of physiological saline. These suspensions were then centrifuged in graduated tubes for ½ hour at 1600 to 1800 R.P.M. The volume of bacterial sediment was noted after which the sediment was resuspended in sufficient physiological saline to make a final 1 per cent by volume suspension. Part of the suspension was removed to use as living vaccine while the remainder was heated at 57°C. for 30 minutes in sealed tubes submerged in a water bath. All heated suspensions proved sterile when planted on media capable of supporting the growth of *H. influenzae suis*.

At the time that the present experiments were conducted no recently isolated strains of *H. influenzae suis* were at hand. The two strains used had both been under cultivation sufficiently long that, while still capable of producing influenza when given intranasally to swine in mixture with swine influenza virus, they no longer transferred with the virus from sick to normal animals by contact. The ability of the bacterium to transfer together with the virus from swine to swine by pen contact is a property possessed by all freshly isolated cultures of *H. influenzae suis* which is lost after a variable period of cultivation on artificial media (5). The experiments to be reported were conducted with non-contagious strains of the bacterium.

Vaccination of Swine with Heated and Living Hemophilus influenzae suis

Each of 8 swine were given 3 intramuscular injections at 8 day intervals of heat-killed *H. influenzae suis*; a second group of 6 swine received injections similarly of living *H. influenzae suis*. The amount of the first dose administered was 1 cc., while the 2 succeeding doses were of 2 cc. each. The heated vaccine caused no apparent reaction in any of the animals. The living vaccine, however, caused a sharp temperature elevation on the day following the second injection.

The vaccinated animals were tested for immunity to swine influenza, 9 to 14 days after their last dose of vaccine, by intranasal inoculation with a mixture of

swine influenza virus and *H. influenzae suis*. After either 3 or 4 days of clinical observation they were killed and autopsied and their respiratory tracts examined for lesions of influenza. Details of the experiments and the outcome of the tests for immunity are given in Table I.

As shown in the table, the results obtained were not clear cut and there was considerable individual variation in the degree of protection afforded. In only one instance, that of swine 1690, was protection against the effects of *H. influenzae suis* apparently complete. The disease seen in this animal was typical, both clinically and at autopsy, of that produced by the virus alone (4); and *H. influenzae suis* could not be cultivated from the respiratory tract. The remaining 7 swine, vaccinated with heated *H. influenzae suis*, developed, when tested for immunity, a swine influenza that was less severe clinically than that shown by any of the 3 control swine. At autopsy, the influenzal pneumonia encountered in the vaccinated pigs was found to involve from 1.5 to 3.5 lobes, whereas, in the control animals, 4 and 5 lobes were consolidated. Virus, demonstrable by mouse inoculation (6), was present in the lungs of all of the pigs, but *H. influenzae suis* could not be grown from the affected lungs of 3 of the 8 vaccinated animals, although it was present higher in the respiratory tract in 2 of the 3 cases. It seems likely that the suppression of *H. influenzae suis* in these 3 swine was an effect of the immunization procedure.

The swine vaccinated with living *H. influenzae suis* differed somewhat from those that had received heated vaccine. When tested for immunity to swine influenza they became severely ill within 24 hours, lay prostrate, and exhibited temperatures of 41°C. or higher. The control swine at this time were only slightly ill, and showed less elevation of temperature. On the 2nd day, however, the vaccinated animals were much improved and no case at this time could have been classified clinically as more than a mild swine influenza. Their temperatures dropped either to normal or to low fever level and remained there. The control swine, on the other hand, became progressively worse and exhibited the signs of typical swine influenza. At autopsy the difference in the extent of pneumonia shown by the vaccinated pigs and the control animals was not striking, and probably in the cases of the last 4 animals in Table I it was negligible. However, the amount of consolidation in the lung of neither control animal was as

TABLE I

Effect of Hemophilus influenzae suis Vaccines on Swine Influenza

Swine No.	Vaccination: Three intramuscular inoculations with	Vaccination: Result	Time between vaccination and immunity test	Test for immunity to swine influenza*: Clinical illness	Findings in lungs at autopsy: Extent of lesions	Findings in lungs at autopsy: Virus	Findings in lungs at autopsy: *H. influenzae suis*
			days				
1663	Heat-killed *H. influenzae suis*	No illness	9	Mild influenza	2†	Present	Absent
1667	" " " " "	" "	9	2 day fever; not definitely ill	1.5	"	Present
1690	" " " " "	" "	9	"Filtrate disease"	0.3	"	Absent
1693	Nil, control for above 3 vaccinated swine			Typical swine influenza	4.3	"	Present
1708	Heat-killed *H. influenzae suis*	No illness	14	Mild influenza	3.5	"	"
1709	" " " " "	" "	14	4 day fever; mildly ill	3	"	"
1710	" " " " "	" "	14	4 " " " "	2.5	"	"
1712	" " " " "	" "	14	4 " " " "	2	"	Absent
1713	" " " " "	" "	14	4 " " " "	3	"	Present
1716	Nil, control for above 5 vaccinated swine			Typical swine influenza	5	"	"
1725	" " " " 5 "	"		" " "	5.3	"	"
1731	Living *H. influenzae suis*	Fever 1 day after 2nd injection	10	Fever and prostration 1st day; mildly ill later	1.5	"	Absent
1735	" " " "	" "	10	" "	1.5	"	"
1737	" " " "	" "	10	" "	1.5	"	"
1732	Nil, control for above 3 vaccinated swine			Severe swine influenza	2.5 (bilateral pleuritis)	"	Present

1730	Living *H. influenzae suis*	Fever 1 day after 2nd injection	9	Fever and prostration 1st day; mildly ill later	2.3	"	Absent
1743	" " " "	" "	9	" "	2.3	"	Present
1745	" " " "	" "	9	" "	1.7	"	Absent
1754	Nil, control for above 3 vaccinated swine			Typical swine influenza	2.3	"	Present

* Intranasal inoculation with mixture of swine influenza virus and *H. influenzae suis*.

† Pneumonia expressed in number of lobes involved. (The swine lung has 7 lobes.)

extensive as is usual in typical swine influenza, although one had a bilateral fibrinous pleuritis. Virus was present in the lungs of all pigs, but *H. influenzae suis* could be grown from the pneumonic lung of only 1 of the 6 vaccinated animals despite its presence higher in the respiratory tracts of all. As in the case of the animals that received heated vaccine, this suppression of *H. influenzae suis* in the lung is considered an effect of the immunization procedure.

None of the sera of the vaccinated swine, drawn just prior to their test for immunity, exerted any neutralizing effect on the swine influenza virus. Neither did they contain agglutinins for *H. influenzae suis.*

DISCUSSION

The results obtained in the present experiments, when considered as a whole, furnish evidence that *H. influenzae suis* given intramuscularly to swine elicits an immune response capable of modifying the course of a later swine influenza infection. Heated vaccine appears to be at least as effective as a living one so far as can be judged from clinical and postmortem findings. However, *H. influenzae suis* was more often completely suppressed in the pneumonic lungs of animals vaccinated with live vaccine than in the lungs of those that had received the heated vaccine. If this suppression of the specific bacterium is really an effect of the immunization procedure, then more protection was achieved by the living vaccine. The severe clinical reaction, with extreme prostration and high fever, occurring within 24 hours of the test for immunity in the swine vaccinated with living organisms, may represent an allergic reaction in which destruction of *H. influenzae suis* occurs in the lung. Certainly the prompt clinical improvement shown by these animals after their initial reaction suggests that the swine influenzas they suffer are not progressive after the first 24 hours, and that the factors responsible for the continued illness of the control swine are no longer operative in them. Their condition corresponds to that seen at the onset of convalescence on the 5th or 6th day post-infection in susceptible swine when, though still carrying anatomical changes caused by influenza, they appear clinically almost normal. In the animals treated with heated vaccine, on the other hand, suppression or destruction of *H. influenzae suis* in the respiratory tracts

appears to be less drastic and, while partial protection is evident from both clinical and postmortem examination, the immediate severe reaction following the test for immunity is avoided. No explanation for this difference in the character of immunity established by heated and living *H. influenzae suis* vaccines is apparent. Agglutinins for *H. influenzae suis* were not demonstrable in the sera of any of the vaccinated swine at the time of the test for immunity to swine influenza.

From the practical standpoint of controlling swine influenza the partial protection afforded swine by the bacterial vaccines is of no immediate value since it is already known that complete protection to the disease can be achieved by means of swine influenza virus vaccines (2, 7). The present studies are of interest only in showing that at least a partial immunity to the bacterial component of the etiological complex responsible for swine influenza can be established, and that this is capable of modifying the course of a later swine influenza infection. Swine influenza virus vaccines remain the method of choice in immunizing swine to swine influenza.

SUMMARY

Either living or heat-killed *H. influenzae suis* vaccines, given intramuscularly to swine, elicit an immune response capable of modifying the course of a later swine influenza infection. The protection afforded is only partial and is in no way comparable to the complete immunity afforded by swine influenza virus vaccines.

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