IMMUNOLOGICAL RELATIONSHIP BETWEEN THE SWINE AND HUMAN INFLUENZA VIRUSES IN SWINE

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(Received for publication, April 26, 1937)

Ferrets or mice recovered from infection with the virus of human or swine influenza are usually immune to infection with the other virus (1–3). In these two animals a complete and frequently fatal disease is produced by either type of influenza virus alone, and there is no evidence that concomitant infection with *Hemophilus influenzae suis* or any other bacterium modifies its course in any constant manner (1, 3–6). Swine, on the other hand, infected with either swine or human influenza virus alone develop but a mild, transient, indefinite illness (filtrate disease) and come down with influenza only when the bacterium, *H. influenzae suis* (7), has accompanied the virus (8–10). It seemed possible that the cross-immunological relationship between swine and human influenza virus found in the simple virus infections of ferrets and mice might not follow in the complex virus-bacterium infections necessary to induce influenza in swine. The present paper reports experiments dealing with the cross-immunization of swine by means of initial infections with either swine or human influenza virus alone or in mixture with the bacterium, *H. influenzae suis*.

EXPERIMENTAL

Infectious Materials Used

Francis' P.R. 8 strain (5) human influenza virus and strain 15 (Iowa, 1930) swine influenza virus were employed in all experiments. Culture 18 (11) *H. influenzae suis* was used to complete the etiological complex with either strain of virus in most cases, although in a few instances this was pooled with cultures 23 and 24, more recently isolated from field cases of swine influenza.

Virus, either the human or the porcine type, was in all experiments prepared in physiological saline as a 10 per cent suspension of lung from swine infected with virus alone. The swine strain had originally been freed of *H. influenzae suis* by

Berkefeld filtration or by serial passage through ferrets or mice. Swine whose infections were to be with virus alone were given from 6 to 10 cc. of the supernatant fluid from sedimented but uncentrifuged suspensions intranasally. Swine whose infections were to be with a mixture of virus and bacterium received, in addition to virus, 0.5 to 1 cc. of a 24 hour horse blood culture[1] of *H. influenzae suis*. The culture was mixed with the virus suspension just prior to its administration intranasally. Variations in the dosage of either virus or bacterium, within the limits used in the present experiments, had no influence on the results obtained.

Immunity to Swine Influenza Induced by Infection with Human Influenza Virus Alone or in Mixture with Hemophilus influenzae suis

Eight swine were inoculated intranasally with a mixture of human influenza virus and *H. influenzae suis*. As noted in Table I, 6 of these animals developed an illness that was clinically characteristic of a mild swine influenza. The remaining 2 came down with an illness which clinically resembled that produced in swine by infection with virus alone, and it is believed that in these *H. influenzae suis* failed to become established with the virus in the respiratory tract. The occasional failure of this bacterium to establish itself with human influenza virus in the swine respiratory tract is well known from earlier work (10).

Nine swine inoculated intranasally with human influenza virus alone developed the mild, indefinite, filtrate disease. 2 other swine receiving human influenza virus alone intranasally twice at 20 day intervals exhibited symptoms of filtrate disease following the first inoculation only.

When the swine had completely recovered from their human influenza infections they were tested for immunity to swine influenza by inoculating them intranasally, together with control swine, with a mixture of swine influenza virus and *H. influenzae suis*. The results of these tests for immunity are outlined in Table I.

As shown in the table, 6 of the 8 swine whose initial infection had been with a mixture of human influenza virus and *H. influenzae suis* proved immune to swine influenza. Of the remaining animals, swine 1820 developed a transient fever but did not appear ill, while the other one, swine 1823, whose initial infection had clinically resembled filtrate disease, was febrile and depressed and exhibited a scattered lobular pneumonia when autopsied on the 3rd day. Swine influenza virus was demonstrated, by mouse inoculation, in the lung of this animal although its presence could not be demonstrated in the turbi-

[1] 0.5 to 1 cc. of sterile defibrinated horse blood added to a plain agar slant. In this medium *H. influenzae suis* grows largely in the blood at the base of the slant with only scant colony formation on the agar surface.

nates. *H. influenzae suis* could not be cultivated from either the lung or terminal bronchi.

Four of the 6 swine that had appeared clinically immune to swine influenza were killed and autopsied on the 3rd or 4th day after inoculation. No lesions of swine influenza were seen in their respiratory tracts. Their lungs appeared normal aside from scant, old, puckering scars in the anterior lobes, evidently residual for their initial human influenza infection. Virus could not be demonstrated by mouse inoculation in the lungs of any of the animals nor in the turbinates of 2 tested. Neither could *H. influenzae suis* be cultivated from their lungs or terminal bronchi. Autopsy thus confirmed the clinical evidence that these 4 swine had been immune to swine influenza. The remaining 3 of the 8 swine initially infected with human influenza virus and *H. influenzae suis* were kept under observation in order later to obtain serum for neutralizing antibody studies.

The results obtained in the swine whose initial infections had been with human influenza virus alone differed from those just described. Only 1 animal, swine 1780, proved completely immune to swine influenza. The remaining 8 developed disease varying clinically from that seen in normal swine infected with swine influenza to that in which the salient features were merely a transient depression with or without fever. 6 of these animals were killed and autopsied on the 3rd or 4th day. One, swine 1729, showed no influenzal pneumonia; 1, swine 1747, showed only a pleuritis; while, in the remaining 4, pneumonias of from 1 to 3 lobes were encountered. These pneumonias were qualitatively like those seen in the control animals but were in most cases less extensive. However, although swine influenza virus was regularly detectable by mouse inoculation in the turbinates and lungs of the control swine, it was either not demonstrated or present only in low concentrations in the turbinates and lungs of the human virus-immune animals. *H. influenzae suis* could be cultivated from the lungs of 4 of the 6 swine autopsied and from the terminal bronchi of all. Its presence in this group of animals was in striking contrast to its uniform absence in the lungs and terminal bronchi of the swine whose initial infection had been with a mixture of human virus and *H. influenzae suis*.

The 2 swine that had been inoculated intranasally twice at 20 day intervals with human influenza virus alone were found clinically

TABLE I

Immunity to Swine Influenza Induced by Infection with Human Influenza Virus Alone or in Mixture with Hemophilus influenzae suis

Swine No.	Human influenza infection		Time between infections*	Test for immunity to swine influenza†				
	Inoculated intranasally with	Result		Clinical illness	Findings at autopsy			
					Lung lesions	Virus in		*H. influenzae suis* in
						Turbinates	Lung	Terminal bronchi	Lung
			days						
1599	Human influenza virus + *H. influenzae suis*	Mild influenza	10	None	Scant and old	Not tested	Absent	Absent	Absent
1605	" "	" "	10	"	" " "	" "	"	"	"
1714	" "	" "	13	"	" " "	Absent	"	"	"
1739	" "	" "	13	"	" " "	"	"	"	"
1823	" "	"Filtrate disease"	13	Fever and depression	Scattered lobular pneumonia	"	Present	"	"
1720	Human influenza virus alone	" "	13	Mild influenza	1 lobe pneumonia	"	Absent	Present	Present
1729	" " " "	" "	13	Depression, no fever	Scant and old	Present	"	"	Absent
1742	" " " "	" "	12	Typical influenza	2 lobe pneumonia	"	Present	"	Present
1746	" " " "	" "	12	Mild influenza	3 lobe pneumonia	Absent	Absent	"	"
1747	" " " "	" "	12	Fever and depression	Bilateral pleuritis	Present	"	"	"
1779	" " " "	" "	12	Depression, no fever	Scant pneumonia	Absent	Present	"	Absent
1662	Nil, control			Typical influenza	5 lobe pneumonia	Not tested	Not tested	"	Present

1664	" "			" "	3 lobe pneumonia	" "	Present	"	"
1693	" "			" "	5 lobe pneumonia	Present	"	"	"
1698	" "			" "	4 lobe pneumonia	Not tested	Not tested	"	"
1784	" "			Mild influenza	3 lobe pneumonia	Present	Present	"	"
1717	" "			Typical influenza	4 lobe pneumonia	"	"	"	"
1763	" "			" "	3 lobe pneumonia	"	"	"	"
1830	" "			" "	5 lobe pneumonia	"	"	"	"
1819	Human influenza virus + *H. influenzae suis*	Filtrate disease	13	None	Not autopsied, see Table III				
1820	" "	Mild influenza	13	Transient fever, not ill					
1821	" "	" "	13	None					
1645	Human influenza virus alone	Filtrate disease	20	Fever and depression					
1657	Human influenza virus alone twice at 20 day interval	Filtrate disease after 1st inoculation	11	None					
1659	" "	" "	11	"					
1750	Human influenza virus alone	Filtrate disease	12	Mild influenza					
1780	" " " "	" "	12	None					

* Sera obtained at end of this interval neutralized the human but not the swine influenza virus in all cases.

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

immune to swine influenza when later tested. They, together with 3 swine receiving a single injection of human influenza virus prior to testing for immunity to swine influenza, were kept under observation in order subsequently to obtain serum for neutralizing antibody studies.

It would appear from these experiments that, while initial infection with a mixture of human influenza virus and *H. influenzae suis* usually immunizes swine to swine influenza, initial infection with the human virus alone usually fails to do so, although it does appreciably alter their susceptibility. That the cross-immunity to swine influenza conferred by a primary infection with the human agent is not associated with demonstrable virus-neutralizing antibodies for the swine virus is indicated by the fact that the sera of all 19 swine studied, obtained just prior to the inoculation test for immunity to swine influenza, failed to neutralize the swine agent. All, however, neutralized the human virus completely.

Technique of the Neutralization Tests.—The neutralization tests recorded throughout this paper were conducted in the usual way in mice (12), employing the supernatant of a 2 per cent suspension of infected mouse lung as virus and mixing this in equal parts with the undiluted sera to be tested. Either 3 or 4 mice, while under ether narcosis, were inoculated in each test by dipping their noses in the virus-serum mixture contained in a slightly tilted Petri dish. Surviving mice were killed on the 7th day and their lungs, together with those of mice dying earlier, were examined for the presence of influenza lesions. Mice which survived 7 days and whose lungs showed no influenzal pneumonia at autopsy were considered to have received a completely neutralizing serum, mice which survived 7 days but whose lungs showed influenzal lesions at autopsy were considered to have received a partially neutralizing serum, while mice which died of an influenzal pneumonia during the period of observation were considered to have received a non-neutralizing serum. The swine and human viruses employed in the neutralization tests were of such virulence as to kill all control mice within 7 days.

Immunity to Human Influenza Infection[2] Induced by Infection with Swine Influenza or Swine Influenza Virus Alone

Six swine inoculated intranasally with a mixture of swine influenza virus and *H. influenzae suis* developed swine influenza. 8 swine inoculated intranasally

[2] In order to simplify terminology, "human influenza infection" is used to indicate an infection with a mixture of human influenza virus and *H. influenzae suis*.

with swine influenza virus alone came down with filtrate disease. Following complete recovery all 14 animals were tested for immunity to human influenza infection by inoculating them intranasally, together with control swine, with a mixture of human influenza virus and *H. influenzae suis*. The results of these tests for immunity are given in Table II.

As shown in the table, all 6 of the swine initially infected with swine influenza proved clinically immune to human influenza infection. 2 of these animals were killed and autopsied on the 4th day after inoculation. No lesions of human influenza infection were seen in their respiratory tracts and their lungs appeared normal aside from old healing lesions in the anterior lobes, residual from the initial swine influenza infections. Virus could not be demonstrated by mouse inoculation in the lungs or turbinates and *H. influenzae suis* could not be cultivated from either the lungs or terminal bronchi. Clinical evidence of immunity was thus confirmed by postmortem findings. The remaining 4 swine in the group were saved for later neutralizing antibody studies.

Of the 8 swine initially infected with swine influenza virus alone, 6 proved clinically immune to later human influenza infection. The remaining 2 became ill, but in neither of these were the postmortem findings characteristic of a human influenza infection. One animal (swine 1778) showed no recent respiratory tract lesions at all, merely an old, unresolved, scattered, lobular pneumonia probably persisting since the initial swine virus infection. The other animal (swine 1673) had a bilateral fibrinous pleuritis and pericarditis and from the exudate *H. influenzae suis* and a streptococcus were cultivated. 2 of the clinically immune animals killed and autopsied 4 days after inoculation showed no lesions of human influenza infection. In the anterior lobes of the lungs of both animals were scant contracted old scars evidently the result of healing swine influenza virus lesions. Virus could not be demonstrated by mouse inoculation in the turbinates or lungs of any of the 4 swine autopsied. The remaining 4 swine in the group, all clinically immune to human influenza infection, were kept under observation for later neutralizing antibody studies.

It is apparent from these experiments that initial infection with both the agents responsible for swine influenza or the swine influenza virus alone usually immunizes swine to human influenza infection, and

TABLE II

Immunity to Infection with Mixture of Human Influenza Virus and Hemophilus influenzae suis Induced by Infection with Swine Influenza or Swine Influenza Virus Alone

Swine No.	Swine influenza infection		Time between infections*	Test for immunity to human influenza infection†					
	Inoculated intranasally with	Result		Clinical illness	Findings at autopsy				
					Lung lesions	Virus in		*H. influenzae suis* in	
						Turbinates	Lung	Terminal bronchi	Lung
			days						
1723	Swine influenza virus + *H. influenzae suis*	Typical influenza	13	None	Old and healing	Absent	Absent	Absent	Absent
1815	" "	Mild influenza	19	"	" "	"	"	"	"
1673	Swine influenza virus alone	Filtrate disease	15	Fever and prostration	Bilateral pleuritis	"	"	"	Present
1727	" " " "	" "	16	None	Scant old scars	"	"	"	Absent
1776	" " " "	" "	12	"	" "	"	"	Present	"
1778	" " " "	" "	12	Transient fever	Scattered old lobular pneumonia	"	"	"	Present
1691	Nil, control			Mild influenza	1 lobe pneumonia	Present	Present	"	"
1672	" "			Filtrate disease	Scant, scattered and lobular	"	"	Absent	Absent
1741	" "			Mild influenza	1.5 lobe pneumonia	"	"	Present	Present
1793	" "			" "	" "	"	"	"	"

1819	" "			Filtrate disease	Not autopsied				
1820	" "			Mild influenza	" "				
1821	" "			" "	" "				
1678	Swine influenza virus + *H. influenzae suis*	Mild influenza	18	None	Not autopsied, see Table III				
1683	" "	Typical influenza	15	"					
1787	" "	" "	15	"					
1801	" "	Mild influenza	19	"					
1665	Swine influenza virus alone	Filtrate disease	15	"					
1668	" " " "	" "	15	"					
1744	" " " "	" "	12	"					
1775	" " " "	" "	12	"					

* Sera obtained at end of this interval neutralized the swine but not the human influenza virus in all cases except that of swine 1744 (see Table III).

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

TABLE III

Influence of Initial Virus Infection upon Subsequent Antibody Response to the Viruses of Human and Swine Influenza

Swine No.	Serum drawn	Serum tested for capacity to neutralize							
		Swine influenza virus				Human influenza virus			
		Extent of pulmonary lesions in mouse No.				Extent of pulmonary lesions in mouse No.			
		1	2	3	4	1	2	3	4
(*a*) Initial infection. Human influenza virus: Reinoculated with swine influenza virus intranasally									
1819	Normal	4+*	4+	4+	4+	4+	4+	4+	4+
	12 days after initial infection	4+	4+	4+	3+	0	0	0	0
	12 days after reinoculation	0	0	0	0	0	0	0	0
1820	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	12 days after initial infection	4+	4+	4+	3+	0	0	0	0
	12 days after reinoculation	2+	1+	1+	0	0	0	0	0
1821	Normal	4+	4+	4+	4+	4+	4+	4+	
	12 days after initial infection	4+	4+	4+	4+	0	1+	0	0
	12 days after reinoculation	0	0	0	0	0	0	0	0
1645	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	19 days after initial infection	4+	4+	4+		0	0	0	
	11 days after reinoculation	0	0	0	0	0	0	0	0
1657	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	31 days after initial infection*	4+	4+	4+	4+	0	0	0	0
	11 days after reinoculation	0	0	0	0	0	0	0	0
1659	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	31 days after initial infection*	4+	4+	4+	4+	0	0	0	0
	11 days after reinoculation	0	0	0	0	0	0	0	0
1750	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	12 days after initial infection	4+	4+	4+	4+	0	0	0	0
	11 days after reinoculation	0	0	0	0	0	0	0	0
1780	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	12 days after initial infection	4+	4+	4+	4+	0	1+	0	0
	11 days after reinoculation	4+	4+	4+	4+	0	0	0	0

* 0 = mouse with no pulmonary lesions at autopsy. 1+ to 4+ = mice with progressive degrees of influenzal pneumonia; 4+ indicates a complete and fatal pneumonia.

TABLE III—*Concluded*

Swine No.	Serum drawn	Serum tested for capacity to neutralize							
		Swine influenza virus				Human influenza virus			
		Extent of pulmonary lesions in mouse No.				Extent of pulmonary lesions in mouse No.			
		1	2	3	4	1	2	3	4
(*b*) Initial infection. Swine influenza virus: Reinoculated with human influenza virus intranasally									
1678	Normal (not obtained)								
	22 days after initial infection	0	0	0	0	4+	4+	4+	4+
	12 days after reinoculation	0	0	0	0	0	0	0	0
1683	Normal	4+	4+	4+		4+	4+	4+	4+
	13 days after initial infection	0	0	0	0	4+	4+	3+	3+
	12 days after reinoculation	0	0	0	0	4+	3+	3+	2+
1787	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	13 days after initial infection	0	0	0	0	4+	4+	4+	2+
	12 days after reinoculation	0	0	0	0	2+	2+	2+	2+
1801	Normal (not obtained)								
	16 days after initial infection	0	0	0	0	4+	4+	4+	4+
	12 days after reinoculation	0	0	0	0	4+	4+	4+	4+
1665	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	14 days after initial infection	0	0	0		4+	4+	4+	4+
	12 days after reinoculation	0	0	0	0	1+	1+	1+	1+
1668	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	14 days after initial infection	0	0	0		4+	4+	4+	3+
	12 days after reinoculation	0	0	0	0	4+	3+	3+	2+
1744	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	12 days after initial infection	0	0	0	0	3+	2+	2+	1+
	11 days after reinoculation	0	0	0	0	3+	2+	2+	2+
1775	Normal	4+	4+	4+	4+	4+	4+	4+	4+
	12 days after initial infection	0	0	0	0	4+	4+	4+	3+
	11 days after reinoculation	0	0	0		4+	4+	4+	3+

that the virus alone is little if any less effective in achieving immunity than is a mixture of virus and *H. influenzae suis*. The cross-immunity to human influenza infection conferred by the porcine agent is not

usually associated with demonstrable virus-neutralizing antibodies for the human virus. Of the 14 swine studied, the serum of only 1 (swine 1744), obtained just prior to the test for immunity to human influenza infection, exerted any neutralizing effect on the human virus. The remaining 13 sera, although neutralizing swine virus completely, were devoid of neutralizing activity for the human agent.

Influence of Initial Virus Infection upon Subsequent Antibody Response to the Viruses of Human and Swine Influenza

In order to determine whether swine would develop neutralizing antibodies for either swine or human influenza virus, when inoculated intranasally with these agents following recovery from an initial infection in which the heterologous virus had been employed, the following experiments were carried out.

Eight swine recovered from infection with either human influenza virus alone or a mixture of human influenza virus and *H. influenzae suis* were reinoculated intranasally with a mixture of swine influenza virus and *H. influenzae suis*. Some proved clinically immune and others not, as recorded in Table I. 11 or 12 days after reinoculation they were bled and the serum then obtained, together with that secured before and following recovery from their initial infection, was tested for the presence of neutralizing antibodies for the swine and human viruses by the usual technique (12).

Eight further swine recovered from infection with swine influenza or swine influenza virus alone were reinoculated intranasally with a mixture of human influenza virus and *H. influenzae suis*. All proved clinically immune, as recorded in Table II. Like those in the preceding group, they were bled 11 or 12 days after reinoculation and the serum obtained, together with that drawn before and following recovery from their initial infection, was tested for neutralizing antibodies against both viruses. The results of the tests of these 2 groups of swine sera are outlined in Table III. Since the development of neutralizing antibodies for either swine or human influenza virus was independent of whether or not *H. influenzae suis* had accompanied the virus in the infection, no distinction is made in the table between the animals initially infected with virus alone and those infected with a mixture of virus and bacterium.

As shown in the table, it was found that the sera of all 8 swine, obtained following recovery from an initial infection with human influenza virus, neutralized the human but not the swine agent. Reinoculation of these animals intranasally with swine influenza virus resulted in the appearance, in sera obtained 11 or 12 days later, of

antibodies neutralizing the swine virus completely in 6 of the 8 cases. In the serum of 1 animal (swine 1820) a weaker titer of swine virus antibody appeared, while in the serum of the remaining animal (swine 1780) no swine virus-neutralizing antibodies were demonstrated. Antibodies developed independently of whether or not the animals exhibited recognizable clinical manifestations of infection following reinoculation with swine influenza.

The results obtained in studies with sera of swine initially infected with swine influenza virus and reinoculated intranasally with the human agent were quite different from those just described. Only 1 (swine 1678) of the 8 swine developed antibodies which completely neutralized the human virus. 2 others (swine 1667 and 1787) developed antibodies which neutralized partially under the conditions of the test. The sera of the remaining 5 swine failed to show a significant increase in neutralizing antibodies for the human virus. Swine 1744, whose serum drawn before reinoculation with the human virus partially neutralized, still only partially neutralized afterwards.

It seems clear from the experiments just described that the swine and human influenza viruses influence the subsequent immunological reactivity of swine in differing fashions. To summarize, swine recovered from infection with swine influenza virus are not only immune to the human influenza virus but usually fail to develop specific virus-neutralizing antibodies for it following intranasal inoculation. Swine recovered from initial infection with human influenza virus, on the other hand, may or may not prove immune to swine influenza, but whether or not immune, usually elaborate swine influenza virus-neutralizing antibodies.

The Antibody Response of Swine Influenza-Convalescent Swine to Human Influenza Virus Administered Intramuscularly

There were two obvious possible explanations for the general failure of swine influenza-recovered swine to develop neutralizing antibodies for the human influenza virus following intranasal inoculation. First, the immunity conferred by a previous infection with the swine virus might be of such a nature as to render the respiratory tract mucosa actually impermeable to the human virus. If this were the case and human virus were completely prevented from invading susceptible

cells, one should not expect an antibody response. Second, previous infection with swine virus might, in some manner, have interfered with or exhausted the mechanism responsible for the elaboration of neutralizing antibodies for the closely related human virus. In this

TABLE IV

Antibody Response of Swine Influenza-Convalescent Swine to Human Influenza Virus Administered Intramuscularly

Swine No.	Serum drawn	Serum tested for capacity to neutralize							
		Swine influenza virus				Human influenza virus			
		Extent of pulmonary lesions in mouse No.				Extent of pulmonary lesions in mouse No.			
		1	2	3	4	1	2	3	4
Initial infection. Swine influenza virus: Reinoculated with human influenza virus intramuscularly									
1893	Normal	4+*	4+	4+		4+	4+	3+	
	13 days after initial infection	0	0	0		1+	2+	1+	
	11 days after reinoculation	0	0	0		1+	0	1+	
1894	Normal	4+	4+	4+		4+	4+	4+	
	13 days after initial infection	0	0	0		4+	4+	4+	
	11 days after reinoculation	0	0	0		0	0	0	
1895	Normal	4+	4+	4+		4+	4+	4+	
	13 days after initial infection	0	0	0		4+	4+	4+	
	12 days after reinoculation	0	0	0		0	0	0	
1897	Normal	4+	4+	4+		4+	4+	4+	
	13 days after initial infection	0	0	0		4+	4+	4+	
	12 days after reinoculation	0	0	0		1+	1+	0	
1809	Normal (not obtained)								
	12 days after initial infection	0	0	0	0	4+	4+	4+	4+
	11 days after reinoculation	0	0	0	0	0	0	0	0

*0 = mouse with no pulmonary lesions at autopsy. 1+ to 4+ = mice with progressive degrees of influenzal pneumonia; 4+ indicates a complete and fatal pneumonia.

event, even though human virus did penetrate the respiratory tract mucosa, it would be incapable of eliciting a specific antibody response. The following experiments were conducted in an attempt to determine the applicability of the second hypothesis.

Five swine were infected in the usual way with swine influenza. After recovery they were reinoculated with human influenza virus, but, instead of administering the virus intranasally as in the experiments outlined in Table III, it was given intramuscularly. The animals exhibited no evidence of illness and after a period of observation of 11 or 12 days were bled. Serum obtained at this time, together with that secured before and after the swine influenza infections, was tested for the presence of neutralizing antibodies for the swine and human influenza viruses. The results of these neutralization experiments are given in Table IV.

As shown in Table IV, 3 of the 5 swine influenza-immune swine, inoculated intramuscularly with human influenza virus, developed antibodies which completely neutralized the human virus; 1 animal, swine 1897, developed antibodies which neutralized partially; while the 5th animal, swine 1893, neutralized the human virus partially both before and after its intramuscular injection.

These experiments indicate that the usual failure of intranasally administered human influenza virus to elicit specific neutralizing antibodies in swine influenza-recovered swine is not due to interference with or exhaustion of the mechanism responsible for antibody elaboration.

DISCUSSION

It has been found that swine recovered from infection with swine influenza or swine influenza virus alone are usually immune to infection with a mixture of human influenza virus and *H. influenzae suis*, and that they rather promptly render human virus, administered intranasally, non-demonstrable. This cross-immunity is not associated with the presence of demonstrable neutralizing antibodies for the human virus in the sera of the immune animals. Furthermore, antibodies for the human virus usually fail to develop even after reinoculation intranasally with that agent. Swine immune to human influenza infection, by virtue of a previous attack of swine influenza, thus behave towards the human virus much like naturally refractory animals in that they are resistant to infection without possessing virus-neutralizing antibodies, they do not permit the establishment in the respiratory tract of virus given intranasally, and they usually fail to develop virus-neutralizing antibodies following intranasal inoculation.

Antibodies against human influenza virus do appear, however, in

the sera of swine influenza-immune swine to which the human virus is given intramuscularly. This indicates that their failure to appear after intranasal inoculation is not due to interference, by previous swine virus infection, with the mechanism responsible for antibody elaboration. Rather it suggests that the failure may have resulted from inability of the virus to penetrate the respiratory tract mucosa deeply enough to produce an antibody response. It seems likely that, in swine, the cross-immunity to human influenza virus established by previous infection with swine influenza virus is the result of an acquired barrier to the entrance of human virus into the respiratory tract mucosa.

The cross-immunity conferred against swine influenza by the human influenza virus differs from that in the reverse direction just discussed, and here the association of *H. influenzae suis* in the initial infection is important. Swine recovered from infection with a mixture of human influenza virus and *H. influenzae suis* are usually immune to swine influenza, while those whose initial infections have been with the human virus alone are usually still susceptible to swine influenza, although they develop milder attacks than the control animals. Furthermore, while the pneumonias exhibited by these non-immune swine at autopsy are qualitatively similar to those seen in swine influenza in fully susceptible animals, swine influenza virus is either not demonstrable or is present only in low concentration in the turbinates and lungs. This finding is in striking contrast to the uniformity with which virus is demonstrable in the lungs and turbinates of the control swine.

Antibodies capable of neutralizing swine influenza virus are not present in the sera of animals recovered from human influenza, but they do appear in the sera of most such swine following reinoculation with swine influenza, and this even in the absence of clinical manifestations of infection. The finding indicates that the immunity to swine virus conferred by previous infection with the human agent is not of such a nature as to give rise to a barrier to virus invasion in the respiratory tract mucosa of the apparently immune host.

The fact that the respiratory tract mucosas of swine still let swine influenza virus through after recovery from infection with the human virus may explain why infection with a mixture of human virus and

bacterium gives a better immunity to swine influenza than does infection with human virus alone. Swine initially infected with a mixture of human influenza virus and *H. influenzae suis* develop an immunity to both agents: immunity to the human virus is evidenced by the appearance of specific neutralizing antibodies, while immunity to *H. influenzae suis* is indicated by the failure of this bacterium to become established in the lower respiratory tract upon reinoculation with swine influenza. Swine initially infected with human influenza virus alone, on the other hand, become immune only to this virus. When later inoculated intranasally with a mixture of swine influenza virus and *H. influenzae suis* the animals immune to both the human virus and *H. influenzae suis* have only the heterologous virus with which to deal. The swine virus in these cases, to judge by the formation of swine virus-neutralizing antibodies, invades the tissues of the respiratory tract and persists for a short time at least. That it is rather promptly inactivated, however, probably through an immunity mechanism established as a result of previous infection with the closely related human virus, is indicated by the fact that, in animals that remain free of symptoms, no swine virus can be demonstrated in the turbinates or lung even 3 days after inoculation. The swine show no clinical or postmortem evidence of this evanescent virus infection and thus, like ferrets and mice, appear to possess a perfect cross-immunity. On the other hand, swine immune only to the human virus cannot usually adequately resist this transitory infection with the swine influenza virus when a concomitant *H. influenzae suis* infection is added. Even here, however, the virus component is rapidly destroyed in the influenzal lesions it has initiated, as evidenced by its complete absence, or presence only in low concentration, in the turbinates and lungs as early as the 3rd day after infection.

To judge from the two instances in which swine were given 2 intranasal injections of human influenza virus alone, repeated inoculations with the human virus enhance the effectiveness of the cross-immunity defense mechanism against swine influenza.

It seems likely, from the experiments discussed, that the cross-immunity shown by swine recovered from infection with the viruses of human and swine influenza, respectively, may be due to different mechanisms. Animals convalescent from swine influenza are immune

to human influenza virus apparently by virtue of the failure of the human agent to get through the lining of the respiratory tract. In the case of swine recovered from infection with human influenza virus, on the other hand, the respiratory tract mucosa still lets the swine influenza virus pass, but here the invading virus is rather promptly inactivated by some unknown defense mechanism evidently established by the earlier human virus infection.

The findings recorded were all obtained in "acute" experiments and it is possible that other results would be obtained when long periods of time intervened between succeeding exposures to infection. Practical considerations, incident to experimental work with swine, have made it impossible to include such long time experiments in the present studies.

SUMMARY

Swine recovered from infection with either swine influenza or swine influenza virus alone are usually not only immune but refractory to human influenza infection. Swine recovered from infection with a mixture of human influenza virus and *H. influenzae suis* are usually immune to swine influenza while those recovered from infection with human influenza virus alone are usually not immune to swine influenza. The possible mechanisms involved in the cross-immunity between the influenza viruses are discussed.

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