

## STUDIES ON IMMUNITY TO SWINE INFLUENZA

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### INTRODUCTION

It has previously been shown (1) that the bacterium, *H. influenzae suis*, and a filtrable virus are essential to the production of influenza in swine. When administered separately intranasally, *H. influenzae suis* is completely non-pathogenic, while the filtrable virus when similarly introduced induces a very mild and scarcely recognizable illness that has been designated as the "filtrate disease" in distinction from swine influenza which results when virus and organism are administered intranasally together. The present paper describes studies dealing with the immunizing properties of each of the etiological components.

### *Infectious Materials Used*

As in our earlier experiments (1, 2), *H. influenzae suis* was grown upon plain agar slants to the condensation water of which had been added approximately 0.75 cc. of sterile defibrinated horse blood. On such media the growth of the organisms was limited largely to the bloody fluid at the base of the slant and a narrow zone of agar immediately above. A stock culture isolated in December, 1928, and designated as 451, has been used in all experiments recorded in this paper. Only the bloody condensation fluid of cultures was used in inoculating the swine in the experiments to be reported.

The swine influenza virus used was either a Berkefeld N or a Berkefeld V filtrate of lung, bronchial lymph nodes, and bronchial exudate from a fresh case of the disease. Animals to be used as a source of virus were killed on the 3rd or 4th day of their illness. Pathological material to be filtered was minced with scissors and ground fine in a mortar with sterile sand. A suspension of approximately 5 per cent was made in infusion broth, pH 7.3, shaken by hand for 10 minutes, and then centrifuged. The supernatant fluid was removed and filtered through a Berkefeld candle, and the resulting filtrate served as a source of virus.

In testing swine for immunity induced by previous inoculations of virus or

TABLE I  
*The Effect of H. influenzae suis and Swine Influenza Virus Administered Separately upon the Subsequent Susceptibility of Swine to Influenza*

Swine No.	Weight at beginning of experiment <i>lbs.</i>	Preliminary treatment		Length of time after preliminary inoculation or exposure <i>days</i>	Inoculation to test immunity		Remarks
		Inoculated intranasally with	Result		Inoculated intranasally with	Result	
922	19	2 cc. 24 hr. culture HIS* + 8 cc. infusion broth	No illness	18	5 cc. 10% suspension dried and glycerolated influenza virus + 0.2 cc. 48 hr. culture HIS	Swine influenza	No immunity
919	15	8 cc. Berkefeld N filtrate of influenza virus	Filtrate disease	18	"	No illness	Immune
920	13	"	"	18	"	"	"
921	17	"	"	18	"	"	"
923	16	2 cc. 24 hr. culture HIS + 8 cc. Berkefeld N filtrate of influenza virus	Swine influenza				To control effectiveness of organism and virus in inducing swine influenza
959	21	Control	Control		5 cc. 10% suspension dried and glycerolated influenza virus + 0.2 cc. 48 hr. culture HIS	Swine influenza	No immunity
1067	45	0.5 cc. 72 hr. culture HIS + 9 cc. defibrinated swine blood	No illness	11	9 cc. light suspension dried and glycerolated influenza virus + 1 cc. 40 hr. culture HIS	"	"
1125	26	Control	Control		"	"	"

1155	50	1 cc. 48 hr. culture HIS + 9 cc. physiological saline	No illness	12	14 cc. light suspension dried and glycerolated influenza virus + 1 cc. 24 hr. culture HIS	Swine influenza	No immunity
1156	56	Control					
1077	21	2 cc. Berkefeld N filtrate of influenza virus	Filtrate disease	31	10 cc. suspension lung, lymph nodes, and bronchial exudate from influenza-sick swine	No illness	Immune
1082	23	Infected by pen exposure to filtrate disease	"	24	"	"	"
1060	45	Control					
897	17	Infected by pen exposure to filtrate disease	Filtrate disease	42	8 cc. suspension lung, lymph nodes, and bronchial exudate from influenza-sick swine	Swine influenza	Immune
951	16	Control					
1130	29	Infected by pen exposure to filtrate disease	Filtrate disease	10	4.5 cc. suspension glycerolated influenza virus + 0.5 cc. 48 hr. culture HIS	Swine influenza	Immune
1136	35	Control					
1070	59	5.5 cc. Berkefeld V filtrate of influenza virus	Filtrate disease	11	5 cc. light suspension dried and glycerolated influenza virus + 0.5 cc. 48 hr. culture HIS + 10 cc. physiological saline	Swine influenza	Immune
1135	40	Control					

\* HIS = *H. influenzae swis.*

organism, where fresh infectious material was not available at the time, glycerolated and dried materials were used. It has previously been shown (1) that the swine influenza virus can be stored for at least 41 days in 50 per cent glycerol or 54 days when frozen and dried by Swift's method (3). *H. influenzae suis*, however, does not survive glycerolation and its survival in dried material is irregular. Therefore in the experiments recorded in this paper in which glycerolated or dried virus was used, small amounts of cultures of *H. influenzae suis* have been added to the suspensions of virus just before using.

*The Effect of H. influenzae suis and Swine Influenza Virus, Administered Separately, upon the Susceptibility of Swine to Influenza*

As shown in Table I, three swine (922, 1067, 1155) which were inoculated intranasally with *H. influenzae suis* showed no evidence of illness following the inoculation and also were fully susceptible to swine influenza when tested later by intranasal inoculation with suspensions containing swine influenza virus and *H. influenzae suis*.

Five swine (919, 920, 921, 1070, 1077) were inoculated intranasally with Berkefeld filtrates containing swine influenza virus and all developed the mild filtrate disease. When inoculated later with the mixture of virus and organism they were found to be completely immune to swine influenza.

There was a possibility that dissolved products of *H. influenzae suis*, which could conceivably be present in infectious Berkefeld filtrates, might account, at least partially, for this development of immunity in filtrate-infected swine. To test this possibility three other swine (897, 1082, 1130) were infected with the filtrate disease by placing them in the same pens with animals inoculated with infectious Berkefeld filtrates. By this method of infection they received virus free from any trace of *H. influenzae suis* protein, and like the swine infected by intranasal inoculation, they were found subsequently to be immune to swine influenza.

The experiments recorded in Table I indicate that an attack of the filtrate disease, whether induced by intranasal inoculation with filtered virus or by exposure, confers an active immunity to swine influenza as induced by the concerted action of *H. influenzae suis* and the filtrable virus of swine influenza. The administration intranasally of *H. influenzae suis* alone induces no demonstrable immunity to swine influenza. These data indicate that the filtrable virus is of primary etiological significance and that *H. influenzae suis* plays only a secondary and contributory rôle in the clinical entity known as swine influenza.

*Neutralization of the Swine Influenza Etiological Complex by Serum of Animals Convalescent from the Filtrate Disease*

It has been previously shown (1) that the blood serum of a hog convalescent from swine influenza when mixed with an infectious suspension and administered intranasally to a susceptible animal was capable of preventing infection. It seemed of interest, therefore, to determine whether both of the etiological components were essential to the

TABLE II  
*Neutralization of the Swine Influenza Etiological Complex by Serum of Animals Convalescent from the Filtrate Disease*

Swine No.	Weight	Inoculated with				Result	Remarks
		Infectious suspension	Filtrate disease convalescent serum				
			Source	Amount			
1128	36 lbs.	5 cc. light suspension dried and glycerolated influenza virus + 0.5 cc. 48 hr. culture HIS	Swine 1077 and 1082 pooled	10 cc.	No illness	Found subsequently to be immune	
1132	29	" "	" "	10	" "	Autopsy negative	
1135 (control)	40	" "	10 cc. physiological saline		Swine influenza	Autopsy typical of swine influenza	

generation of this neutralizing property of convalescent serum, or whether, as in the active immunization just discussed, the filtrable virus alone was sufficient.

Filtrate disease convalescent serum was obtained by bleeding two hogs, from the tail, 24 and 31 days after their infection with the swine influenza virus. These sera were freed from bacteria by Seitz filtration and then combined in equal quantities. The procedure was as follows: To 5 cc. of a light suspension of dried and glycerolated swine influenza virus was added 0.5 cc. of the bloody condensation

fluid of a 48 hour culture of *H. influenzae suis*. 10 cc. of the filtrate disease convalescent serum was added to this mixture, and after standing at room temperature for 1 hour, it was injected intranasally into the test swine, 15.5 cc. into each animal. The control animal received the same amount of infectious suspension to which 10 cc. of sterile physiological saline had been added. The results are recorded in Table II.

The potency of the infectious suspension was evidenced by the clinical and autopsy picture presented by the control. The neutralization was apparently complete for Swine 1132 which developed no clinical evidence, and, when autopsied on the 7th day after inoculation, showed no pathological evidence of swine influenza. Swine 1128 also at no time appeared ill but on the 3rd day after inoculation it had a temperature of 40°C. This is the lower limit of what we have considered a fever temperature in swine. 2 weeks after its initial inoculation it was found to be immune to swine influenza. It thus seems likely that in this animal the neutralization was not quite complete, otherwise the animal would not only have shown no illness but have remained fully susceptible to infection with swine influenza. A smaller dose of infectious suspension or a larger dose of convalescent serum would probably have resulted in a mixture as neutral for Swine 1128 as the one employed was for Swine 1132.

As in the experiments dealing with the immunity to swine influenza conferred by an attack of the filtrate disease, the neutralization of infectious suspensions, capable of inducing characteristic swine influenza, by serum from swine convalescent from the filtrate disease again indicates the primary etiological significance of the filtrable virus and the secondary contributory rôle of *H. influenzae suis*.

#### *Failure of the Swine Influenza Virus to Induce Illness when Administered Intramuscularly*

In these experiments only glycerolated swine influenza virus was used. The cultures of *H. influenzae suis* were of the type used in the experiments previously described in this paper. Glycerolated virus was prepared as follows:

Portions of atelectatic or pneumonic lung of approximately hickory nut size and pieces of the edematous bronchial lymph nodes of somewhat smaller size were taken from swine infected with influenza that had been killed on the 3rd day of their illness. These pieces of tissue were placed in 50 per cent glycerol-physiological salt solution and stored at refrigerator temperature for at least 6 days before use in these experiments. To prepare infectious suspensions from tissues stored in this

way, pieces were cut with sterile scissors from the stored material. These were washed in three changes of sterile physiological saline and then ground in a mortar with sand and physiological saline to make approximately a 5 per cent suspension. Such suspensions were allowed to stand undisturbed for a few minutes and the supernatant fluid, when decanted, served as the infectious suspension.

Six swine were inoculated intramuscularly with glycerolated virus. Four of these animals were converted artificially into carriers of *H. influenzae suis* by intranasal inoculation with cultures of this organism. Experiments, which will be reported in detail later, demonstrated that swine receiving *H. influenzae suis* in this way carry the organisms in their respiratory tracts for at least 3 days. The organisms thus carried, while innocuous in themselves, maintain their full potential pathogenicity because when virus alone is administered to such animals swine influenza instead of the filtrate disease results. A carrier state was established in the four above mentioned swine because, should the virus have proven pathogenic when administered intramuscularly, swine influenza as induced by organism and virus together would have been easier to recognize than the filtrate disease induced by the virus alone. In this way the presence of *H. influenzae suis* in the respiratory tract served as an indicator for the invasion of the respiratory tract by swine influenza virus. The data for the six swine inoculated intramuscularly with glycerolated virus together with those for the control swine receiving the virus intranasally are recorded in the first four columns in Table III. It is there shown that while the three controls which received glycerolated influenza virus and *H. influenzae suis* intranasally all developed swine influenza, none of the six swine inoculated with the virus intramuscularly developed any recognizable illness in spite of the fact that four of them were carrying *H. influenzae suis* in their respiratory tracts. One of these animals, Swine 1146, killed 4 days after inoculation, was completely negative at autopsy for any pathology of swine influenza. Of the remaining five, saved to test for immunity, three were given two subsequent intramuscular inoculations of glycerolated influenza virus and showed no reaction or evidence of illness following either of these.

The above data, summarized in the left portion of Table III, indicate that swine influenza virus given intramuscularly is incapable of inducing filtrate disease, or swine influenza, in animals converted artificially into carriers of *H. influenzae suis*. The suggestion derivable from these experiments is that the swine influenza virus is effective in inducing disease only when introduced directly into the respiratory tract, and in this respect is similar to certain other viruses, some of which regularly infect only when introduced into the epidermis (dermatotropic viruses) and others only when introduced directly into nervous tissue (neurotropic viruses).

TABLE III  
The Effect of Intramuscular Injection of Swine Influenza Virus

Swine No.	Weight <i>lbs.</i>	Inoculation and route	Result	Inoculation to test immunity			Remarks
				Length of time after last inoculation <i>days</i>	Inoculated intranasally with	Result	
1146	51	10 cc. glycerolated influenza virus i.m.* and 0.5 cc. 40 hr. culture HIS in 10 cc. physiological saline i.n.	No illness			Autopsy negative	
1147	61	10 cc. glycerolated influenza virus i.m. and 2 similar subsequent inoculations controlled by Swine 1154	" "	14	14 cc. light suspension dried and glycerolated influenza virus + 1 cc. 24 hr. culture HIS	Immune	
1148	63	10 cc. glycerolated influenza virus + 0.5 cc. 40 hr. culture HIS i.n.	Swine influenza			Control for infectivity of virus used on Swine 1146 and 1147 when administered i.n.	
1149	77	0.5 cc. 48 hr. culture HIS + 4.5 cc. physiological saline i.n., 10 cc. suspension glycerolated influenza virus i.m., and 2 similar subsequent inoculations with glycerolated influenza virus i.m.	No illness	14	14 cc. light suspension dried and glycerolated influenza virus + 1 cc. 24 hr. culture HIS	Immune	
1150	73	" "	" "	14	" "	"	



1154	40	10 cc. suspension glycerolated influenza virus + 0.75 cc. 48 hr. culture HIS i.n.	Swine influenza			Control for infectivity of virus used on Swine 1149 and 1150 when administered i.n.
1156	56				14 cc. light suspension dried and glycerolated influenza virus + 1 cc. 24 hr. culture HIS	Control for immunity shown by Swine 1147, 1149, and 1150
1088	79	0.5 cc. 44 hr. culture HIS + 10 cc. physiological saline i.n., and 10 cc. suspension glycerolated influenza virus i.m.	No illness	15	10 cc. suspension dried and glycerolated influenza virus + 0.5 cc. 44 hr. culture HIS	Immune
1100	57	10 cc. suspension glycerolated influenza virus i.m.	" "	15	" "	"
1172	33	0.5 cc. 44 hr. culture HIS + 10 cc. suspension glycerolated influenza virus i.n.	Swine influenza			Control for infectivity of virus used on Swine 1088 and 1100 when administered i.n.
1175	19				10 cc. suspension dried and glycerolated influenza virus + 0.5 cc. 44 hr. culture HIS	Control for immunity shown by Swine 1088 and 1100

\* i.m. = intramuscularly; i.n. = intranasally. HIS = *H. influenzae suis*.

*Immunity Following the Intramuscular Administration of Swine  
Influenza Virus*

The five animals mentioned above, which were saved to test for immunity, were inoculated intranasally with *H. influenzae suis* mixed with dried and glycerolated swine influenza virus. Swine 1147, 1149, and 1150 had received three intramuscular injections of glycerolated virus prior to their test for immunity while Swine 1088 and 1100 had received only one. All five were found to be completely immune to infection with material which in control swine induced clinically and pathologically characteristic swine influenza.

These experiments are summarized in the right portion of Table III and indicate that swine influenza virus given intramuscularly immunizes hogs against swine influenza without inducing any evidence of illness. So far as can be judged, a single intramuscular injection of glycerolated swine influenza virus confers just as satisfactory an immunity as do three injections.

It is not believed that intranasal inoculation with *H. influenzae suis* contributed to the immunization, in view of the fact that immunity developed whether it was administered or not. Swine 1147 and 1100 which received no *H. influenzae suis* developed as satisfactory an immunity as did the three animals which received the organism intranasally.

DISCUSSION

Evidence derived from the experiments reported in this paper indicates that, of the two components essential to the production of influenza in swine, the filtrable virus is of primary importance while *H. influenzae suis* plays only a secondary or contributory rôle. *H. influenzae suis* administered alone intranasally to swine induced neither illness, as had previously been established (2), nor immunity to swine influenza. The filtrable virus of swine influenza, on the other hand, while capable alone of inducing only the mild filtrate disease, established a solid immunity to swine influenza as induced by the mixture of virus and organism. These findings are supported by the observation that convalescent serum from swine that had suffered only the filtrate disease was capable of neutralizing the combined etiological complex of organism and virus. These results accord with the expected action of two agents of unequal etiological importance but both essential for the production of a disease.

The swine influenza virus showed a certain tissue specificity in that it was found to be incapable of inducing illness when administered intramuscularly to swine although it was uniformly infective when introduced into the respiratory tract. This fact suggests that the swine influenza virus bears a relationship to tissues of the respiratory tract like that of dermatotropic viruses to the skin or of neurotropic viruses to the central nervous system.

It was furthermore of interest, and perhaps of practical value, to note that swine inoculated intramuscularly with the swine influenza virus, while failing to become ill, nevertheless developed an immunity to swine influenza. Under the conditions of laboratory experimentation, intramuscular inoculation of swine with glycerolated swine influenza virus constituted a safe and satisfactory method of immunization. It is possible that this observation can be applied in developing a method of immunization against the disease for use in the field.

#### SUMMARY

Of the two etiological components of swine influenza, only the filtrable virus possessed immunizing properties. *H. influenzae suis*, while essential to the production of the disease, played only a secondary and contributory rôle and, alone, conferred no immunity. Serum of swine convalescent from the filtrate disease neutralized the swine influenza etiological complex of organism and virus. Intramuscularly administered swine influenza virus was incapable of inducing illness but did render hogs immune to swine influenza. It is suggested that a specific relationship, as regards infectivity, exists between the swine influenza virus and the tissues of the respiratory tract.

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