IMMUNIZATION EXPERIMENTS WITH SWINE INFLUENZA VIRUS

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In earlier experiments (1) it was shown that swine influenza virus, administered intramuscularly, immunized pigs to swine influenza and achieved this result without inducing evidence of infection. It was pointed out that this method of immunization might be of practical value.

The discovery by Smith, Andrewes, and Laidlaw that ferrets (2) and mice (3) are also susceptible to swine influenza virus has made it possible to compare the immunity produced by various methods in these small animals with that similarly produced in the natural host. The present experiments were conducted in an effort to determine the effect of dosage, route of administration, and animal source upon the efficacy of swine influenza virus in immunizing swine, ferrets, and mice.

EXPERIMENTAL

Preparation of Virus for Use as Vaccine

The strain 15 (Iowa, 1930) swine influenza virus was used in all experiments. It will be designated swine, ferret, or mouse virus in this paper to indicate its immediate animal source and the only species other than swine through which it has passed. All mouse virus used had been transferred serially at least five times in mice, and all ferret virus at least fifteen times in ferrets.

To prepare virus for use as vaccine, weighed amounts of infected lung which had been in glycerol in the refrigerator for from 3 days to a month were ground with sand to make 5 per cent suspensions in physiological salt solution. These were allowed to sediment for 10 minutes and the supernatant fluid removed by pipette was employed as the vaccine. All virus suspensions were prepared on the day on which they were to be used.

Titration of Swine Influenza Virus Used to Vaccinate

The approximate number of mouse-infecting doses of virus per cubic centimeter of vaccine was estimated in some of the experiments. While these figures are not exact, they furnish an idea of the relative amounts of virus administered during the period of immunization. They were obtained as follows: Etherized mice were inoculated intranasally as previously described (4) with dilutions of 5 per cent virus suspensions, ranging at intervals of 10, to 1:10,000. 3 or 4 mice were inoculated with each dilution. All mice surviving on the 6th day were killed with chloroform and their lungs, as well as the lungs of those which died earlier, were examined for influenzal lesions. The highest dilution of virus causing definite lung lesions in one or more mice inoculated was taken as the virus titer. From this the number of mouse-infecting doses of virus per cubic centimeter of 5 per cent suspension was calculated on the assumption that approximately 0.1 cc. (4) of suspension entered the respiratory tract of each mouse inoculated. Thus a suspension whose highest infectious dilution was 1:100 would contain 100 mouse-infecting doses of virus for each 0.1 cc. or 1000 per cc. These approximate values are recorded in two of the following tables.

Active Immunization of Swine to Swine Influenza

While it was known that swine virus administered intramuscularly actively immunized swine to swine influenza (1), it seemed of interest to determine whether ferret virus and mouse virus would achieve a similar result.

A number of swine were given two subcutaneous or intramuscular inoculations, 8 days apart, of swine influenza virus from various animal sources. They were tested for immunity to swine influenza 15 or 33 days after their last immunizing dose of virus by the intranasal instillation of a mixture of swine influenza virus and *H. influenzae suis* (5). After a 4 day observation period following the immunity test, during which their temperatures were recorded morning and evening, they were killed by chloroforming or bleeding. Their respiratory tracts were examined at autopsy for lesions of influenza and the lungs, and in some cases turbinates, were tested for virus by inoculation into mice. Blood serum obtained from each pig before and after immunization was tested in mice for virus-neutralizing antibodies by a method already described (6). The results of the immunization experiments in swine are given in Table I.

As shown in Table I, 7 swine which received intramuscular or subcutaneous injections of swine influenza virus from ferrets, mice, or swine were found immune to swine influenza when tested later by intranasal inoculation with a mixture of swine influenza virus and *H. influenzae suis* (5). 2 control swine similarly inoculated developed

TABLE I

The Immunization of Swine to Swine Influenza

Swine No.	Vaccination			Results of tests for immunity		Virus in respiratory tract at postmortem as tested by mouse inoculation								Neutralization of swine influenza virus by swine sera							
						Turbinates				Lung				Virus + serum drawn before immunization				Virus + serum drawn immediately before immunity test			
						Mouse				Mouse				Mouse inoculated with mixture				Mouse inoculated with mixture			
	Source of virus	No. and route of inoculations (each inoculation 10 cc.)	Interval between last inoculation and immunity test	Clinical illness	Lung lesions at autopsy	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
			days																		
15-30	Swine	2—intramuscular	33	None	None					0*	0	0		4+	4+	4+		0	0	0	0
15-53	Mouse	2— "	33	"	"					0	0	0		3+	2+	4+		±	1+	0	
15-61	Ferret	2— "	33	"	"									4+	4+	4+		0	0	0	1+
15-73	Nil (unvaccinated control)			Swine influenza	4 lobe† pneumonia																
16-40	Mouse	2—subcutaneous	15	Transient malaise—no temperature elevation	None	0	0	0	0	0	0	0	0	4+	4+	4+	4+	0	1+	1+	2+
16-51	"	2— "	15	None	"	2+	1+	2+		0	0	0	0	4+	4+	4+	4+	0	1+	0	1+
16-52	"	2—intramuscular	15	"	"	0	0	0	0	0	0	0	0	4+	3+	4+	4+	1+	0	0	0
16-58	"	2— "	15	"	"	0	0	0		0	0	0	0	4+	4+	4+	4+	0	0	0	0
16-62	Nil (unvaccinated control)			Swine influenza	5 lobe pneumonia																

* 0 = mouse with no pulmonary lesions at autopsy.

± to 4+ = mice with progressive degrees of influenzal pneumonia; 4+ indicates a complete and fatal pneumonia.

† The swine lung is comprised of 7 lobes.

swine influenza that was typical both clinically and at autopsy. The ferret and mouse viruses appeared to be as effective in immunizing swine as was that derived from swine.

No virus could be demonstrated in the lungs of the 6 swine tested although it was found in the turbinates of one of them. Previous experiments have shown that virus is regularly and abundantly present in the turbinates, tracheal exudate, and lungs of susceptible swine killed on the 3rd or 4th day of an influenza infection (7). The immunized animals were thus not only refractory to infection but had also, with one exception, inactivated or destroyed the virus administered in testing for immunity. In the exceptional animal, virus established itself in the nose but failed to invade the lung.

Antibodies neutralizing swine influenza virus appeared in the sera of all animals during the course of immunization. It was estimated, without recourse to titration, that these were of lower titer than those resulting from an attack of the disease.

Active Immunization of Ferrets

Smith, Andrewes, and Laidlaw (8) attempted to immunize ferrets to swine and to human influenza virus by repeated subcutaneous injections of each virus. According to a personal communication, ferrets were the immediate animal source of the virus used. Of the 11 ferrets included in their experiments, 2 were found completely resistant later to the test dose of virus given by intranasal or intrapulmonary inoculation under ether narcosis. The remaining animals developed either nasal symptoms or fever much like the controls. They differed from the controls, however, in that they showed no lung lesions at autopsy. It was concluded that in these animals a partial immunity, sufficient to protect the lungs from virus attack, had been established.

In the present experiments an attempt was made to immunize ferrets to swine influenza virus by the subcutaneous or intraperitoneal injection of ferret, mouse, or swine virus.

2 cc. doses of 5 per cent infected lung suspension were administered either once or twice, at 8 day intervals, to each ferret. The animals were tested for immunity, 15 to 41 days after their last immunizing injection, by intranasal inoculation under ether narcosis with 1 cc. of a 5 per cent suspension of swine influenza virus derived from ferret lung. After an observation period of from 4 to 7 days following the

TABLE II

The Immunization of Ferrets to Swine Influenza Virus

Ferret No.	Vaccination: Source of virus	Vaccination: No. and route of inoculations (each inoculation 2 cc.)	Vaccination: Mouse-infecting doses of virus per cc.	Vaccination: Interval between last inoculation and immunity test	Results of tests for immunity: Clinical illness	Results of tests for immunity: Lung lesions at autopsy	Virus in respiratory tract at postmortem as tested by mouse inoculation: Turbinates, Mouse 1	Turbinates, Mouse 2	Turbinates, Mouse 3	Lung, Mouse 1	Lung, Mouse 2	Lung, Mouse 3
				days								
4-6	Ferret	2—subcutaneous		15	0*	0†						
4-9	"	2— "		15	0	0						
5-7	"	2— "		15	0	0	0‡	0	0			
5-8	"	2— "		15	0	0	0	0	0			
7-3	"	2— "	1000	33	0	0						
5-3	"	1— "		15	0	0						
5-5	"	1— "		23	0	0	0	0	0			
6-0	"	1— "		23	0	0	0	0	0			
8-0	"	1— "	1000	33	++	++						
5-6	Swine	2— "		15	++	+						
6-1	"	2— "		15	+++	+++						
8-2	"	2— "	1000	33	++	+++						
7-9	"	1— "	1000	41	++	+++						
8-3	"	1— "	1000	41	++	++						
9-3	"	2— "	1000	15	++	++	4+	4+	3+	2+	2+	2+
9-0	"	2—intraperitoneal	1000	15	+	+++	4+	4+	2+	3+	2+	3+
9-1	"	2— "	1000	15	0	0	4+	3+	3+	0	0	0
9-2	"	2— "	1000	15	0	0	4+	3+	2+	0	0	0

* *Clinical illness:* 0 = none.
\+ = clinical picture that of mild influenza.
++ = an influenza of average severity.
+++ = severe. Most of the ferrets with this degree of illness would probably have died.

† *Lung lesions:* 0 = none detectable at autopsy.
\+ = influenzal pneumonia involving less than ¼ of lung at postmortem.
++ = influenzal pneumonia involving from ¼ to ½ of lung at postmortem.
+++ = influenzal pneumonia involving from ½ to ¾ of lung at postmortem.

‡ *Mouse inoculations:* 0 = no pulmonary lesions at autopsy.
± to 4+ = progressive degrees of influenzal pneumonia. 4+ indicates a complete and fatal pneumonia.

TABLE II—*Concluded*

Ferret No.	Vaccination: Source of virus	Vaccination: No. and route of inoculations (each inoculation 2 cc.)	Vaccination: Mouse-infecting doses of virus per cc.	Vaccination: Interval between last inoculation and immunity test	Results of tests for immunity: Clinical illness	Results of tests for immunity: Lung lesions at autopsy	Virus in respiratory tract at postmortem as tested by mouse inoculation: Turbinates, Mouse			Lung, Mouse		
							1	2	3	1	2	3
				days								
7-5	Mouse	2—subcutaneous	1000	33	+	+						
7-6	"	2— "	1000	33	+	+						
7-7	"	1— "	1000	41	++	++						
8-1	"	1— "	1000	41	++	+++						
9-7	"	2— "	10,000	15	++	0	1+	2+	2+	0	0	0
9-4	"	2—intraperitoneal	10,000	15	0	0	1+	1+	±	0	0	0
9-5	"	2— "	10,000	15	0	0	0	0	0	0	0	0
9-6	"	2— "	10,000	15	0	0	0	0	0	0	0	0
4-8	Nil (unvaccinated control)				+++	+++						
6-2	"	" "			++	++						
7-8	"	" "			++	+++						
8-4	"	" "			+++	+++						
8-7	"	" "			+++	+++						
9-9	"	" "			++	+++	3+	2+	2+	4+	4+	3+
10-0	"	" "			++	++	4+	2+	2+	4+	2+	3+
10-1	"	" "			++	++	3+	3+	2+	4+	4+	3+

immunity test, during which their temperatures were recorded morning and evening, they were killed by chloroforming. Their respiratory tracts were examined at autopsy for evidence of infection (9) and in some cases the turbinates and lungs were tested for virus by inoculation into mice. The results of attempts to immunize ferrets to swine influenza virus are given in Table II. The experiments included were not all conducted simultaneously.

As shown in Table II, 8 of 9 ferrets that had received one or two subcutaneous injections of ferret virus were rendered immune to swine influenza virus. Little if any immunity, however, was established by the similar administration of swine or mouse virus. 6 ferrets that had received either one or two injections of swine virus, and 4 ferrets that had received one or two injections of mouse virus subcutaneously, were not immune and differed little or not at all from the control

animals with respect to illness and lung lesions exhibited following their test infection. One ferret (No. 9-7) that had received two subcutaneous injections of mouse virus developed an influenza that appeared typical clinically, but at autopsy its lungs were normal. It is apparent from the above experiments that, when given subcutaneously, ferret virus is superior to that from either mice or swine in immunizing ferrets to swine influenza virus. No reason for this superiority of homologous over heterologous virus is evident.

The advantage of ferret over swine or mouse virus was less apparent when the immunizing inoculations were given intraperitoneally. All 3 ferrets that had received mouse virus into the peritoneal cavity and 2 of 3 of those similarly inoculated with swine virus were rendered clinically immune to swine influenza virus. These experiments indicate that the route by which heterologous swine influenza virus is administered to ferrets determines, to a marked degree, its effectiveness in producing immunity.

The results of the tests for virus in the turbinates and lungs of a number of the ferrets, given in the last column of Table II, indicate that the lungs of immunized animals, which appeared normal at autopsy, were also free from detectable virus. However, the turbinates of some of the ferrets that had shown no clinical symptoms contained sufficient virus to infect mice. It is probable that these ferrets had been less effectively protected than those in which virus failed to become established in the turbinates following the test for immunity.

The 14 ferrets in Table II which showed varying degrees of immunity may be grouped into three classes: those immune and free from demonstrable virus; those immune which had virus in the turbinates, and the single ferret (No. 9-7) which, though not clinically immune, developed no lung lesions and had virus only in its turbinates. The majority of ferrets in the experiments reported by Smith, Andrewes, and Laidlaw (8) would belong in the last group.

Active Immunization of Mice

Smith, Andrewes, and Laidlaw (8), and Francis and Magill (10) have reported the immunization of mice to human influenza virus by means of repeated doses of virus given subcutaneously, intradermally, or intraperitoneally, or by a combination of these routes.

The following experiments were conducted in an effort to define the conditions required for the immunization of mice to swine influenza virus. Preliminary experiments had suggested that mice behaved towards homologous and heterologous swine influenza virus much as did the ferrets described in the preceding section. It seemed likely, therefore, that the question of immunization with swine influenza virus from various animal sources could be investigated more thoroughly in mice than in ferrets. Moreover, since the infection produced by swine influenza virus in mice is both highly fatal and noncontagious (4), the efficacy of immunization procedures in this species may be determined by survival alone and the extreme isolation precautions essential with ferrets or swine are unnecessary.

Mice 3 to 5 weeks old and weighing from 10 to 15 gm. at the beginning of the immunization procedure were used. 0.2 cc. doses of 5 per cent infected lung suspension were administered, either once, or repeatedly at 8 day intervals, to each mouse subcutaneously or intraperitoneally as recorded in Table III. The animals were tested for immunity to mouse lung swine influenza virus (either a 2 per cent or 5 per cent suspension) administered intranasally under ether narcosis (4) 14 or 30 days after their last immunizing dose of virus. The control mice, acquired from stock at the same time as those to be vaccinated and kept in the same isolation room, quite regularly succumbed to this amount of virus within 7 days. All mice dying were autopsied in order to establish that death had been the result of an influenza virus pneumonia. Survival was taken as the criterion of immunity. The results of attempts to immunize mice to swine influenza virus by various procedures are recorded in Table III.

The four experiments presented in Table III are not strictly comparable for, while the amount of infected lung suspension used to vaccinate was kept constant, the virus content of these suspensions varied from approximately 100 to 10,000 mouse infecting doses per cc. Within individual experiments, however, the results reflect quite clearly the effectiveness of one immunization procedure as compared with others in the same experiment, and even between experiments certain broad comparisons can be made. Of 83 control mice infected in the four experiments, 79 died, indicating the virulence of the virus and the severity of the test for immunity.

Swine virus administered subcutaneously was definitely the least effective of any of the immunization procedures tried; only 5 of 63 mice (8 per cent) thus treated survived the test dose of virus and these 5 survivors were all in Experiment 4 in which an unusually virus-rich

TABLE III

The Immunization of Mice to Swine Influenza Virus

Experiment No.	Vaccination				Results of tests for immunity
	Source of virus	No. and route of inoculations (each inoculation 0.2 cc.)	Mouse-infecting doses of virus per cc.	Interval between last inoculation and immunity test	
				days	
1	Swine	2—subcutaneous	1000	30	0/19*
	Ferret	2— "	1000	30	3/18
	"	2—intraperitoneal	1000	30	8/10
	Mouse	2—subcutaneous	1000	30	6/12
	"	1— "	1000	30 and 38	9/19
	"	2— " (diluted 1:10)	100	30	3/16
	Mice recovered from intranasal infection with swine virus				8/8
	Unvaccinated control mice				0/19
2	Swine	2—intraperitoneal	100	14	11/18
	Ferret	2— "	100	14	12/20
	Mouse	2— "	1000	14	10/18
	"	2— " (diluted (1:10)	100	14	11/20
	Mice recovered from intranasal infection with swine virus				6/6
	Unvaccinated control mice				1/20
3	Swine	3—subcutaneous	100	14	0/20
	"	3—intraperitoneal	100	14	8/18
	Mouse	3—subcutaneous	100	14	11/14
	"	3—intraperitoneal	100	14	10/11
	Unvaccinated control mice				2/20
4	Swine	3—subcutaneous	10,000	14	5/24
	"	3—intraperitoneal	10,000	14	23/24
	Mouse	3—subcutaneous	10,000	14	19/23
	"	3—intraperitoneal	10,000	14	21/21
	"	1—subcutaneous	10,000	30	11/24
	"	1—intraperitoneal	10,000	30	12/25
	"	3—subcutaneous (with 10 per cent swine serum)	10,000	14	16/18
	"	3—intraperitoneal (with 10 per cent swine serum)	10,000	14	21/23
	"	3—subcutaneous (diluted 1:10)	1000	14	15/24
	"	3—intraperitoneal (diluted 1:10)	1000	14	13/23
	Unvaccinated control mice				1/24

* The numerator represents the number of mice that survived the immunity test; the denominator the number of mice in the group tested.

vaccine had been employed. Ferret virus given subcutaneously also failed to induce an appreciable degree of immunity.

Swine virus given intraperitoneally, on the other hand, produced a fair degree of immunity; 42 of 60 mice (70 per cent) thus treated survived the test dose of virus. In Experiment 4, in which a swine lung vaccine rich in virus had been used, 23 of 24 mice survived. Ferret virus was also a better immunizing agent when given intraperitoneally, 20 of 30 mice (66 per cent) thus treated surviving the immunity test.

Mouse virus administered two or three times proved the best immunizing agent for mice and the intraperitoneal route held only a slight advantage over the subcutaneous route. 36 of 49 mice (73 per cent) that had received mouse virus subcutaneously and 41 of 50 mice (82 per cent) that had received it intraperitoneally survived the test infection. Single injections of mouse virus given either subcutaneously or intraperitoneally produced an immunity that was inferior to that following multiple injections. Only 17 of 43 mice (40 per cent) that had received a single subcutaneous dose of mouse virus and 12 of 25 mice (48 per cent) that had received a single intraperitoneal injection survived the test dose of virus. The importance of dosage of virus administered in establishing immunity is indicated by the two groups of mice receiving multiple inoculations of 0.2 cc. amounts of 0.5 per cent instead of the usual 5 per cent mouse virus. Only 18 of 40 mice (45 per cent) receiving multiple injections of this dilute virus subcutaneously and 24 of 43 mice (56 per cent) receiving it intraperitoneally survived the test dose of virus. From this it would appear that multiple injections of 0.5 per cent mouse virus were only slightly, if at all, superior to single injections of 5 per cent mouse virus in immunizing mice.

Laidlaw and Dunkin (11) suggested that the multiplicity of antigens contained in heterologous dog distemper vaccine interfered with the antibody response to formolized virus and thus accounted for its inability to immunize. It seemed possible that this explanation might also account for the failure of swine virus given subcutaneously to immunize mice to swine influenza virus. However, the addition of normal swine serum to mouse virus did not appreciably alter its capacity to immunize mice (Experiment 4 of Table III), suggesting that some more complex explanation was applicable here.

Risk of Infection during Immunization with Swine Influenza Virus

A small number of mice succumbed during the period they were receiving their immunizing injections of swine influenza virus. These were carefully autopsied in an effort to determine the cause of death. In most instances intestinal infections with an accompanying diarrhea were responsible. In a few, however, pneumonia was encountered. The lungs of such animals were tested for the presence of swine influenza virus by mouse inoculation, but in no instance was it demonstrated. None of the ferrets or swine became ill during the course of immunization and their temperatures, recorded daily, remained within normal limits. The present experiments thus afford no evidence that the administration of swine influenza virus subcutaneously, intraperitoneally, or intramuscularly, entails any risk of infection.

Experience in some unpublished immunization experiments conducted among swine on farms in eastern Iowa, however, suggests that under certain conditions immunization with swine influenza virus may be a hazardous procedure.

In the field experiments referred to, 1635 swine on 55 different Iowa farms were given one or more intramuscular injections of glycerolated swine influenza virus. 3603 other swine on these same farms were left uninoculated to serve as controls should an epizootic of swine influenza later appear. Wherever feasible the vaccinated swine were kept isolated from the remainder of the drove for a period of from 10 days to 2 weeks. In a number of instances, however, there were no facilities for isolation and it was necessary to keep the inoculated swine in the same yards with uninoculated animals. In two such droves swine influenza appeared shortly after swine influenza virus had been administered intramuscularly to a portion of the animals.

Drove 1 contained 223 swine. Early in August, 12 days after 23 of these animals had received an intramuscular injection of swine influenza virus, swine influenza appeared in the drove. On the 4th day following onset all save 30 animals were typically ill of influenza. Among these 30 apparently normal swine were 20 of the 23 that had received virus intramuscularly 16 days earlier. So far as could be determined, there was, at the time, no other swine influenza in eastern Iowa to which this outbreak could be traced. Furthermore, it was early August, fully 2 months before swine influenza ordinarily becomes prevalent in the Middle West. The length of time (12 days) elapsing between vaccination and the appearance of disease in the swine eliminated from consideration the possibility that they had become infected by virus accidentally spilled in the yards at the time of vaccination. The most probable source of infection seemed to be the animals to

which swine influenza virus had been administered intramuscularly. It is believed, although it cannot be proved from the data at hand, that virus spread from the intramuscular site of inoculation and invaded the respiratory tract of one or more of these animals. From here it was transmitted rapidly by contact among the 200 susceptible swine in the drove. Either the swine first infected, or some of those to which the virus was transmitted very early, must have been carriers of *H. influenzae suis* for the disease developing in the drove was swine influenza (caused by the combined action of virus and *H. influenzae suis* (5)), and *H. influenzae suis* was recovered from the pneumonic lung of one of the fatal cases. 20 of the 23 vaccinated animals failed to develop influenza at the time the remainder of the herd became ill, probably because the 12 days elapsing between their inoculation and the outbreak of the disease had been sufficient for the establishment of immunity. There is considerable likelihood, based on experience with droves in which inoculated animals were kept isolated for 2 weeks after vaccination, that had the 23 vaccinated animals in this herd been kept separate from the 200 noninoculated swine, no illness would have appeared in either group of animals.

The second drove, in which influenza appeared shortly following the intramuscular administration of swine influenza virus, contained 195 swine. 4 days after 95 of these animals had been vaccinated, influenza appeared in the drove. So far as could be observed all animals became ill. The source of infection is believed similar to that in drove 1, although here the interval between injection and onset of illness was so short that infection from premises contaminated with virus at the time the animals were inoculated could not be eliminated. Insufficient time had elapsed for the development of immunity in the vaccinated animals although the owner was of the opinion that the first cases appeared in unvaccinated swine. As in the case of drove 1, this outbreak occurred in August, but a year later, and it could not be traced to an outside source of infection.

The swine influenza in the two herds just discussed is believed to have been caused by the virus used to vaccinate. The examples cited are considered illustrative of the hazard entailed in the introduction of a "live" virus vaccine into only a portion of a densely crowded susceptible population. To judge from the laboratory experiments with mice, ferrets, and swine and the field experiments with swine, the use of "live" swine influenza virus as a prophylactic agent may be less dangerous to the recipient of the virus than it is to other susceptible individuals with which the recipient may come in contact during the course of immunization.

DISCUSSION

The immunization experiments described indicate that ferrets and mice are similar in their reactions towards swine influenza virus ad-

ministered as a vaccine. With both species only the homologous virus proved an effective immunizing agent when given subcutaneously; ferret or swine virus given by this route to mice, and mouse or swine virus similarly administered to ferrets, established little or no active immunity. These failures were not due to inability of swine influenza virus to immunize when introduced into subcutaneous tissues, because the homologous virus, given by this route, proved effective in both ferrets and mice. Neither were they entirely the fault of the virus suspensions employed because the heterologous virus immunized almost as well as the homologous when given intraperitoneally.

In the case of swine the route of inoculation or the source of the virus used to vaccinate was of little importance, for active immunity followed subcutaneous or intramuscular injection of either homologous or heterologous virus.

The mechanism whereby swine influenza virus, introduced intraperitoneally, intramuscularly, or subcutaneously succeeds in establishing an immunity capable of protecting the highly susceptible tissues of the respiratory tract is unknown. Specific virus-neutralizing antibodies resulting from vaccination may contribute to the immunity, although they can scarcely be held entirely responsible since their presence is not necessarily synonymous with complete active immunity, as shown by Smith, Andrewes, and Laidlaw (8). A possibility which may be entertained only to be discarded is that virus spreads from the site of inoculation to the respiratory tract in minute quantities insufficient to produce clinically recognizable disease but resulting in subclinical infections and subsequent immunity. Against this possibility are two observations brought out in the present experiments: the superiority, as immunizing procedures, of multiple over single virus injections, and the superiority of homologous over heterologous virus given subcutaneously. If immunity were merely the result of subclinical infection, it is not apparent why it should be greatly influenced by number of injections, route of inoculation, or animal source of virus administered. The above arguments are effective in the cases of the mouse and the ferret. They may not, however, apply to swine for, with this species, virus from any susceptible host administered either subcutaneously or intramuscularly confers immunity and the evidence of earlier experiments (1) indicates that a

single intramuscular injection of virus is sufficient to immunize effectively. In spite of the absence of good evidence to the contrary, there is little to indicate that swine influenza virus given intramuscularly to swine, regularly induces immunity by virtue of its invasion of the respiratory tract and its establishment there of a low grade and unrecognized infection. The two droves of swine mentioned, in which influenza appeared shortly after virus had been administered intramuscularly, probably acquired their infections from virus used in the attempted immunization. They thus afford evidence that under certain conditions the virus may spread to the respiratory tract. However, they probably represent exceptional instances, because none of the swine investigated under laboratory conditions showed evidence of illness during immunization and over 1500 animals vaccinated in field experiments remained normal. It thus seems likely that swine, as well as ferrets and mice, can acquire an immunity to swine influenza virus following its administration by unusual routes, without the actual infection of tissues in which it causes disease manifestations.

SUMMARY

1. Swine influenza virus obtained from the lungs of infected ferrets or mice, when administered intramuscularly or subcutaneously, immunizes swine to swine influenza.

2. Ferrets, which have received subcutaneous injections of swine influenza virus obtained from the lungs of infected ferrets, are immune to intranasal infection with this virus. Similar injections with virus from the lungs of infected mice or swine do not immunize.

3. Mice can be immunized to intranasal infection with swine influenza virus by the subcutaneous injection of virus obtained from the lungs of infected mice, but not by similar injection with virus from the lungs of infected ferrets or swine. Repeated injections induce greater immunity than a single one.

4. Intraperitoneal inoculation of both mice and ferrets with swine influenza virus immunizes them to intranasal infection and it appears to make little or no difference whether the virus used as vaccine is obtained from the lungs of infected mice, ferrets, or swine.

5. Field experiments in which swine influenza followed the intramuscular administration of virus are cited as examples of the hazard

involved in the use of this means of immunization in a densely crowded susceptible population.

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