SWINE INFLUENZA

V. Studies on Contagion

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In experiments published earlier (1–3) it has been shown that both a filtrable virus and the organism, *H. influenzae suis*, are etiologically essential to the production of influenza in swine. All five strains of the disease studied have been highly contagious. Animals infected by pen exposure to cases of the disease developed typical influenza identical with that produced by inoculation intranasally with virus and organism. Furthermore, in swine infected by contact both the virus and the organism could be demonstrated as having transferred.

During the spring and summer of 1931, when a strain of the disease was being passed through swine every 6 weeks to preserve it for subsequent experimental work, a change in its contagious character occurred. It is the purpose of this paper to describe and interpret the change.

A Change in the Contagious Character of a Strain of Swine Influenza

The change in contagion occurred in a strain of the disease in which the etiological components were Virus 15, obtained originally from Iowa in December, 1930, and *H. influenzae suis* Culture 451, the bacterial component of the first strain of swine influenza obtained from Iowa in November, 1928. This combination of virus and organism had been used in inducing influenza in swine during intensive work with the disease throughout the winter of 1930–31. During this time it regularly caused, when administered intranasally to swine, an influenza that was characteristic in all respects and that was known to be fully contagious as late as March, 1931.

The general plan followed for preserving the virus during intervals between experiments was to store it in the refrigerator either in 50 per cent glycerol or dried by Swift's method (4). During the spring and summer of 1931, when the change in contagion to be discussed took place, the virus was stored only in the dried state. The bacterial component of the etiological complex, *H. influenzae suis*, which does not regularly survive such storage, was maintained by weekly transfer on plain agar slants containing 0.75–1 cc. of sterile defibrinated horse blood at their bases. The usual procedure at each 6 weeks passage was to prepare an approximately 5 per cent suspension of the dried virus and administer 10 cc. of this suspension mixed with 1 cc. of the bloody condensation fluid from a 24 to 48 hour culture of *H. influenzae suis* intranasally to each animal to be infected. Clinically and pathologically characteristic swine influenza developed in the swine inoculated at each passage. The animals were sacrificed on the 3rd or 4th day following infection and pathological lung, bronchial lymph nodes, and bronchial exudate were saved to dry by Swift's method to furnish virus for the next passage. Cultures of *H. influenzae suis* were not saved from the passage animals. A stock laboratory strain of the organism was used throughout in conjunction with the passaged virus.

In September, 1931, during the time that the disease was being maintained by passage at 6 week intervals, an attempt was made to pass it by contact. Instead of contracting swine influenza, as was to be expected from previous experience with the disease, the contact animal developed only a very mild, afebrile, and transient illness similar to that described in an earlier paper (3) and called the "filtrate disease" because it is produced by the intranasal infection of swine with the swine influenza virus alone.

In a series of seven subsequent contact experiments in which normal swine were exposed to animals suffering from the apparently typical influenza induced by Strain 15 swine influenza virus and *H. influenzae suis* Culture 451 no cases of influenza occurred. Instead, all of the exposed swine developed, after an incubation period of from 2 to 6 days, only a very mild and transient illness which clinically and pathologically resembled the filtrate disease. Bacteriological examination of their respiratory tracts failed to reveal the presence of *H. influenzae suis*, further supporting the view that we were dealing with the filtrate disease and not merely with an unusually mild form of swine influenza. The illness contracted by the animals infected by contact was shown to be transmissible in series by pen exposure, and two swine allowed to recover were found later to be solidly immune to swine influenza as

induced by intranasal inoculation with virus and *H. influenzae suis* (5). The above facts, considered collectively, were in accord with the view that the mild illness, contracted by animals exposed to swine influenza as induced by Strain 15 virus and *H. influenzae suis* Culture 451, was filtrate disease and was caused by the transfer of virus without the corresponding transfer of the organism to the exposed animals. That the organism still maintained its ability, when once established in the swine respiratory tract, of acting with the virus in inducing characteristic swine influenza was shown by the regularity with which infections could be produced by the intranasal inoculation of swine with organism and virus.

It was evident that sometime after March, 1931, when the strain of swine influenza under discussion was last known to be fully contagious, a change had taken place in one or both of the etiological agents to alter the contagious character of the complete disease in the manner described. In order to determine definitely whether the failure of *H. influenzae suis* Culture 451 to establish itself in the swine respiratory tract under conditions of infection by contact was due to some change in its own properties or to some change in the character of the virus with which it was associated, a fresh epizootic field strain of swine influenza was obtained.

Experiments with a Fresh Field Strain of Epizootic Swine Influenza

A widespread outbreak of swine influenza which occurred among the swine on exhibition at the Iowa State Fair early in September, 1932, furnished fresh infectious material. Lung, bronchial exudate, and bronchial lymph nodes from an animal killed on the 3rd day of a typical but severe illness were brought back to the laboratory on ice. Cultures, revealing large numbers of *H. influenzae suis* in the lung and bronchial exudate had been made before leaving Iowa. A normal pig inoculated intranasally with a suspension prepared from the pathological material brought back, and fortified by the addition of the cultures that had been isolated before leaving Iowa, developed characteristic swine influenza. This fresh field strain of the disease proved to be fully and typically contagious, and *H. influenzae suis* was isolated in pure primary culture from the lung and bronchial exudate of the pig infected by contact. The new virus was designated Strain 18, and the associated bacillus, *H. influenzae suis* Culture 18.

The means were now available for determining which of the two etiological components of the old stock strain of swine influenza was

TABLE I

Differences in the Contagious Character of Swine Influenza Induced by Old and Recently Procured Strains of Infectious Material

Experiment No.	Swine No.	Inoculated intranasally with: Swine influenza virus, strain No.	Inoculated intranasally with: *H. influenzae suis*, culture No.	Clinical picture	Autopsy findings	*H. influenzae suis* in: Lung	*H. influenzae suis* in: Bronchial exudate	Remarks
1	1213	Infectious material from naturally occurring case of swine influenza (Strain 18—1932)		Swine influenza	Typical	Mixed culture	Mixed culture	Obtained at Iowa State Fair—Sept. 2, 1932
	1195	Contact with Swine 1213		Severe swine influenza	Moderate	Pure culture	Pure culture	
2	1212	18 (1932)	18 (1932)	Swine influenza	Extensive	Pure culture	Mixed culture	
	1251	Contact with Swine 1212		Swine influenza	Extensive	Mixed culture	Mixed culture	
3	1283	18 (1932)	18 (1932)	Mild swine influenza	Typical	Pure culture	Pure culture	
	1286	Contact with Swine 1283		Mild swine influenza	Moderate	Absent	Mixed culture	
4	1192	18 (1932)	451 (1928)	Swine influenza	Typical	Pure culture	Pure culture	
	1250	Contact with Swine 1192		Filtrate disease	Few	Absent	Absent	
5	1282	18 (1932)	451 (1928)	Mild swine influenza	Moderate	Mixed culture	Mixed culture	
	1289	Contact with Swine 1282		Filtrate disease	Few	Absent	Absent	
6	1191	15 (1930)	451 (1928)	Mild swine influenza	Moderate	Mixed culture	Pure culture	
	1231	Contact with Swine 1191		Filtrate disease	Few	Absent	Absent	

7	1135	15 (1930) | 451 (1928)	Swine influenza	Typical	Pure culture	Mixed culture	
	1134	Contact with Swine 1135	Filtrate disease	Few	Absent	Absent	
8	1217	15 (1930) | 18 (1932)	Swine influenza	Extensive	Pure culture	Mixed culture	
	1222	Contact with Swine 1217	Swine influenza	Extensive	Mixed culture	Pure culture	
9	1277	15 (1930) | 18 (1932)	Swine influenza	Typical	Pure culture	Pure culture	
	1244	Contact with Swine 1277	Fatal swine influenza	Edematous pneumonia	Pure culture	Pure culture	Died on 2nd day of illness

responsible for the change in its contagious character. This was done by substituting the individual components of the freshly obtained and fully contagious field strain of the disease for corresponding components of the old stock strain. Representative experiments in which this was done are outlined in Table I.

In the experiments given in Table I, the virus used was in all instances filtered through Berkefeld candles, adding broth cultures of *B. prodigiosus* as the test organism. All filtrates were cultured on media capable of supporting the growth of both *B. prodigiosus* and *H. influenzae suis* and all virus samples recorded were bacteriologically sterile. The cultures of *H. influenzae suis* used in the experiments were grown in sterile defibrinated horse blood at the bases of plain agar slants, and all Strain 18 cultures were transferred frequently enough, before use, to free them of any swine influenza virus carried over mechanically from the infectious material used as a source. The Strain 18 culture when administered alone intranasally to swine was shown to be completely incapable of inducing any clinical evidence of illness or pathological alteration detectable at autopsy.

The data presented in Table I indicate that Strain 18 virus and Strain 18 culture, when mixed and administered intranasally to swine, induced characteristic swine influenza, fully contagious for normal animals. Strain 15 virus mixed with Strain 18 culture and administered intranasally to swine also produced a fully contagious swine influenza. However, when Culture 451 was substituted for Culture 18, with either Strain 15 or 18 virus, the disease produced by intranasal inoculation, while clinically and pathologically characteristic of swine influenza, was not fully contagious for normal animals. Instead of swine influenza, these animals infected by contact developed the mild filtrate disease. It was thus apparent that the change in the contagious character of the old stock strain of swine influenza had been due to some biological alteration in Culture 451 and that Virus 15 was in no way responsible. Experiments are now being carried on to determine some significant difference between Culture 18 and Culture 451 which will account for the latter's failure to transfer with the swine influenza virus in contact infections.

The Production of Swine Influenza by Contact in Animals Experimentally Converted into Carriers of H. influenzae suis

A small group of experiments performed earlier acquire particular interest following establishment of the fact that the change was in the

bacterial and not the virus component of the etiological complex. These will be reported at this time because they may be of some significance in the problem of respiratory infections in general and also because they reinforce the conclusion already reached that the contact infection in animals exposed to the old stock strain of swine influenza represented an infection in which *H. influenzae suis* had not transferred with the virus.

In a preliminary experiment it had been determined that when a pig, which had been inoculated intranasally 3 days previously with *H. influenzae suis*, of itself innocuous, was given by the same route a small amount of Berkefeld filtrate of swine influenza virus, it promptly developed characteristic swine influenza. A control animal which received only the virus developed the mild filtrate disease. It was concluded from such an experiment that in carriers of *H. influenzae suis*, inoculation with the virus alone was sufficient to induce swine influenza. This fact was utilized in attempting to determine the nature of the mild illness contracted by pigs exposed to cases of the old stock strain of swine influenza. Experiments were conducted in which carriers of *H. influenzae suis* Culture 451 were placed in contact with cases produced by inoculation with the old stock strain of swine influenza. Protocols of these experiments are outlined in Table II.

In two of the experiments recorded in Table II, pigs that were not carriers of the organism were included. In Experiments 2 and 3 the animals to be infected by exposure were not introduced into the pens until 24 hours after the inoculation of the animals to whose disease they were to be exposed; while in Experiment 1 the animals to be exposed were put into the pen with the infected animal soon after its inoculation.

In all three experiments carriers of *H. influenzae suis*, when exposed to the old stock strain of swine influenza, developed, after incubation periods of from 24 to 48 hours, a characteristic but unusually severe swine influenza. All three of the animals infected in this way showed at autopsy an edematous pneumonia of the type seen in severe and usually fatal cases of swine influenza. One of the animals died on the 4th day of its illness and the remaining two were extremely sick when sacrificed on the 3rd and 4th day. As in similar experiments performed earlier, the swine that were not carriers of *H. influenzae suis* developed only filtrate disease following exposure to cases of the old stock strain of swine influenza. These three experiments suggest that the disease developing in carriers of *H. influenzae suis* after contact with cases of swine influenza is more severe than that induced by

TABLE II

Contagion of Swine Influenza for Animals That Are Carriers of H. influenzae suis

Experiment No.	Swine No.	Mode of infection	Clinical picture	Autopsy findings	*H. influenzae suis* in		Remarks
					Lung	Bronchial exudate	
1	1125	HIS* + dried and glycerolated influenza virus i.n. † Jan. 11	Swine influenza	Typical	Mixed culture	Mixed culture	
	1123	HIS alone i.n. Jan. 9 Contact Swine 1125 Jan. 11	Negative Swine influenza	Water-logged type of pneumonia	Absent	Mixed culture	Marked pleuritis and pericarditis yielding pure cultures of HIS
	1122	Contact Swine 1125 Jan. 11	Mild filtrate disease	Few	Absent	Absent	Disease typical of that induced by virus alone
2	1063	HIS + dried and glycerolated influenza virus i.n. Jan. 18	Swine influenza	Typical	Pure culture	Mixed culture	
	1062	HIS alone i.n. Jan. 18 Contact Swine 1063 Jan. 19	Negative Swine influenza	Water-logged type of pneumonia	Absent	Pure culture	Marked pleuritis and pericarditis. Animal died on 4th day of illness

3	1135	HIS + dried and glycerolated influenza virus i.n. Feb. 22	Swine influenza	Typical	Pure culture	Mixed culture	
	1127	HIS alone i.n. Feb. 20 Contact Swine 1135 Feb. 23	Negative Swine influenza	Water-logged type of pneumonia	Mixed culture	Mixed culture	Extensive pleuritis yielding pure cultures of HIS
	1134	Contact Swine 1135 Feb. 23	Mild filtrate disease	Few	Absent	Absent	Disease typical of that induced by virus alone

* HIS = *H. influenzae suis* Culture 451.

† i.n. = intranasally.

intranasal inoculation with mixtures of virus and *H. influenzae suis*. With so small a series of cases no conclusion is drawn at this time.

No controls for the pathogenicity of *H. influenzae suis* alone were included in the experiments given in Table II because Culture 451, which was used, had been tested repeatedly, both in previous years and recently, and found, with the exception of a test made very shortly after its isolation in 1928, to be uniformly incapable of inducing illness that could be confused with influenza (2). It has, furthermore, been tested since the experiments recorded in Table II were conducted and found to be still completely non-pathogenic for swine. Swine that were converted into carriers of *H. influenzae suis* were under observation and exhibited no evidence of illness or elevation of temperature to a fever level not only for the 24 to 72 hours elapsing between the time of inoculation with the organism alone and their exposure to the disease, but also during the 24 to 48 hour latent periods between their exposure and the onset of their illness. They were therefore recorded as negative under the heading of "clinical picture" in Table II.

The results described above accord with the conclusion already reached that the contact infection in pigs exposed to the old stock strain of swine influenza is one in which *H. influenzae suis* is unable to establish itself with the virus in the respiratory tracts of the exposed animals. The resulting illness, identical with the filtrate disease, is due to infection with the swine influenza virus alone.

DISCUSSION

The experiments reported in this paper may prove to be of interest in the general problem of respiratory infections in that they demonstrate three types of infection possible with an etiological complex made up of a virus and a bacterium. The first of these types is that seen in the epizootic disease in which both virus and *H. influenzae suis* transfer from sick to normal animals by exposure. The second and third types, while not known to exist under field conditions, are none the less possible. They are exemplified by the experiments given in Table II. In these two types only the virus transfers by contact from sick to normal animals and the severity and type of the resultant disease are dependent upon whether or not the infected animal is a carrier of *H. influenzae suis*. If it is not, it develops only a very mild and transient illness which, however, is transmissible to other swine. If it is already a carrier of *H. influenzae suis*, infection with the virus produces true swine influenza. Thus, in a hypothetical swine popula-

tion in which some of the animals were carriers of *H. influenzae suis* of the type of Culture 451, introduction of the swine influenza virus would result in two kinds of disease clinically quite different: swine influenza in the carrier animals, and an extremely mild and poorly defined illness in the remainder. In this population it would not be impossible for a carrier to develop a fatal infection from exposure to a very slightly sick non-carrier, and, in turn, the infection transferred from the fatally ill carrier animal to one that was not a carrier might be so slight as to be scarcely recognizable. If now, in this hypothetical population, there was one animal carrying an *H. influenzae suis* of the type of Culture 18, capable of transferring with the virus by contact, the disease would undoubtedly proceed as epizootic swine influenza, infecting each new case with both virus and organism regardless of whether or not it had previously been a carrier.

SUMMARY

A strain of swine influenza has been observed to change from a condition of full contagiousness, in which both *H. influenzae suis* and the swine influenza virus were transferred by pen contact, to one of only partial contagiousness, in which the virus alone was transferred, resulting in the mild filtrate disease instead of swine influenza in animals infected by contact. Swine that had been experimentally converted into carriers of *H. influenzae suis* developed swine influenza following contact with animals infected with the altered strain of the disease. Experiments in which the etiological components of a freshly obtained and fully contagious strain of swine influenza were substituted for the corresponding components of the altered strain of the disease revealed the fact that the change in the contagious character of the latter was due to an alteration in the bacterial component of the etiological complex and that the virus component was in no way responsible.

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