ANTIGENIC RELATIONSHIP OF BRITISH SWINE INFLUENZA STRAINS TO STANDARD HUMAN AND SWINE INFLUENZA VIRUSES*

THE USE OF CHICKEN AND FERRET ANTISERA IN RED CELL AGGLUTINATION

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The antigenic relationships of strains of the human and swine influenza viruses have presented a puzzling problem for investigation. Soon after studies on this subject were undertaken, it became evident that some of the apparent antigenic differences determined by a variety of methods were not significant. The differences were considered to be quantitative rather than qualitative, which led to the inclusion of numerous human strains in one group designated as Type A (1). As other strains from man were isolated, they were either included in the accepted Type A group or, on the basis of distinct antigenicity, placed in another category (Type B) with other strains of like antigenic nature.

Swine influenza in the United States was first described by Dorset, McBryde, and Niles (2) in 1922, and its specific virus was isolated by Shope (3) in 1931. A similar disease has been recognized clinically in Great Britain and on the Continent for some years. Although clinical, pathological, and contributing bacterial factors are different in the disease as it occurs in America and in Europe, virus has been demonstrated in outbreaks abroad having properties much like the strains of swine influenza virus of American origin. A survey of the situation, under the title of "Piglet influenza or infectious pneumonia," has been published by Lamont (4). From epizootics in swine of North Ireland and of Cambridge, England, virus strains have been isolated by Lamont and Shank (4), Blakemore and Gledhill (5), and Glover and Andrewes (6). The work to be reported here on swine viruses from Cambridge and North Ireland is a continuation of studies undertaken in 1940–41 by one of us with Dr. R. E. Glover and Dr. C. H. Andrewes, at the National Institute for Medical Research, London.

Using the inhibition of chicken red cell agglutination by specific anti-influenza serum, as first described by Hirst (7), we have studied the antigenic relationships of the British swine strains to Type A (PR 8, WS) and Type B (Lee) human strains and the Shope swine virus. In addition to the use of specific antisera from ferrets, we have employed the sera of barnyard fowl injected with influenza virus, which we have found capable of specifically inhibiting hemagglutination by the influenza virus.

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Materials and Methods

Viruses.—The Cambridge, North Ireland, PR 8, WS, and Lee viruses were brought from England, and the Shope virus was obtained from Dr. G. K. Hirst of the International Health Division of The Rockefeller Foundation. All viruses were passaged in embryonated eggs and the allantoic fluid of eggs thus infected was employed in the analytic tests. The methods of egg inoculation and harvest of virus were as employed by Hirst (7). From time to time the virus content of infected fluid was determined by intranasal inoculation of mice.

Sera.—The ferret sera, kindly supplied by Dr. Glover, were obtained on the 17th day after the ferrets had been infected by the nasal route.

The chicken sera were prepared by the intraperitoneal injection of adult fowl with virus in infected allantoic fluid. The amount injected was determined by the ability of the virus to agglutinate chicken red cells, the virus content of all inocula being the same. The chickens received one injection and were bled, for the purposes of the work to be reported, 15 days later. All sera were inactivated by being heated at 56°C. for 30 minutes.

Sera of normal chickens served as controls and were regularly incapable of inhibiting agglutination by virus, in concentrations equal to and greater than those of test sera. The possible interference of isohemagglutinins in the fowl sera was controlled by suitable examination; no instance of non-specific agglutination of chicken red cells by normal or immune fowl sera was encountered.

Red Blood Cells.—Chicken erythrocytes were obtained from a local poultry market. The blood was collected in 2 per cent sodium citrate and strained through gauze, and the cells were washed at least three times. Thereafter, the cells were packed by centrifugation at 900 R.P.M. for 8 minutes and a 2 per cent suspension of packed cells was employed in all tests.

Methods.—The techniques of chicken red cell agglutination by influenza virus and agglutination inhibition by specific anti-influenza sera, as described by Hirst (7), were employed with the following minor modifications: (1) the tubes used were 10 cm. long and had an internal diameter of 1 cm.; (2) in reading the tests, the highest dilution corresponding to tube 4 (0.62 per cent suspension of red cells) of the standards was taken as the end-point in both the agglutination and the agglutination-inhibition tests; (3) whenever it was not possible to get the end-point (tube 4), the titer was assumed to be half the difference of the dilutions in the tubes on each side of the apparent end-point.

EXPERIMENTAL RESULTS

The results of the tests using *ferret* sera (Table I) showed a close antigenic relationship between the Cambridge, North Ireland, PR 8, and WS strains. Cross-inhibition reactions in high titers were observed, but not to titers obtained with homologous antigens and antisera. Among these four strains, it was noted that there was no constancy in the reciprocal relation between viruses and sera. This was most marked with the Shope virus; its serum inhibited agglutination by heterologous strains in high titer, but the virus was little affected by heterologous antisera. The agglutinative action of the

Lee (Type B) virus was found to be uninhibited by heterologous sera even in low dilutions; Lee antiserum from the ferret was not available for test.

Antisera produced in *chickens* proved effective in inhibiting influenza virus agglutination of chicken red cells. The inhibiting titers were high and comparable to those obtained with ferret sera. The chicken sera likewise had

TABLE I

*Titers of Ferret Sera against Human and Swine Influenza Viruses**

Virus	Serum					
	PR 8	WS	Ca	NI	Shope	Lee
PR 8	**3,072**	768	192	384	128	—
WS	512	**6,144**	256	768	256	—
Ca	512	3,072	**6,144**	384	512	—
NI	1,536	3,072	768	**6,144**	768	—
Shope	<64	128	96	96	**768**	—
Lee	<64	<64	96	<64	<64	—

Ca = Cambridge. NI = North Ireland.

* Expressed as the reciprocal of the highest serum dilution inhibiting chicken red cell agglutination by influenza virus.

TABLE II

*Titers of Chicken Sera against Human and Swine Influenza Viruses**

Virus	Serum					
	PR 8	WS	Ca	NI	Shope	Lee
PR 8	**6,144**	384	3,072	12,000	256	96
WS	192	**1,536**	1,024	6,144	128	48
Ca	256	256	**6,144**	3,072	64	48
NI	256	768	1,536	**24,000**	192	48
Shope	64	64	192	768	**3,072**	<32
Lee	32	<32	<64	<64	<64	**1,024**

Ca = Cambridge. NI = North Ireland.

* Expressed as the reciprocal of the highest serum dilution inhibiting chicken red cell agglutination by influenza virus.

the capacity of neutralizing virus as determined by the standard mouse technique, and cross-neutralization tests in mice gave results much like those obtained in cross-inhibition of agglutination of red cells by virus.

The fowl antisera (Table II) demonstrated a group antigenic relationship among the British swine and Type A human strains, similar to that found by the use of ferret immune sera. Again, the reciprocal reactions of sera and viruses were not constant, but there was a higher degree of regularity in the cross-relationships with chicken than with ferret sera. The action of the

Shope antiserum on other viruses and of heterologous antisera on the Shope virus was relatively slight. These low heterologous titers against the Shope virus obtained with chicken sera correspond to the titers seen with the ferret sera. Another observation is that, of the five specific fowl antisera, the Shope and PR 8 had the lowest heterologous titers. The antigenic distinctiveness of the Lee virus was again demonstrated by the lack of cross-reactivity of the Lee virus and serum with the heterologous sera and viruses.

Tables I and II summarize the experimental data. The figures given are of one series of examinations and are representative of two and, in some instances, three series of tests on the same materials, in which there was close agreement. The experiments were so arranged that all antisera of the same animal origin were examined together at one time; in other series of tests, both ferret and chicken sera were included for comparison.

DISCUSSION

Hirst (7) has shown how antigenic differences and associations of influenza viruses could be demonstrated by the method of inhibition of chicken red cell agglutination by the influenza virus. It was our purpose to extend the application of the technique, not only by the antigenic analysis of various standard strains but also by the orientation of two recently isolated swine strains in respect to the standard human and swine viruses.

By the use of this method, it was clearly brought out that the Type A and Shope viruses are not closely related antigenically and that Type B (Lee) is sharply set off from all others. These findings are in essential agreement with the results obtained by Hirst.

The viruses from swine influenza in Britain showed an interesting association with the Type A human varieties (WS and PR 8), the four strains forming a rather homogeneous antigenic group. A closer relation was demonstrated by the use of serum prepared against the WS strain (also of Britain) than with the antiserum of PR 8 (of Puerto Rican origin). The Shope virus, on the other hand, proved to be more distantly related to both the human and the British swine strains.

These findings are consistent with the results reported by Glover and Andrewes (6) of vaccination and serum neutralization tests, and lend support to their statement: "Our immunological studies are incomplete, but at present they seem to indicate that British pig strains form a less compact group than the American strains and serologically are different from them. It is by no means certain that they are clearly separated from human viruses as are Shope's strains."

Chicken antiserum, prepared by single injections of specific viruses, proved to be a useful agent for these analyses. The chicken as a source of specific antiserum had the advantage of furnishing a large amount of blood, being

easily handled, and not being subject to natural infection with the viruses under study. The results of using fowl serum were comparable with those obtained by the use of ferret serum, with the exception that anti-Shope fowl serum showed a sharper distinction between that virus and other strains than the anti-Shope ferret serum. Further work on this point of difference in the action of antisera from various animals is being conducted.

SUMMARY

The antigenic relationships of Type A (PR 8, WS) and Type B (Lee) human strains and the Shope and British (Cambridge, North Ireland) swine strains were studied by specific antiserum inhibition of chicken red cell agglutination by the influenza virus.

The Cambridge and North Ireland strains were found to be closely related to the Type A strains and differentiated from the Shope virus.

The distinctive antigenicity of the Lee strain of Type B was confirmed.

Specific antibodies were developed in chickens following single intraperitoneal injections of influenza virus. Inhibition tests yielded results, in the antigenic analysis of the influenza viruses examined, comparable to those obtained with ferret antisera.

Specific inhibition of hemagglutination by influenza virus proved an effective method for the study of strain relationships.

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