THE INFECTION OF MICE WITH SWINE INFLUENZA VIRUS

By RICHARD E. SHOPE, M.D.

(From the Department of Animal and Plant Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

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Andrewes, Laidlaw and Smith (1), and Francis (2), have succeeded in infecting white mice with a virus isolated from human cases of influenza. The descriptions of the disease produced in the two laboratories indicate a complete agreement in results. The former workers further observed that the virus of swine influenza also was transmissible to mice and produced in these animals a disease similar in all respects to that caused by the virus of human origin.

Because of the obvious advantages of the use of mice over either ferrets or swine for certain phases of work with swine influenza, it has seemed advisable to study the mouse disease thoroughly. It appeared important to know whether mice could be infected directly from swine, as ferrets can (3 and 4), whether the virus is modified for swine by mouse passage and whether observations concerning the mouse disease might be directly applicable to the swine disease. Furthermore, study of swine influenza infection of mice offered certain advantages over similar studies with the virus of human origin because with the swine virus it is possible to revert, when occasion requires, to the natural host. The present paper confirms and extends the observations of Andrewes, Laidlaw and Smith (1) regarding the infectivity of swine influenza virus for white mice.

EXPERIMENTAL

Method Used in Inoculating Mice Intranasally.—Through a personal communication from Drs. Andrewes, Laidlaw and Smith, prior to a description of their technic, it was learned that white mice could be infected with swine influenza virus. Consequently the method of inoculation developed in this laboratory differs from theirs.

Mice to be infected were etherized in a glass jar until they fell on their sides.

Their noses and mouths were then immersed in the virus suspension, contained in one side of a slightly tilted Petri dish. They were kept thus submerged for 3 or 4 seconds and during this time inspired from 7 to 10 times. It was important to ascertain that the animals' respirations continued during the entire time their noses were in the virus suspension. By measuring the volume of suspension before and again at the end of experiments in which large series of mice were infected from the same container, it was found that each mouse removed an average of 0.14 cc. of suspension. Since much of the suspension adhered to the fur about the nose and mouth, the average dose of virus gaining entrance to the respiratory tract by this method of infection probably lay somewhere between 0.05 cc. and 0.1 cc. This dosage is slightly greater than that used by Andrewes, Laidlaw and Smith in most of their experiments. The method of inoculation just described has been carefully controlled and found to be harmless in itself.

Attempts to produce recognizable disease in unanesthetized mice have been uniformly unsuccessful owing obviously to the fact that the unanesthetized animals hold their breath during the time that their noses are submerged in the virus suspension. While mice treated in this way develop no clinical evidence of illness and show no pulmonary lesions when autopsied 4 to 6 days later, they may become immunized and resist later infection with virus administered in the usual fashion.

In the experiments to be described, the virus suspensions employed in inducing infections were, unless otherwise specified, the supernatant fluid from sedimented but uncentrifuged 5 per cent suspensions of lungs from infected animals. In infecting mice or ferrets, swine influenza virus alone was employed; while in infecting swine the virus was mixed with a small amount of a culture of *H. influenzae suis* (5) in order to produce typical swine influenza (6). Animals which died or were killed on the 3rd or 4th day following inoculation were found to furnish the most satisfactory virus.

The Production of Disease in White Mice by Intranasal Inoculation with Infectious Material from Cases of Swine Influenza

Two field strains of the virus have been tested for their ability to produce disease in mice. Since they differed somewhat in initial pathogenicity for mice, they will be discussed separately. Strain 15, obtained from Iowa in December, 1930, and maintained for study in this laboratory by serial transfer through swine at least once every 90 days, proved regularly pathogenic for mice. There was nothing to indicate that a preliminary adaptation period was essential to the acquisition of full virulence of this strain for mice. Passage directly from swine to mice induced a disease clinically identical with that already described by Andrewes, Laidlaw and Smith (1).

The incubation period ranged from 24 to 48 hours. The first symptoms were loss of appetite and malaise. Infected mice huddled in a corner of their cages and their coats were roughened. By the following day exaggerated respiratory movements were apparent and sounds similar to fine crepitant râles could be heard by listening over the cage. Deaths occurred as early as the 3rd day and usually by the 8th day all mice had succumbed. Animals sacrificed on the 3rd or 4th day exhibited plum-colored areas of pulmonary consolidation involving from 1/4th to 3/4ths of the total lung volume. Animals allowed to proceed to death exhibited, as a rule, a complete pneumonia indicating that the lung lesions were progressive in character. The pathological picture was identical with that already described (1 and 2). The mortality rate among mice infected with fresh unglycerolated Strain 15 virus obtained directly from swine approaches 100 per cent.

Strain 20 virus was obtained from Iowa in December of 1934 and, so far, has behaved differently from Strain 15 in its initial pathogenicity for mice. This strain has undergone only three serial transfers in swine since being brought to the laboratory. Mice inoculated with Strain 20 virus directly from swine show little evidence of illness. Their fur may become a bit rough on the 3rd to 6th day after infection, but they do not become seriously ill and none die. If they are sacrificed on the 4th day, the plum-colored areas of pulmonary consolidation, characteristic of influenza virus infection in mice, are seldom seen. The lungs either appear normal or are very slightly hyperemic and exhibit one or two small areas of consolidation. However, serial passage from these mice soon yields a virus that is as regularly lethal for mice as Strain 15. Strains 15 and 20 are immunologically identical as judged by cross-protection and cross-neutralization tests. Also both produce a characteristic pneumonia in ferrets (4). The difference in initial pathogenicity for mice may be in some way referable to the prolonged and frequent serial transfer of Virus 15 through swine. It will therefore be of interest to observe whether Virus 20, after more serial transfers through swine, acquires the ability to cause fatal initial infections in mice.

A record of some experiments in which Strain 15 and Strain 20 virus were transferred serially in mice is given in Table I. The virus used in initiating these experiments was either from swine or ferrets. The swine virus had undergone no ferret or mouse passages since coming to the laboratory. The ferret virus had been derived originally from swine but had been submitted to no mouse passages. Virus for infections beyond the first mouse passage was derived from the lungs of mice dead or killed on the 4th day postinfection. Mice sacrificed for virus were, of course, not included in the record of the experiments outlined in Table I.

As shown by the table, Strain 15 virus produced fatal infections in

mice from the 1st passage. Strain 20 virus, on the other hand, required at least 2 mouse passages to bring it to full pathogenicity for mice. It was at first thought that this might be due merely to a difference in the virus content of infected swine and mouse lungs. To eliminate this possibility Strain 20 virus was transferred serially 4 times through mice until it was fully pathogenic for this species. It was then passed through a pig and virus recovered from the swine lung was used to infect mice. All of 6 mice inoculated with this

TABLE I

The Serial Passage of Swine Influenza Virus in White Mice

Source of virus	Strain of virus	Serial passages in white mice											
		1st			2nd			3rd			4th		
Lung		No. inoculated	No. dying	Survival	No. inoculated	No. dying	Survival	No. inoculated	No. dying	Survival	No. inoculated	No. dying	Survival
				days			*days*			*days*			*days*
Swine 1574 (fresh)	15	5	5	5–7	5	5	4–6	9	9	3–8			
Swine 1574 (glycerolated)	15	6	4	6–8	9	9	4–7	5	5	5–8	7	7	4–6
Ferret 66 (fresh)	15	3	3	3–4									
Swine 1610 (fresh)	15	4	4	4–8									
Swine 1601 (fresh)	15	5	5	3–6									
Swine 1616 (fresh)	15	5	3	7–12									
Swine 1550 (glycerolated)	20	6	0		5	0		5	3	6–13	7	7	4–9
Swine 1575 (fresh)	20	6	0		5	4	5–8	9	9	4–7	5	5	6–8
Ferret 86 (fresh)	20	5	0		8	7	4–6	6	5	3–7			
Swine 1624 (fresh)	20	8	0		7	1	6	6	6	4–7			

swine passage virus succumbed typically in between 4 and 7 days. This experiment indicated that an actual increase in virulence of Strain 20 for mice had occurred and that the change was not reversed by one back-passage through swine.

Bacteriology and Filtration Experiments

Bacteriological study of the pneumonic lungs of mice dead following infection with the virus has not suggested that any single bacterial component played a rôle in the mouse disease. Usually the lungs have been sterile. When bacteria were encountered there was nothing to

indicate that they had enhanced the severity of the disease and seldom was the same organism recovered from animals of two succeeding serial transfers. No single bacterial form has been found with any degree of constancy. It was of interest to note that, while *H. influenzae suis*, essential to the production of influenza in swine (6), was present in the swine material used in initiating many of the mouse infections it failed to become established in mice.

Bacteriologically sterile Berkefeld N filtrates of suspensions of the lungs of either infected swine or mice have been found fully capable of infecting mice when administered in the customary fashion. Usually mice infected with filtrates have succumbed. However, in some instances the illness in the filtrate-infected animals has been less severe than in the controls receiving unfiltered suspension. This was undoubtedly due to the loss of some virus by adsorption during filtration, because mice of the succeeding passage have regularly succumbed. From these experiments it is apparent that in mice the swine influenza virus is capable of inducing an extensive and fatal pneumonia unaided by secondary bacterial invaders. The mouse disease thus differs materially from that seen in swine in which not only the virus but a bacterium, *H. influenzae suis*, are etiologically essential (6).

Immunity Conferred by Infection

Mice surviving infection with swine influenza virus cannot be reinfected for a period of at least a month. It is not known how much longer their immunity may endure. The production of an extensive pneumonia is not essential to immunization. Mice that have been infected with Strain 20 virus directly from swine and that develop no pneumonia are apparently as solidly immune to Strain 15 or 20 virus as are animals that survive only after a prolonged and stormy pneumonia convalescence.

Failure of Contact Transfer in Mice

Normal mice placed in the same cages with those infected with swine influenza virus have in no instance become recognizably ill. Furthermore, mice exposed in this way have proven fully susceptible to infection when later inoculated intranasally with virus under ether anesthesia.

In one experiment bread soaked in swine influenza virus of full pathogenicity was fed to a group of 10 mice four times during the course of 6 days. The animals ate of the mixture and the shavings used as bedding became moist with it. None of the mice became ill, and 14 days after the last virus feeding all were tested for immunity by intranasal inoculation with virus. All proved fully susceptible and died as promptly as their controls. These experiments confirm the observation of Andrewes, Laidlaw and Smith (1) that swine influenza virus does not produce a readily communicable disease in mice.

Failure of the Virus to Infect Mice When Administered Subcutaneously or Intraperitoneally

Mice, inoculated subcutaneously or intraperitoneally with 0.2 cc. amounts of virus known to be fatally pathogenic by nose, exhibited no clinical evidence of illness and were completely negative when autopsied 4 to 6 days following inoculation. This is in accord with the experience of Andrewes, Laidlaw and Smith (1) and indicates a tropism of the virus for respiratory tract tissues similar to that seen in swine (7). Immunity following repeated subcutaneous or intraperitoneal administrations of virus will be discussed in a later paper.

The Infection of Swine and Ferrets with Mouse-Passaged Virus

It has previously been reported (4) that 16 serial transfers of the swine influenza virus in ferrets failed to alter its pathogenicity for swine. Since that time 4 more serial ferret passages have been tested. The hog receiving 19th passage virus mixed with *H. influenzae suis* failed to become typically ill. However, swine inoculated intranasally with 20th, 23rd and 24th ferret passage virus together with *H. influenzae suis* developed characteristic swine influenza. From this it would appear that 24 serial transfers of the virus in ferrets had not altered its pathogenicity for swine.

Similarly, prolonged serial passage of swine influenza virus in mice has exerted no appreciable influence on its virulence or infectivity for swine. Fourth, 8th, 16th, 23rd, 36th, 41st, 45th and 53rd mouse passage Strain 15 virus mixed with *H. influenzae suis* has been administered intranasally to swine. All 8 pigs inoculated developed characteristic swine influenza indistinguishable in any respect from that induced by similar inoculation with virus of swine origin. No significant lengthening of the incubation period, decrease in clinical

severity or diminution in the extent of the pathological alterations encountered at autopsy was observed in these swine. The virus was also found to be fully pathogenic for ferrets after 16 and 46 mouse passages. Virus 20, after 4 mouse passages, proved fully pathogenic for a hog when administered intranasally with *H. influenzae suis*. These experiments indicate that prolonged serial passage of swine influenza virus in mice does not attenuate it for swine. They moreover seem to prove that the agent responsible for the disease in mice is actually the swine influenza virus.

Further evidence as to the identity of the virus causing disease in mice with the swine influenza virus was furnished by cross-neutralization experiments. In these, virus of swine origin that had at no time been submitted to mouse passage, and convalescent serum from swine infected with such virus were used. It was found that such convalescent swine sera neutralized mouse passage virus in either mice or swine as well as swine virus in mice. Furthermore, sera from recovered mice neutralized swine virus. It would thus seem established that the agent causing the disease in mice is the swine influenza virus and not some intercurrent infectious agent acquired during serial mouse passage.

Titration of Swine Influenza Virus in Mice

In certain types of experiments exact knowledge as to the minimal infectious dose of a virus is desirable. It was hoped that, with the mouse available as a test animal, accurate quantitative experiments with swine influenza virus might be possible. With this end in view mouse passage, ferret passage and swine passage virus were titrated in mice. From a group of four such experiments conducted in November of 1934, using 3 mice per dilution, it was found that mouse passage virus was active in a final dilution of 1:20,000 (on the basis of wet lung weight), and that ferret and swine passage virus were only slightly if at all less active. There was no further occasion to titrate the virus until February of 1935. At this time the final infecting dilution was found to be 1:2000. In April and May titrations, conducted in the same manner as above, indicated that the final infecting dilution for both mouse and swine passage virus was 1:200. The mice in all the titration experiments were from the same stock and all were used

within a week of the time weaned, being thus of approximately the same age. There is no explanation apparent for the wide variation found in the minimal infectious dose of the virus. It is suggested that it may be a seasonal variation but only titrations throughout several years can establish this. From this standpoint it is of interest that November, the month in which swine influenza virus appeared to be most highly infectious for mice, is also the month in which the middle western swine epizootics most frequently appear. The experiments indicate, aside from this possible epidemiological interest, that titrations of virus, to be valid for a given experiment, must be done at the same time as the experiment for which the data are intended.

Immunological Relationship of Swine and Human Influenza Virus

It has been noted by Smith, Andrewes and Laidlaw (3 and 8) that the infection of ferrets with either swine or human influenza virus confers considerable reciprocal protection against the other virus. Cross-neutralization tests, however, have shown that, while the two viruses are related, they are not identical. Francis (9) found that the sera of neither ferrets nor swine recovered from swine influenza were capable of neutralizing his PR 8 strain of the human virus although they were known to be effective against the swine virus. In like manner, Laidlaw, Smith, Andrewes and Dunkin (10) have noted that the serum of a horse, hyperimmunized against the swine influenza virus, was highly active against its homologous virus but did not neutralize their human strains in the dilutions tested. Neutralization in the opposite direction was, however, somewhat better, for the serum of a horse hyperimmunized against the human virus neutralized swine virus in the lower dilutions.

Experiments conducted in this laboratory are in agreement with the work just cited. Mice immunized by Francis against his PR 8 and Philadelphia strains of the human virus were found resistant to a dose of swine influenza virus that killed all controls in the experiment. In like manner, mice immunized against swine influenza virus in this laboratory were found resistant when tested with a dose of PR 8 human virus that proved fatal for 5 out of 6 controls. Serum from a horse hyperimmunized by Laidlaw, Smith, Andrewes and Dunkin (10) against their W.S. strain human virus and from a rabbit hyper-

immunized by Francis against his PR 8 human virus proved capable of completely neutralizing swine influenza virus for mice. The control mice in these experiments received swine influenza virus mixed with normal horse or normal rabbit serum and all died on the 3rd and 4th day following inoculation.

DISCUSSION

The disease produced in mice by infection with swine influenza virus resembles that in ferrets (3 and 4). It differs materially from that induced or occurring naturally in swine. In mice and ferrets the virus administered intranasally suffices to produce an extensive and often fatal pneumonia. In swine, however, a severe illness, characteristic of the naturally occurring influenza in this species, ensues only when the virus is administered in company with a bacterium, *H. influenzae suis* (6). The mouse and the ferret must therefore be considered as highly artificial hosts in that in neither species is the disease etiologically a complete replica of swine influenza; there is no evidence that *H. influenzae suis* or any other organism contributes significantly to their illness.

The virus infection in mice appears to be non-contagious, while the ferret disease is communicable (3), and influenza in swine is highly contagious. This difference is a useful one from an experimental standpoint for with mice the time and space consuming practise essential to isolation is unnecessary.

Evidence of adaptation of swine influenza virus to mice was noted with one of the two strains studied. Strain 20 virus, while initially infectious for mice, required several serial mouse passages to bring it to full pathogenicity for this species. Strain 15 virus, however, required no adaptation to mice. It killed quite regularly even in its first serial mouse passage, and repeated transfers in mice have not noticeably enhanced its activity for this species. There was no evidence that prolonged serial passage of swine influenza virus in mice attenuated it for its natural host.

It seems clear from the present experiments that the swine influenza virus is a stable one, so far as its three known hosts are concerned; for prolonged passage through ferrets has not altered its pathogenicity for mice or swine, and its infectivity and virulence for

ferrets or swine are unaffected by repeated serial transfers in mice. It can be transferred at will from any one of its known animal hosts to any other, and no significant alteration in its properties, other than an enhancement in the virulence of Strain 20 for mice by serial passage in this species, has been noted. The swine influenza virus would thus appear to differ significantly from strains of the human influenza virus so far studied, both as concerns its initial pathogenicity for ferrets and its infectivity for mice. The experience with human influenza virus has indicated that it undergoes an adaptation during early passages in ferrets. Francis (2) noted that in etherized ferrets his PR 5 human virus strain did not produce pneumonia until its 6th serial passage. He remarked that the disease then developing more closely resembled that produced in etherized ferrets by swine influenza virus (4) than the classical disease first described by Smith, Andrewes and Laidlaw (3). The latter workers have since found that serial passage of their human virus in anesthetized ferrets also eventually results in the appearance of pneumonia in animals inoculated in this way (11). It would thus appear that only after a number of serial passages in ferrets does the human influenza virus acquire the ability, possessed by the swine influenza virus from the very outset, of producing pneumonia in ferrets.

Another initial difference in the two viruses that disappears after the human strain has been transferred several times, has to do with its infectivity for mice. Human influenza virus is said not to be infectious for mice until after transfer serially through ferrets (2 and 11), while the swine influenza virus requires no intervening ferret passages to become established in mice. This difference is an interesting one for it suggests that passage of human influenza virus through ferrets alters it in such a way that it becomes more like the swine influenza virus and less like the virus originally obtained from the human patient. The acquisition by human influenza virus, upon ferret passage, of pathogenic properties for ferrets and mice similar to those possessed from the outset by swine influenza virus, suggests that the human virus undergoes changes as a result of passage in animals that the swine influenza virus has perhaps already undergone.

Laidlaw (11) has recently suggested that the swine influenza virus may represent the virus of the human pandemic of 1918 which at that

time in some way became established in swine and has since persisted as the cause of an epizootic disease in this species. If this should be the case, the initial differences, aside from the immunologic ones, between it and recently isolated human strains may be those due to "fixation" by prolonged sojourn in a foreign host.

SUMMARY

The experiments confirm the earlier observation of Andrewes, Laidlaw and Smith that the swine influenza virus is pathogenic for white mice when administered intranasally. Two field strains of the swine influenza virus were found to differ in their initial pathogenicity for mice. One strain was apparently fully pathogenic even in its 1st mouse passage while the other required 2 or 3 mouse passages to acquire full virulence for this species. Both strains, however, were initially infectious for mice, without the necessity of intervening ferret passages. There is no evidence that bacteria play any significant rôle in the mouse disease though essential in that of swine, and fatal pneumonias can be produced in mice by pure virus infections. Mice surviving the virus disease are immune to reinfection for at least a month. In mice the disease is not contagious though it is notably so in swine. The virus, while regularly producing fatal pneumonias when administered intranasally to mice, appears to be completely innocuous when given subcutaneously or intraperitoneally. Prolonged serial passage of the virus in mice does not influence its infectivity or virulence for swine or ferrets. It is a stable virus so far as its infectivity is concerned, and can be transferred at will from any one of its three known susceptible hosts to any other.

In discussing these facts the stability of the swine influenza virus has been contrasted with the apparent instability of freshly isolated strains of the human influenza virus. Though the mouse is an unnatural host for the virus it is, nevertheless, useful for the study of those aspects of swine influenza which have to do with the virus only.

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