J. Hyg., Camb. (1965), **63**, 479
Printed in Great Britain

Serological epidemiological studies with influenza A viruses

By G. C. SCHILD and C. H. STUART-HARRIS
The University of Sheffield Virus Research Laboratory, Lodge Moor Hospital, Sheffield 10

(*Received* 3 *May* 1965)

The first studies of the age distribution of antibodies in man against human and swine influenza viruses were made in Britain in 1935 (Andrewes, Laidlaw & Smith, 1935) and in the U.S.A. in 1936 (Francis & Magill, 1936; Shope, 1936). In 1953 a large-scale survey of such antibodies was reported by Francis, Davenport & Hennessy (1953), using sera from Michigan pooled by age group. The results of these tests showed that antibodies against serologically different viruses were present in different amounts in persons of different ages. Each family of influenza viruses thus gave a characteristic antibody distribution by age, and antibodies were not found in pooled sera from persons born after the last recorded isolation of a particular virus family. As a result of these findings Davenport, Hennessy & Francis (1953) formulated the hypothesis that childhood infection by a particular serological family of influenza A viruses imprints upon the antibody-forming mechanism a pattern which is recalled later by antigenic stimuli from serologically different influenza viruses. With swine influenza virus, antibodies were found in high titres in sera from persons over 30 years of age in 1953. Similar results were obtained with sera from Sheffield collected in 1954 (Davenport, Hennessy, Stuart-Harris & Francis 1955), and the findings were thought to support the view suggested by Laidlaw (1935) that though swine influenza virus had never been recovered from human cases of influenza, it or a virus sharing its major antigen was responsible for the 1918 pandemic of influenza.

However, Isaacs (1957), using Nigerian sera, and Masurel & Mulder (1962), with sera from the Netherlands, both reported that they had found low titres of swine virus antibody in individuals under the age of 30 and that the titres rose gradually from the first year of life to a plateau in those of 35 years of age or over. The alternative explanation formulated to explain the existence of low levels of antibody was that swine virus antibodies in human sera might be formed as a result of repeated infections by human influenza viruses sharing minor antigens with swine influenza virus. Such an hypothesis failed to explain the fact that children of 12 or over in Britain and the U.S.A. showed good amounts of swine antibody in 1935, whereas there was a lack of antibody under 12 in 1935 and up to the age of 30 in 1953. However, the early work was done with neutralization tests on individual sera and the later studies were with the haemagglutination-inhibition test on pooled sera.

It was decided that a repetition of the tests with swine influenza virus using individual sera newly collected from many persons of different ages would be helpful in assessing these two hypotheses. Sera collected between 1961 and 1964, 7 or

more years after the first study in Sheffield, were tested by haemagglutination-inhibition and in some instances by tissue culture neutralization methods and the results are here reported. Representative strains of the influenza A, A1 and A2 virus families were used and tests were made with two viruses of equine influenza recovered from horses and two strains of duck influenza virus.

MATERIALS AND METHODS

Sera

The sera from individuals aged 6 months to 60 years were collected in 1961 and 1962 from patients in the Children's Hospital, Sheffield (6 months to 15 years), and the City General Hospital (5–60 years of age). Serum collections made before inoculations of poliovirus vaccine were available from school-children aged 12–16 years, from a teacher's training college (17–19 years) and from employees of the West Riding County Council (18–45 years). Altogether, 524 sera were collected and these were approximately evenly distributed by sex and age.

Sera were also available from forty male employees at an engineering works who volunteered to give blood in 1952 and again in 1963. Their ages in 1952 were between 29 and 52 years.

In 1964 a separate collection of sera was made from aged persons (60–90 years) resident in Fir Vale Infirmary or patients in the Royal Hospital, Sheffield. One hundred and twenty-two sera were collected from fifty-six males and sixty-six females approximately equally distributed in the various decades.

All sera were stored in sealed screw-capped vials at $-20°$ C.

Viruses

A/Swine (Shope Sw. 15, 1930), A/PR 8 (1934), A1/FM 1 (1947) and A 2/Singapore/1/57 were stock strains adapted to growth in the allantoic cavity. A/Equine/Prague/56, A/Equine/Miami/63, A/Anatum/Prague/56 and A/Anatum/England/62 were kindly provided by Dr H. G. Pereira, World Influenza Centre, Mill Hill, London. Pools of virus were made by allantoic inoculation of 10-day embryonated eggs with 10^{-3} or 10^{-4} dilutions of virus. After incubation at 35° C. for 48 hr. the allantoic fluids were harvested, pooled and stored in sealed ampoules at $-70°$ C.

Haemagglutination-inhibition (HI) tests

These were carried out in 'Perspex' trays by a standard technique (World Health Organization, 1953) using 8 haemagglutinating units of virus (50% end-point). Before testing, all sera were treated to remove non-specific inhibitors by *incubating overnight at 37° C. with 5 vol. of cholera filtrate (N.V. Philips Roxane)* and heating at 56° C. for 1 hr. HI titres were read from the pattern of haemagglutination and expressed in terms of the initial serum dilutions. Ferret antisera prepared against A/Swine, A/PR 8, A1/FM 1 and A 2/Singapore/1/57 were used as standards. No cross-reactions were detected between these strains using potent ferret antisera. Comparisons of HI titres in paired sera from 1952 and 1963 were always made in a single test with identical reagents.

Neutralization tests (A/Equine/Miami/63)

Equal volumes of inactivated sera diluted 1/5 and virus dilution (100 TCID 50/0·1 ml.) were incubated together at 20° C. for 30 min. before inoculation into two to four rhesus monkey kidney tissue cultures (0·2 ml./tube). Cultures were incubated at 36·5° C. for 3 days and virus growth was then detected by haemadsorption with fowl erythrocytes (Vogel & Shelokov, 1957). Sera showing neutralization at 1/5 were titrated in twofold serial dilutions to determine the end-points. All tissue cultures were maintained in medium 199.

RESULTS

Antibodies in sera from persons of 1–60 years of age

The age distribution of antibodies to the four families of influenza virus A2, A1, A and swine are shown in the accompanying figures (Figs. 1–4). In each figure the solid line represents the proportion of sera at the stated ages in which the haemagglutination-inhibition (HI) test indicated the presence of antibody at a titre of 1 in 9 or more (initial dilution). The geometric mean titres (dotted line) were also calculated from the reciprocals of the haemagglutination-inhibition titres and sera negative at 1 in 6 (the lowest dilution tested) were given the arbitrary rating of 3. The curves for percentages of positive sera and geometric mean titres were closely parallel for each virus. Antibody to A2 virus (Fig. 1) was present in more than 20% of the sera at each age but the highest mean titres occurred at 6–11 years of age. With increasing age the titres fell and reached their lowest levels in persons aged 36–50 again rising, however, to a second peak at 51–60 years of age. With the A1 virus (Fig. 2), antibody was not found in sera from children aged 8 or less but from 9 onwards increasing amounts were detected. Peak titres were reached at 12–20 years of age with a second peak at ages 50–60 and low levels in those aged 31–40. With the A/PR8 virus (Fig. 3), sera from persons aged 15 or less contained no antibody, peak titres occurred at ages 21–30 and a second minor peak occurred at ages 41–50.

These results confirmed the absence of antibodies to A and A1 viruses in sera from those born more recently than 1946 and 1953 respectively. As the last recorded A and A1 epidemics in England occurred respectively in 1943 and 1956 a reasonable agreement exists between the disappearance of a particular virus family from the community and absence of antibody from children's sera. Secondly, the peak titres to A and A1 viruses occurred in those aged 5–10 years more than the youngest persons in whom antibodies were detected. This suggests that the maximum antibody response occurs in the 5–15 age group during the prevalence of a particular virus family as indeed was found with the A2 virus (Fig. 1). This general age distribution of influenza antibodies thus supports the doctrine formulated by *Davenport et al. in 1953.*

Figure 4 shows the distribution of antibodies to swine virus. These were not found in children aged 11 or less in 1961. Negative or a low percentage of positive results (2–8%) were found between 12 and 30 years of age. After 30 years of age

Fig. 1

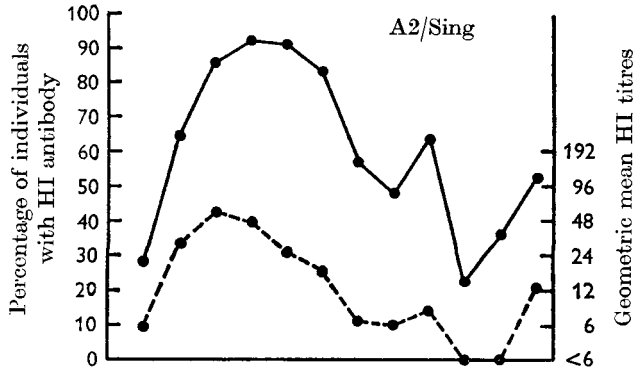


Fig. 2

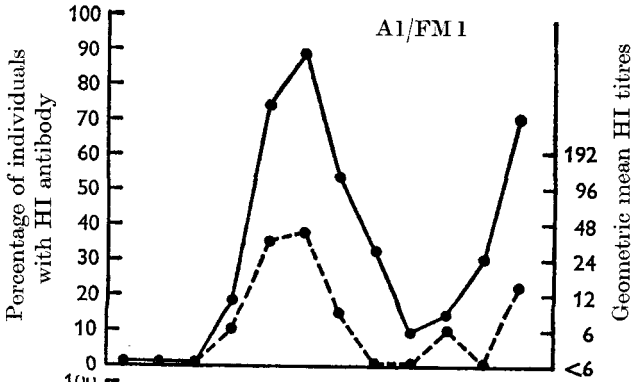


Fig. 3

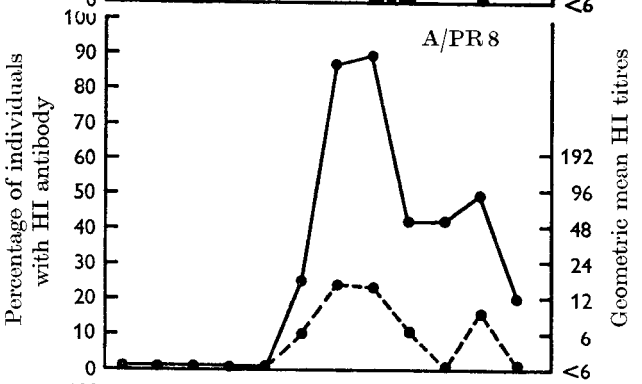
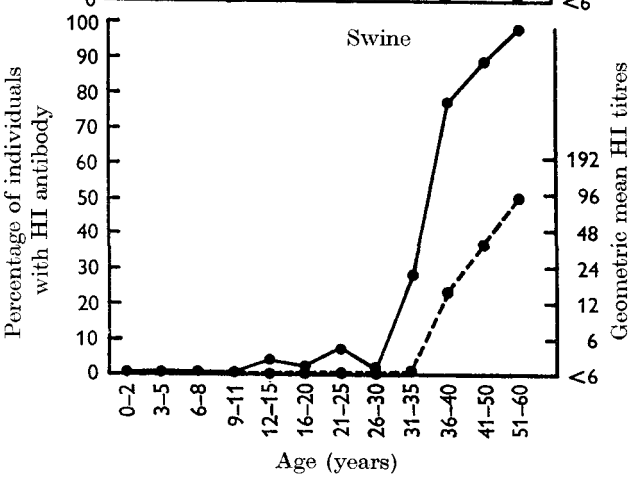


Fig. 4



Figs. 1–4. For legend, see foot of facing page.

https://doi.org/10.1017/S0022172400045368 Published online by Cambridge University Press

the proportion of sera with antibody rose rapidly and peak titres were found in the age group 51–60 years where approximately 100 % of sera contained antibody. These results must be compared with those obtained using pooled sera in Sheffield in 1954 in which no antibody was detected in persons aged 31 or less. It seems probable that tests with individual sera might at that time have revealed antibody in younger persons but it is unlikely that a moderate percentage of positive sera would have been missed. There was, therefore, no obvious change in the age distribution curve for swine antibodies in 1961 compared with that in 1954, whereas the curves for both A and A 1 viruses appeared to have shifted to the right in this period of time. A possible reason for this difference between swine and A and A 1 viruses is considered below.

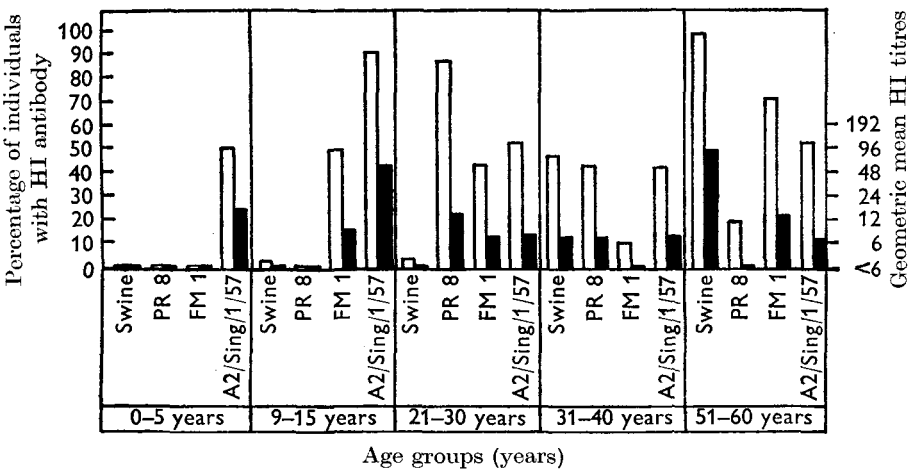


Fig. 5. Summary of the distribution of HI antibodies to swine, A, A 1 and A 2 viruses in various age groups of the population in 1961–62. Open columns = percentage of individuals with HI antibodies. Solid columns = geometric mean HI titres.

Figure 5 shows, in summarized form, the geometric mean titres and proportion of positive sera in selected age groups with each of the four viruses. It is seen that, apart from the swine virus, the findings with the various serological families fit in with the theory that a particular cohort of persons acquires antibodies in childhood to the virus circulating in the community at that time. These antibodies persist even though the particular virus family is replaced by a serologically different strain. A recall of the childhood antibodies formed by infection with swine virus might result from minor shared antigens possessed by the various human viruses. The existence of antibodies to swine influenza virus has been explained by the view that this virus or one sharing its major antigen was the cause

Figs. 1–4. Proportion of individuals with HI antibody and geometric mean HI titres to various strains of influenza A virus in serum specimens collected in 1961–62. Continuous lines = percentage of individuals with HI antibodies; broken lines = geometric mean HI titres. Fig. 1. A 2/Singapore/1/57 virus. Fig. 2. FM 1 virus. Fig. 3. PR 8 virus. Fig. 4. Swine virus.

https://doi.org/10.1017/S0022172400045368 Published online by Cambridge University Press

of the 1918 pandemic of influenza. The fact that the curve of swine virus antibodies did not alter much between 1954 and 1961 and that a low percentage of positive sera was detected in young adults in 1961 demanded further inquiry.

Changes in influenza antibodies between 1952 *and* 1963

Paired sera collected in 1952 and again in 1963 from forty adult males living near Sheffield and who were aged 40–63 in 1963 were tested against each of the four viruses used above. Table 1 shows the mean HI antibody titres for the men in

Table 1. *Comparison of mean HI antibody titres in* 1952 *and* 1963 *in the same individuals*

Ages	No. and year of serum specimen	Geometric mean HI titres A2/Sing/1/57	FM1	PR8	A/Swine
29–38	15 1952	< 3	5·2	8·3	22
40–49	15 1963	9·1	5·4	10	54
39–48	19 1952	< 3	17	3·4	120
50–59	19 1963	10	16	4·3	158
49–52	6 1952	< 3	8·7	3	69
60–63	6 1963	59·8	16·6	4·6	151
All ages	40 1952	< 3	10	4·6	55
	40 1963	13	10	5·9	100

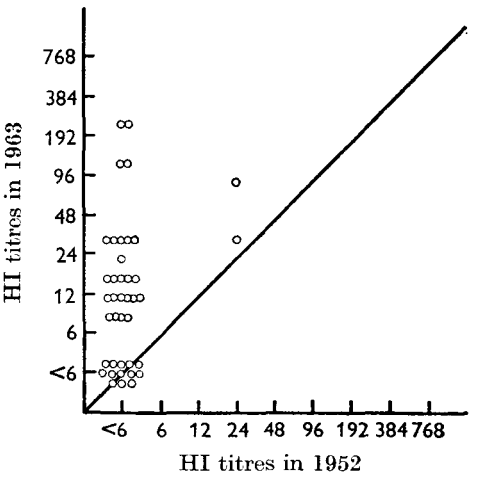


Fig. 6

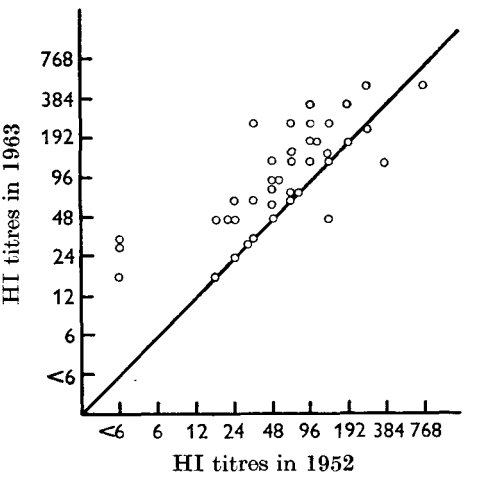


Fig. 7

Figs. 6–7. Changes in HI antibody titres to A2/Singapore/1/57 (Fig. 6) and swine virus (Fig. 7) in the same forty individuals over an 11-year period (1952–63).

various age groups and Figs. 6 and 7 show the actual titres obtained with A2 and swine virus in individual sera in the two years. Two sera collected in 1952 from persons then aged 34 and 43 contained antibody to A2 virus and the rest showed no inhibition at a dilution of 1/6. The subsequent acquisition of A2 virus antibody by twenty-five more of the forty persons was doubtless due to the circulation of this

https://doi.org/10.1017/S0022172400045368 Published online by Cambridge University Press

virus in the epidemics of 1957, 1959 and 1961. Probably some of the thirteen persons whose sera were negative to A 2 virus in 1963 may have had A 2 antibody at some time before 1963 but subsequently lost it. With A 1 virus which caused outbreaks of influenza in the years 1953/54 and 1955/56, some of the forty persons first sampled in 1952 had gained antibody by 1963 but others had lost such antibody. Only in those aged 60–63 in 1963 was there a rise in the mean titres to A 1 virus compared with the titres in 1952 and the means of the other persons were unchanged. With A virus a slight change in mean titres occurred and there were both gains and losses of antibodies in different persons. The overall effect was slight. However, the results with swine virus shown in Table 1 and Fig. 7 were different from those with either the A or A 1 viruses. There was in fact a net gain in mean titres of antibodies to swine virus in persons of all ages and only two individuals underwent a twofold decrease in titre of antibodies between 1952 and 1963. Three persons who had no swine virus antibody in 1952 had developed this by 1963. These changes furnished evidence that the several waves of influenza virus infection to which these forty adults were exposed in the 11-year period between the collections of sera had reinforced their previous antibodies to swine virus. No similar effect was apparent with the A (PR 8) virus so that it seemed probable that the swine virus shares some antigenic grouping either with the A 2 or the A 1 virus family. If this is so, then the lack of shift in swine virus antibodies in the population referred to above (Fig. 4) is explained by the recruitment of antibodies probably by the action of shared antigens. At the same time the lack of decrease in the mean titres to both A and A 1 viruses between 1952 and 1963 in the group of forty persons just described is evidence of the remarkable stability of antibodies to former influenza viruses in the adult population as a whole. It seems that the hypothesis of antigenic recall of antibodies acquired in childhood formulated by Davenport *et al.* (1953) is supported by these findings, but that evidence also exists in favour of the view that minor antigens shared by serologically different virus families also influence the antibody levels found in the population.

Antibodies to equine influenza viruses in sera from aged persons

While the above work was in progress, it was learnt from Prof. Davenport that antibodies to the 1963 but not to the 1956 strains of equine influenza virus had been found in a proportion of sera from aged persons in Michigan. A new collection of sera from persons in Sheffield 60–90 years of age was, therefore, made in 1964 and HI tests were performed with the same swine and human viruses as before and also with the Prague (1956) and Miami (1963) strains of equine influenza virus and two strains of duck influenza virus (A/Anatum/Prague/57 and A/Anatum/England/62).

In the sera from aged persons and also 300 serum specimens from individuals aged 11–55 years no HI antibodies were detected with A/Equine/Prague/56 virus or the two duck strains of influenza virus. Using the A/Equine/Miami/63 virus, which is serologically distinct from A/Equine/Prague/56, antibodies were detected in the HI test in 12–14 % of sera from persons over 70 years of age. The titres were

low and ranged from 1/6 to 1/48. No HI antibodies were found to this virus in 31 sera from persons aged 60–69, nor were any detected in 300 sera from persons aged 11–55 years. To confirm the serological independence of A/Equine/Miami/63 HI tests were carried out using immune ferret antisera with high homologous titres to A/Swine, A/PR8, A1/FM1 and A2/Singapore/1/57. No cross-reactions with A/Equine/Miami/63 were detected. The antibody patterns to the human and swine influenza viruses with the same sera were similar in those containing equine virus antibodies and in those without such antibodies.

Fig. 8. Proportion of sera from aged people with neutralizing antibody to A/Equine/Miami/63 virus at titres of 1 in 5 or greater.

In order to confirm that the inhibition of the 1963 equine virus by sera from aged persons was due to the presence of antibody, neutralization tests were performed in rhesus monkey kidney tissue culture. All sera in which HI antibody was detected contained neutralizing antibody to A/Equine/Miami/63. The distribution of neutralizing antibody with age (Fig. 8) was generally similar to that of HI antibody but 22 % of those aged 65–69 showed antibody at 1/5 or greater. Neutralizing antibody was not detected in 175 sera from individuals aged 11–55 years but was found in a higher percentage of those over 70 than had been detected by HI test. The tissue culture neutralization technique thus seemed to be a more sensitive method of detecting antibody than the HI test.

Finally, Fig. 9 shows the age distribution of antibody to the human, swine and Miami 1963 strains of influenza A virus in the sera from aged persons. The proportion of positive sera and the geometric mean titres to swine, A, A1 and A2 viruses of sera from persons aged 60–69 were similar to those of sera from persons aged 51–60. Over the age of 70, however, sera were less often positive with swine virus and the titres declined in amount, whereas with the other viruses the sera remained much the same as in those under 70. The reason for this decrease in antibody to swine virus could not at first be explained. Separation of the results in women from those in men showed, however, a remarkable difference (Table 2). Over the age of 80, 91 % of male sera contained swine virus antibodies but only 27 % of female sera gave positive inhibition of swine virus. A similar trend was detected in males and females between 70 and 80 years of age (88 % of males and

60 % of females had antibody). No such sex difference was found in sera from persons under 60 years of age, nor did sera from males and females over 60 give different percentage results with other viruses.

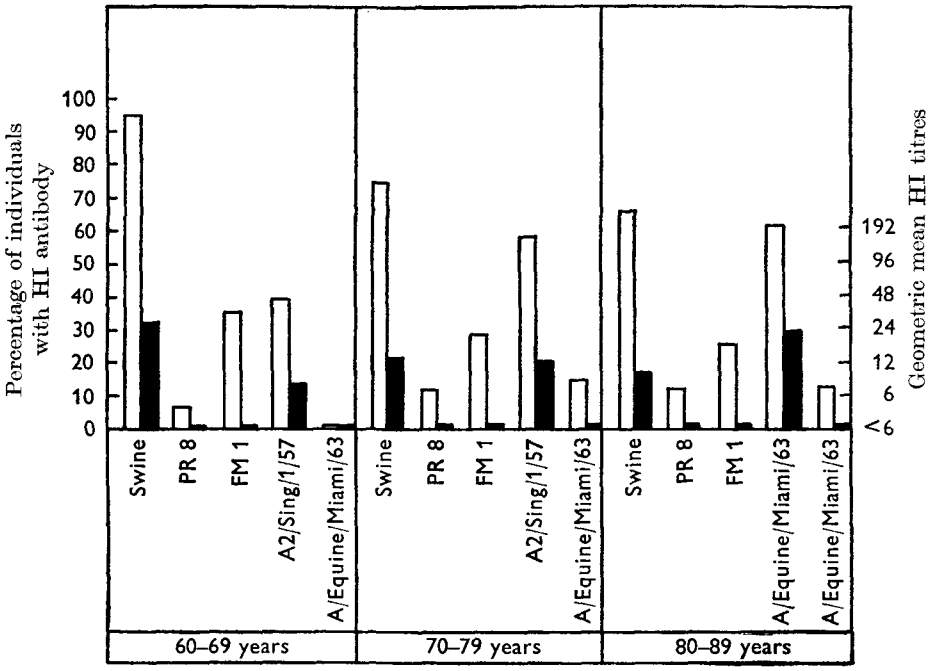


Fig. 9. Distribution of HI antibodies to various influenza A viruses in serum specimens collected in 1964 from aged persons. Open columns = percentage of individuals with HI antibodies. Solid columns = geometric mean HI titres.

Table 2. *Comparison of the proportion of aged males and females with antibody to swine virus in* 1964

Age range (yr.)	Total no. of sera tested	Nos. of sera with HI antibody to swine virus at titres of 1/9 or greater		Percentage of males with antibody	Percentage of females with antibody	Overall percentage positive sera
		Males	Females			
40–59	63	28/29*	32/34	96·6	94·2	95·3
60–69	32	15/15	15/17	100	88·2	93·8
70–79	42	15/17	15/25	88·2	60	71·4
80–89	44	20/22	6/22	90·9	27·3	59·1

* Numerator = number of positive sera; denominator = number of sera tested.

DISCUSSION

The general hypothesis that serum antibodies to influenza viruses not only give information concerning infection by viruses currently circulating in the community but also indicate the antigenic nature of the viruses of former epidemics is not new. Shope's findings (1936) of swine virus antibodies in a high

https://doi.org/10.1017/S0022172400045368 Published online by Cambridge University Press

proportion of sera from young and older adults but not from children led him to argue that this virus or one of similar antigenic composition had been widely prevalent in the recent past. He agreed therefore with Laidlaw (1935) that the swine influenza virus probably represented a surviving form of the human pandemic virus of 1918. All the recent evidence from previous studies and from those reported above are concordant with this hypothesis. The view that the small percentage of adults now under 30 years of age possessing swine virus antibodies represents those in whom antigens of human viruses have produced a heterologous response to swine virus, does not afford an adequate explanation for the existence of a high percentage of adults over 30 with such antibody at the present time. The recent work of Drescher *et al.* (1962*a*, *b*) using the photometric serological technique which permits the separation of specific from cross-reacting antibody is of interest in this connexion. Application of the technique to sera from persons of different ages by Davenport *et al.* (1964) has shown that the swine virus antibody in adults over 40 years of age is specific in its reaction, whereas that from younger persons has the character of cross-reacting antibody. The finding of a shift in age distribution of swine virus antibodies between 1935 and 1953 both in the U.S.A. and in Britain (Davenport *et al.* 1953; Davenport *et al.* 1955) is therefore concordant with the opinion that such antibodies are related to the 1918 epidemic. The lack of any further change in age distribution of swine virus antibodies between 1954 and 1961 in Sheffield may well be due to a cross-relationship between the antigens of swine and A 2 human influenza virus. Such a relationship was suggested by Masurel & Mulder (1962) and evidence in favour of it was obtained by Harboe (1963) in sera from ferrets subjected to repeated infection by various influenza viruses.

The work reported above has thrown no further light on the possible relationship between the A 2 influenza virus and that causing the 1890 epidemic. Mulder & Masurel (1958) were the first to suggest that the A 2 virus was antigenically related to the virus of the 1890 pandemic and their evidence for this was the discovery that in sera collected during the period just before the 1957 A 2 epidemic, antibody to this virus was detected in a small percentage of persons in the Netherlands over the age of 70. Davenport & Hennessy (1958) obtained similar findings in the U.S.A. and so did Davoli & Corsi (1957) in Italy. Yet even Mulder & Masurel (1958) showed that persons immunized with inactivated A 1 vaccine in the pre-Asian era sometimes developed antibody to A 2 virus in the post-vaccination phase. Similar results were obtained in Sheffield (Clarke, Heath, Sutton & Stuart-Harris, 1958), but only occasional instances were found of pre-epidemic A 2 antibody in unvaccinated persons and there was no tendency for concentration of positive specimens in the small number of old persons who were examined. Nevertheless, the results obtained with pre-epidemic 1957 sera by Davenport *et al.* (1964) using the Drescher technique clearly show that the antibody to A 2 virus found in sera before the occurrence of the epidemic was specific in its reaction. It is difficult to escape the conclusion that the A 2 virus antigen had been experienced antigenically by the population before 1957 and possibly in 1890.

The results with the 1963 equine influenza virus are similarly suggestive that this virus is antigenically related to that causing human infection more than 65

years ago. Our own findings confirm those of Davenport (personal communication) and show that antibody capable of neutralizing the virus is certainly present in a proportion of English persons over 65 years of age. Some of the positive sera were sent to Dr Drescher in Berlin who has informed us that the sera react in a specific manner to his test. There is no epidemiological evidence suggesting that equine influenza is a disease at all related to human infection and the serological findings therefore remain unexplained. Davenport *et al.* (1964) use the term 'serological archaeology' for the type of work which led to this discovery and doubtless there is much more yet to be discovered about the viruses of former human influenza epidemics if the appropriate antigens could be located. Certainly it would be unwise to speculate concerning the exact time-relationships between past virus infections and epidemics. It should not be forgotten that aged persons are survivors from a much larger number of persons of their particular cohort. Differences due to chance will almost certainly occur in antibody distributions in the aged therefore. Such a chance selection of survivors may account for the sex difference found in swine virus antibodies in aged Sheffield residents. More work with sera from persons living in other areas is required before deductions are possible.

SUMMARY

Determinations were made of the age distribution of antibody to swine virus and representatives of the various families of human influenza A virus in 1961–62 collections of human sera and paired sera from forty individuals taken in 1952 and 1963:

(*a*) The existence of cohorts of the population, each with a dominant antibody type related to strains of virus first encountered in childhood, was confirmed.

(*b*) The basic epidemiological pattern was similar to that previously detected in 1954. However, it seemed that antibody to swine virus had been reinforced but not antibody to A and A 1 strains.

(*c*) Neutralizing and HI antibodies to A/Equine/Miami/63 virus were detected only in the sera of older people (65 years or over) collected in 1964. No antibodies were found to A/Equine/Prague/56 or two duck viruses.

(*d*) Relatively constant levels of antibody to A, A 1 and A 2 viruses were present in sera from aged persons but antibody to swine virus diminished with age. This could be attributed to a lack of swine antibody in the older females.

The authors are indebted to Mrs Dorothy Edey, A.I.M.L.T., for excellent technical assistance. We also wish to thank Dr C. W. Potter of the University of Sheffield, Dr P. Howard of the Royal Hospital, Sheffield, Dr S. S. Missan of the City General Hospital, Sheffield, and Dr R. W. Elliott, County Medical Officer for the West Riding of Yorkshire for their kind help in obtaining sera.

REFERENCES

ANDREWES, C. H., LAIDLAW, P. P. & SMITH, W. (1935). Influenza: observations on the recovery of virus from man and on the antibody content of human sera. *Br. J. exp. Path.* **16**, 566.

CLARKE, S. K. R., HEATH, R. B., SUTTON, R. N. P. & STUART-HARRIS, C. H. (1958). Serological studies with the Asian strain of influenza A virus. *Lancet*, i, 814.

DAVENPORT, F. M. & HENNESSY, A. V. (1958). The clinical epidemiology of Asian influenza. *Ann. intern. Med.* **49**, 493.

DAVENPORT, F. M., HENNESSY, A. V., DRESCHER, J., MULDER, J. & FRANCIS, T., Jr. (1964). Further observations on the relevance of serologic recapitulations of human infection with influenza viruses. *J. exp. Med.* **120**, 1087.

DAVENPORT, F. M., HENNESSY, A. V. & FRANCIS, T., Jr. (1953). Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus. *J. exp. Med.* **98**, 641.

DAVENPORT, F. M., HENNESSY, A. V. STUART-HARRIS, C. H. & FRANCIS, T., Jr. (1955). Epidemiology of influenza: comparative serological observations in England and the U.S.A. *Lancet*, ii, 469.

DAVOLI, R. & CORSI, O. B. (1957). Anticorpi verso virus dell'influenza 'A-Asia' nella popolazione normale. *Sperimentale*, **107**, 358.

DRESCHER, J., HENNESSY, A. V. & DAVENPORT, F. M. (1962*a*). Photometric methods for the measurement of haemagglutinating viruses and antibody. I. Further experience with a novel photometric method for measuring haemagglutinin. *J. Immunol.* **89**, 794.

DRESCHER, J., DAVENPORT, F. M. & HENNESSY, A. V. (1962*b*). Photometric methods for the measurement of haemagglutinating viruses and antibody. II. Further experience with antibody determinations and the description of a technique for analysis of viral mixtures. *J. Immunol.* **89**, 805.

FRANCIS, T., Jr., DAVENPORT, F. M. & HENNESSY, A. V. (1953). A serological recapitulation of human infection with different strains of influenza virus. *Trans. Ass. Am. Physns*, **66**, 231.

FRANCIS, T., Jr. & MAGILL, T. P. (1936). The incidence of neutralizing antibodies for human influenza virus in the serum of human individuals of different ages. *J. exp. Med.* **63**, 655.

HARBOE, A. (1963). HI antibodies in ferrets cross-infected with distantly related influenza viruses. *Acta path. microbiol. scand.* **57**, 211.

ISAACS, A. (1957). Antibodies to influenza viruses in the sera of Nigerians. *J. Hyg., Camb.*, **55**, 485.

LAIDLAW, P. P. (1935). Epidemic influenza: a virus disease. *Lancet*, i, 1118.

MASUREL, N. & MULDER, J. (1962). Studies on the content of haemagglutination-inhibiting antibody for swine influenza virus A. *Verh. Inst. prev. Geneesk.* **52**, 1.

MULDER, J. & MASUREL, N. (1958). Pre-epidemic antibody against the 1957 strain of Asiatic influenza in the serum of older persons living in the Netherlands. *Lancet*, i, 810.

SHOPE, R. E. (1936). The incidence of neutralizing antibodies for swine influenza virus in the sera of human beings of different ages. *J. exp. Med.* **63**, 669.

VOGEL, J. & SHELOKOV, A. (1957). Adsorption—haemagglutination test for influenza virus in monkey kidney tissue culture. *Science*, **126**, 358.

WORLD HEALTH ORGANIZATION; EXPERT COMMITTEE ON INFLUENZA (1953). *Tech. Rep. Ser. Wld Hlth Org.* no. 64.

Note added in proof:

Since this communication was submitted for publication workers in the U.S.A. have reported finding HI antibody to a 1963 strain of equine influenza A virus in sera collected in 1958 from older persons in Michigan (Minuse, E., McQueen, J. L., Davenport, F. M. & Francis, T., Jr. (1965). Studies of antibodies to 1956 and 1963 Equine influenza viruses in horses and man. *J. Immunol.* **94**, 563). The peak incidence of this antibody was in persons born between 1880–1890.

Further, Dr Masurel (personal communication) wishes us to state that he has detected similar antibodies in the sera of older persons in Leiden.