SEROLOGICAL STUDIES OF SWINE INFLUENZA VIRUSES

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Most of the work with swine influenza virus has been carried out with strain 15, recovered originally in Iowa in 1930. Prior to 1937 this strain was, from time to time, superficially compared with swine influenza viruses obtained in different epizootic outbreaks, and no evidence to indicate immunological heterogeneity among the various strains was detected. Judgment of the identity of the viruses being compared was usually based upon their ability to produce cross-immunity in swine, though some cross-neutralization tests with sera of recovered swine or ferrets failed to detect strain differences either. Swine influenza viruses compared in this way with strain 15 or with one another and considered on the basis of the results obtained to be immunologically identical were strain 14 (Iowa, 1930), strain 17 (Iowa, 1931), strain 18 (Iowa, 1932), strain 19 (Iowa, 1933), strain 20 (Iowa, 1934), and strain 23 (Ohio, 1935).

During the early years of work with human influenza virus, investigators recovered strains from patients in different epidemics and widely separated localities. These viruses from man were assumed, largely on the basis of cross-immunity tests in ferrets, to be immunologically identical. In 1936, however, Magill and Francis (1), using virus-neutralizing serum prepared in a non-susceptible host (rabbit), obtained evidence that their Puerto Rico and Philadelphia strains differed antigenically. Later Burnet (2), Andrewes (3), and Andrewes, Smith, and Stuart-Harris (4) demonstrated serological differences among other strains of human influenza virus. Recently the question of immunologic variation among the large number of strains of human influenza virus now available for study has been thoroughly investigated by Magill and Francis (5, 6) in this country and by Smith and Andrewes (7) in England. The conclusions reached in both investigations were that there is great immunological diversity among strains of human influenza virus and that the virus is antigenically complex. Smith and Andrewes believed that their experiments indicated the existence of at least 4 major antigenic com-
ponents among the 28 strains of virus they studied. They classified the strains, on the basis of their content of the 4 major antigens, into 3 main categories, namely, highly specific strains, relatively non-specific strains, and intermediate strains. Magill and Francis classified their 24 strains into 6 groups as determined by serological similarities or differences and pointed out that the strains which most closely resembled one another were, in general, those from the same epidemic of influenza. Serologically different strains were, however, also recovered from the same epidemic.

These observations concerning serological diversity among strains of the human influenza virus raised the question of whether or not similar variations existed among strains of the swine influenza virus recovered in different epizootics. The experiments reported in this paper were conducted in an attempt to answer the question.

**Materials and Methods**

*Strains of Virus.*—The human influenza viruses employed in the present experiments were strains WS, PR8, and Oakham, recovered respectively from cases of epidemic influenza in 1933, 1934, and 1937. The swine influenza viruses used were strain 15 (Iowa, 1930), strain 20 (Iowa, 1934), strain 23 (Ohio, 1935), strain 24 (Nebraska, 1936), strain 28 (Iowa, 1936), strain BC (New Jersey, 1936), and strain 29 (Iowa, 1937).

All strains of virus studied serologically were well adapted to white mice before use in the present experiments and were of such pathogenicity that the supernatant of a 1 per cent infected lung suspension killed all mice inoculated intranasally in less than 5 days. Virus suspensions both for use in neutralization experiments and for the immunization of rabbits were prepared from glycerolated infected mouse lungs.

*Serum.*—The swine sera were obtained by tail or heart bleeding 11 to 13 days after infection with swine passage swine or human influenza virus mixed with a small amount of a culture of the bacterium *Hemophilus influenzae suis* (8). The swine furnishing the sera were thus in early convalescence.

The rabbit sera were obtained by marginal ear vein bleeding on the 10th and 13th days after intraperitoneal injection with 7 cc. of a 5 per cent suspension of mouse lung infected with either swine or human influenza virus. The 10th and

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1 I am indebted to Dr. C. H. Andrewes for the WS strain, Dr. Thomas Francis, Jr., for the PR8 strain, and Dr. C. H. Stuart-Harris for the Oakham strain.
13th day bleedings from each rabbit were pooled for use in the neutralization tests. This method of immunization differs somewhat from that employed by Magill and Francis (5) in that they bled their rabbits on the 8th day, and they graded their immunizing dose to correspond roughly with the titer of the virus strain being used.

All sera, both from swine and rabbits, were filtered through Seitz pads prior to storage in the refrigerator until used.

Neutralization Tests.—The neutralization tests were conducted in white mice by the technique regularly used in this laboratory (9). The supernatant of a 2 per cent suspension of glycerolated infected mouse lung was employed as virus, and this was mixed in equal parts with the undiluted sera to be tested. The mixtures were stored for 2 hours in the refrigerator prior to their administration to white mice. 3 etherized mice were inoculated, in testing each serum-virus mixture, by dipping their noses in the inoculum contained in a slightly tilted small Petri dish. The mice were observed for 10 days; all dying were examined at postmortem; and on the 10th day, surviving mice were autopsied and the degree of pulmonary involvement was noted.

Because of the numbers of tests involved, all virus strains could not be studied at one time. The general plan followed, therefore, was to test all of the swine and rabbit sera against each of the strains of virus in turn. With the exception of strain 20, all of the swine influenza viruses were of roughly the same pathogenicity for mice, and the amount of virus administered in each test amounted to between 10 and 100 minimal fatal doses. Strain 20 possessed a slightly lower pathogenicity for mice, and the dilution used in the neutralization tests corresponded roughly to 10 minimal fatal doses. Two of the human influenza viruses, strains PR8 and WS, were of approximately the same pathogenicity as the majority of the swine strains, while the Oakham strain, at the time it was used, roughly corresponded in titer with strain 20 swine influenza virus. No effort was made to titrate the number of minimal fatal doses of virus more closely than by decimal dilutions. In each individual neutralization experiment 5 groups of control mice receiving virus mixed with normal rabbit or swine serum were included, and all of the mice in these groups succumbed of influenza during the 10 day period of observation.

RESULTS

The results obtained with convalescent swine sera are shown graphically in Chart 1 and those with immune rabbit sera in Chart 2. In
the two vertical columns to the left of each chart are listed the animals supplying the antisera together with the strains of virus against which the antisera were prepared. The strain of virus used in neutralization tests with the various sera is given at the top of each of the other vertical columns.

As shown in Chart 1 all swine convalescent sera, regardless of the
**Chart 2.** Cross-neutralization tests in mice with sera of immunized rabbits. Designation of results same as Chart 1.
strain of swine influenza virus from which the animals supplying the sera were convalescent, neutralized all strains of the swine influenza virus. In like manner, the 3 human viruses tested were neutralized by the sera of swine recovered from infection with either the WS or PR8 strains of human influenza virus. Between the human and the swine strains the serological relationship found to exist was variable; only one of the human virus antisera (swine 99) had any appreciable neutralizing effect on any of the swine viruses. In the reverse direction, however, most of the swine virus antisera partially neutralized the WS and Oakham strains. The PR8 strain was neutralized partially by only one of the swine virus antisera. These findings taken alone would indicate that each strain of swine influenza virus was serologically like all of the other swine strains in the present experiments. The 3 human viruses would also have to be considered alike on the basis of the results with the human virus antisera. However, consideration of the neutralization tests with the human viruses and swine virus antisera makes it evident that the Oakham and WS strains behave quite differently from the PR8 strain, and it would seem that these two strains are immunologically more closely related to swine influenza virus than is the PR8 strain. The important feature of the data given in Chart 1, so far as they concern the present experiments, is that no evidence is furnished to indicate serological heterogeneity among the 7 strains of swine influenza virus under study.

The results with virus-neutralizing rabbit sera recorded in Chart 2 are not as clear cut and constant as were those with swine sera. Among the swine influenza viruses, strains 15 and BC produced potent antibodies in rabbits both for themselves and for all heterologous swine strains as well, but were, as a rule, neutralized only partially or not at all by antisera prepared against the heterologous swine viruses. Strain 20, on the other hand, was readily neutralized by sera prepared against all of the other swine strains and the PR8 human strain but itself produced antibodies poorly or not at all for the heterologous swine viruses. Strain 29 resembled strain 20, though here one of the 3 rabbits used (rabbit 53) produced fairly good neutralizing antibodies for heterologous strains. The 3 remaining swine strains resembled strains 15 and BC in that they produced antibodies in
rabbits effective at least partially against all the other swine viruses but differed in that neutralization of the heterologous viruses was seldom complete as with the strain 15 and BC antisera. There are exceptions to this attempted classification, obvious from consideration of Chart 2. This suggests that at least some of the differences noted may be more dependent upon variations among the individual rabbits used than among the strains of swine influenza virus under study.

The rabbit antisera more effectively differentiated between the swine viruses and the WS and Oakham strains of human influenza virus than had the swine antisera. With the exception of PR8 antisera against strain 20, there was little cross-neutralization between swine and human strains. Furthermore, the rabbit antisera rather clearly differentiated between the PR8 and WS strains of human virus, something the swine antisera had failed to do.

**DISCUSSION**

It is difficult to reconcile the results obtained with swine convalescent sera and those obtained with sera of immunized rabbits as to their relative significance in denoting serological homogeneity or heterogeneity among the strains of swine influenza virus studied. If the results with swine convalescent sera were the only ones available, it would be simple to conclude that the 7 swine viruses were serologically alike and possessed the same general antigenic composition and pattern. If, on the other hand, only the results with sera of immunized rabbits were to be considered, it would be necessary to recognize the existence of antigenic variations among the swine influenza viruses. Thus, from the rabbit serum results, strains 15 and BC, which appear antigenically alike, differ from strains 20 and 29 in that they are not neutralized by antisera prepared against strains 20 and 29. Antisera prepared against 15 and BC do, however, neutralize strains 20 and 29. The remaining 3 strains lie intermediate between these two groups, though resembling strains 15 and BC most closely in their serological behavior. The classification which rabbit antisera seem to have made among the strains of swine influenza virus studied corresponds, in a way, with that into which Smith and Andrews (7) grouped their human viruses. Strains 20 and 29 could be designated, according to this arrangement, as "specific" strains in
that they produce antibodies that are largely effective against only the homologous strains. Strains 15 and BC would correspond to Smith and Andrewes' "non-specific" or "master" strains, viruses which produce antibodies effective against the whole group of swine influenza viruses. The remaining viruses, strains 23, 24, and 28, would be classified as "intermediate" strains, though resembling the "non-specific" strains more closely than the "specific." There are, however, several individual exceptions to this rather general classification. For instance, rabbit 53, immunized with strain 29, developed antibodies that neutralized heterologous swine strains almost as broadly as sera prepared against 15 or BC. This serum also neutralized the WS strain human influenza virus completely, the only one of the anti-swine virus rabbit sera to be completely effective against any of the human viruses. In like manner, the antisera of rabbits 79 and 55 prepared respectively against strains 24 and 20 were unusual, when compared with antisera of other rabbits immunized with the same viruses, in their capacity to neutralize heterologous strains of swine influenza virus.

It is not believed that the various differences among the swine viruses, detectible by antisera prepared in rabbits, are due to differences in antibody titers of individual rabbit sera used, because frequently the differences are in the wrong direction to be accounted for in this way. Rather it would seem that rabbit antisera actually detect strain differences that are not reflected in convalescent sera of the natural host animal. Such differences are probably of no practical importance so far as the natural disease, swine influenza, is concerned and have an academic interest only in that they indicate a variation in the antibody response to the virus of a susceptible and a non-susceptible host.

Since, in the natural host of swine influenza, all strains of the virus give rise to an antibody response indicative of antigenic homogeneity, the question is raised as to whether the swine serum or the rabbit serum results should be more seriously considered in arriving at a decision as to whether the swine influenza virus strains studied are serologically alike or different. There can be no doubt that in rabbits the various virus strains give rise to antibodies with differing virus affinities. However, in the rabbit, swine influenza virus exhibits no evidence of pathogenicity and is probably not infective in the sense
in which that term is usually applied to indicate invasiveness and persistence of an infective agent in a susceptible host. In all probability, swine influenza virus acts in a manner analogous to that of any other invasively inert, antigenic substance in eliciting a specific response in rabbits. Thus if the swine influenza virus is antigenically complex, as Magill and Francis' (5) and Smith and Andrewes' (7) findings indicate the human influenza virus to be, then one might anticipate that the first antibody response of rabbits would be to the dominant or most readily accessible of the swine influenza virus antigens. In swine, on the other hand, where immunity follows actual multiplication of the virus within the host, invasion of susceptible cells by the virus, and finally, destruction or inactivation of virus at the time of recovery, one might expect an immunological host response to all of the various antigens comprising the virus. It seems entirely possible that the apparent discrepancies between the swine and rabbit serum findings may be accounted for by this difference in the mechanism whereby the virus-neutralizing antibodies are produced in a non-susceptible animal, the rabbit, on the one hand, and in a susceptible host, the swine, on the other. On such a basis, antisera prepared by the infection of swine with virus would be considered to reflect the entire antigenic content or composition of the virus, while antisera prepared by the injection of virus, infectively inert for rabbits, into these animals would be thought of as reflecting the arrangement, within the virus, of the components responsible for mouse pathogenicity. Such an explanation of the findings would orient the apparently discrepant results obtained with swine and rabbit antisera. The conclusion to be reached under this interpretation would be that the various strains of swine influenza virus studied are similar in their antigenic composition but that they vary among themselves either in the arrangement of their common antigenic components or in the situation, within the virus, of the components responsible for their mouse pathogenicity.

CONCLUSIONS

1. Cross-neutralization tests with sera from swine recovered from infection with swine influenza indicated the serological identity of 7 strains of swine influenza virus obtained from different sources.

2. Cross-neutralization tests with sera from rabbits, immunized
to swine influenza virus, exposed serological differences among the same 7 swine influenza virus strains. Two strains appeared to be serologically similar and were characterized by the ability to produce effective homologous virus-neutralizing sera which were, however, poor or ineffective against the heterologous virus strains. Two other strains were also serologically similar but produced antibodies effective not only against themselves, but against all heterologous strains as well. The remaining 3 strains were intermediate in their ability to produce heterologous virus-neutralizing antibodies.

3. The human influenza viruses included, especially strains WS and Oakham, were most effectively differentiated serologically from the swine influenza viruses by rabbit antisera.

4. The suggestion is advanced that swine antisera express the antigenic composition of the swine influenza viruses, while rabbit antisera reflect either their antigenic arrangement or the arrangement of the components responsible for their mouse pathogenicity. On this interpretation the 7 strains of swine influenza virus studied would be considered to have similar antigenic compositions but differing antigenic structures.

5. The serological differences among strains of the swine influenza virus, detectible by rabbit antisera, are probably of no practical significance so far as the natural disease, swine influenza, is concerned.

BIBLIOGRAPHY