J. Hyg., Camb. (1969), **67**, 551
Printed in Great Britain

The influence of antigenic variation on influenza A2 epidemics

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(*Received* 14 *March* 1969)

The pattern of epidemics due to influenza A2 virus can be determined by several means: by following changes in sickness benefit claims, mortality rates and general practitioner consultation rates (Miller & Lee, 1969) and by accumulating laboratory evidence of the presence of influenza viruses in the population. Over the past decade information from these various sources has been found to correlate very closely and a clear picture can be drawn of the epidemics which have occurred every winter but three since 1957. The factors which allow such epidemics to develop are only partly understood but there are two which can be measured and which certainly play a part. One of these is the antigenic structure of the prevalent virus and the other is the antibody directed against it in the exposed population. Both these factors are unstable because of the antigenic variation which influenza A virus undergoes from time to time and because serum antibody is not necessarily maintained at high titre against the original infecting strain and, indeed, may become undetectable against a sufficiently changed new variant. Attempts have been made to assess the importance of these factors in explaining the repeated epidemics due to influenza A2 virus which have occurred in Britain since 1957.

MATERIALS AND METHODS

Virus strains

Strains of influenza virus isolated in public health and other laboratories by the inoculation either of fertile hens' eggs or of primary monkey kidney cell cultures were received at the Virus Reference Laboratory, Colindale, and after further passage were examined by haemagglutination-inhibition with specific ferret antisera. Prototype strains of influenza A2 variants were kindly supplied by Dr H. G. Pereira of the World Influenza Centre, Mill Hill.

Sera

Sera for the antibody survey were collected in October 1968 from the staff of a large London hospital for another study, and were kindly supplied to us by Dr G. ffrench. From this source 366 sera were obtained from persons aged between 16 and 67. For the younger age groups recourse was made to sera sent in for

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antistreptolysin O estimations at the end of 1968, and also to sera from children living in the south of England taken in 1968 for the investigation of pre- and post-natal rubella infection and kindly given by Dr E. Vandervelde.

Sera were stored at $-30°$ C until tested. Haemagglutination-inhibition tests were done as described by Pereira, Pereira & Law (1964).

RESULTS

Influenza viruses

Over the 11-year period since influenza A 2 virus first appeared, isolations of this virus have been made every winter with the exception of the winters 1959–60, 1961–2 (when only influenza B appeared) and 1966–7.

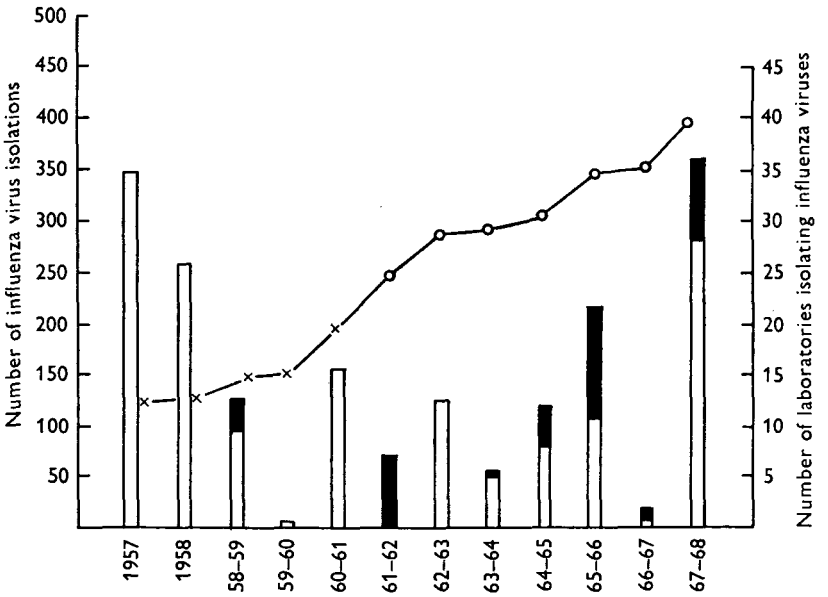

Fig. 1. Laboratories isolating influenza viruses and number of virus isolations, 1957–68. □ = Influenza A2 isolations; ■ = influenza B isolations; × = laboratories using eggs; ○ = laboratories using monkey kidney.

Virus isolations usually extended from the end of December of one year to February or March of the next year. There have been two exceptions to this; one in 1957, when the epidemic began in September and continued with only a short break right through the winter to April 1958, and the other in the 1967–8 winter, when virus isolations were made unusually early, at the end of November of 1967, and continued through to March 1968.

The number of influenza A and B viruses which have been isolated and examined is shown in Fig. 1. Over the 11-year period this has varied considerably. The number does not reflect the extent of any epidemic, for not only has the number of laboratories undertaking virus diagnostic work increased but also techniques have been simplified, permitting a wider sampling of clinical cases.

The influenza A2 viruses isolated in each epidemic were tested against antisera to the original A2 strains and against representative antisera to each successive variant as these were detected. The first antigenic changes were noted in 1961 (Isaacs, Hart & Law, 1962) followed by reports of further differences in 1962–3 by Morris *et al.* (1963) and Weinberger, Buescher, McCown & Gauld (1963) and in 1964 by Pereira *et al.* (1964). The antigenic composition remained relatively stable from 1964 until 1967, when a strain was isolated in Tokyo differing recognizably from those which had circulated during the previous 3 years. This variant, A2/Tokyo/3/67, was isolated in outbreaks of influenza in many countries in the world that year. In Britain, in the epidemic of the winter of 1967–8, it accounted for about one-fifth of the influenza viruses isolated, the remainder being antigenically similar to the earlier strains. Another variant A2/Hong Kong/1/68, even further altered, was isolated in 1968 first in Hong Kong and Singapore and rapidly spread to many parts of the world.

Table 1. *Comparison of influenza A2 viruses by haemagglutination-inhibition*

	Ferret sera					
Virus	A2 Singapore 1/57	A2 England 43/63	A2 England 12/64	A2 England 68/68	A2 Tokyo 3/67	A2 Hong Kong 1/68
A2/Singapore/1/57	**5120**	2560	120	1280	< 10	160
A2/England/43/63	1280	**5120**	240	2560	320	120
A2/England/12/64	160	1280	**320**	5120	120	20
A2/England/68/68	640	2560	640	**5120**	60	120
A2/Tokyo/3/67	160	320	40	240	**480**	< 10
A2/Hong Kong/1/68	640	160	20	640	< 10	**2560**

The antigenic differences between all these variants are shown in Table 1. The difference between the 1957 and the 1963 strains was demonstrable but of small degree. With the 1964 prototype strain it was found that while its antiserum inhibited the earlier A2 viruses moderately well, the strain itself was only poorly inhibited by antiserum to the 1957 strain.

From 1964 small alterations occurred until 1968, when the Tokyo variant appeared in Britain, and this strain, while clearly an A2 virus, showed a more marked shift. This antigenic difference is magnified in Table 1 because of the low homologous titre. Both this strain and the later Hong Kong/68 strain showed a further shift away from the earlier A2 viruses, although they still had components common to them. It is striking that the two most recent variants show no cross-reactivity, one with the other.

An interesting finding related to the growth properties of the successive variants of influenza A2 virus has been the gradual increase in difficulty of isolating and propagating these viruses in fertile hens' eggs. In 1957 the method commonly used for the isolation of influenza virus was the inoculation of such eggs. Viruses were often detected on the first passage and could be grown to high titre on further passage. Primary monkey kidney cell culture had largely replaced egg isolation by 1961 but strains thus isolated could be passaged with ease in eggs. In later years it

became more difficult to isolate strains in eggs and also to passage monkey kidney isolates in eggs. By contrast the Hong Kong/68 variant has been easy to isolate in eggs, yielding high titres of haemagglutinin and behaving more like the original A2 strains than those which have been circulating in the intervening years.

Influenza antibodies

A considerable proportion of the population became infected during the widespread epidemics in 1957, 1958 and 1959 and small surveys after these three epidemics indicated that over half the serum samples examined contained antibody to the Singapore/1/57 strain of influenza A2 virus. The quiet winter of 1959–60 was followed, however, by an extensive outbreak in 1960–1, an outbreak which further increased the proportion of the population with antibody. The antigenic changes detected in the virus at this time were only slight. In the succeeding years the epidemics which occurred were less extensive despite the further antigenic shift found in the A2 viruses isolated in 1964.

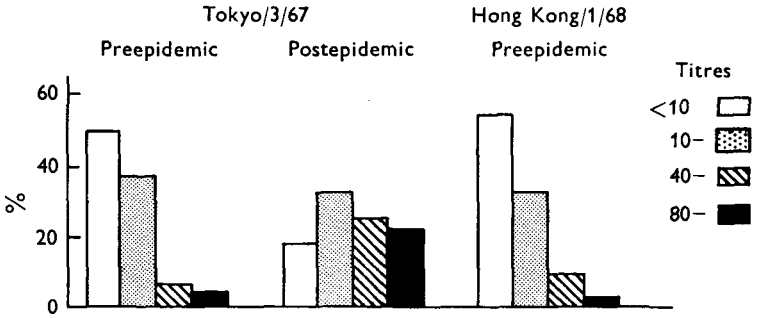

Fig. 2. H.I. antibody to influenza A2 variants, all ages.

A more comprehensive survey of antibody undertaken in 1966 (Pereira, Chakraverty, Pollock & Pope, 1967) again indicated the presence of antibody in a substantial proportion of the population and accordingly epidemics due to the strain then circulating seemed unlikely. The winter of 1966–7 was free from influenza but in the next winter of 1967–8 there was an epidemic of some size. In this epidemic viruses of two antigenic varieties were isolated; one of these was similar to the strains which had circulated during the previous year, the other, which occurred less frequently, was similar to the newer variant A2/Tokyo/3/67. Retrospective studies made on sera taken before the appearance of this variant indicated a possible explanation for this epidemic. The results of these tests are shown in Fig. 2. It can be seen that the proportion of people with high antibody titres to the Tokyo/67 variant was small before the epidemic, whereas 2 years later after the epidemic, the proportion with high titres had greatly increased. The pre-epidemic pattern of antibody to the Tokyo/67 variant was clearly similar to the pattern of antibody to the Hong Kong/68 variant in sera taken before this strain appeared in Britain.

The A2 variant isolated in Hong Kong in 1968 displays an antigenic difference

from the previous A2 strains even more marked than did the Tokyo variant of 1967. The occurrence of outbreaks of influenza in many parts of Asia following its first appearance suggested that antibody to this strain must be low or absent in these populations and sera recently collected in England from people of different ages were therefore tested to estimate the proportion with antibody in Britain. Previous studies (Pereira *et al.* 1967) had shown that very small differences were demonstrable in the antibody pattern in people from widely separated areas in this

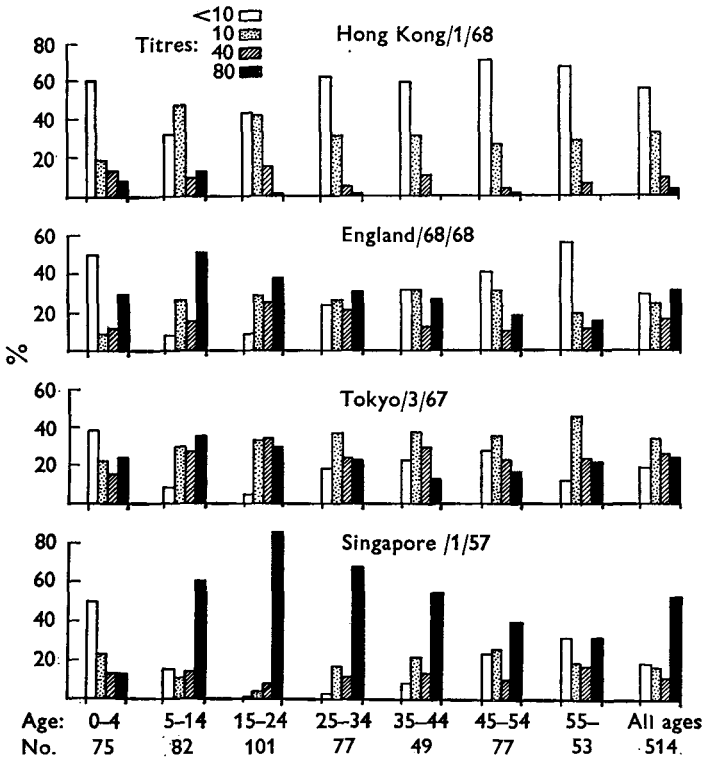

Fig. 3. H.I. antibody to influenza A2 variants among different age groups.

country, so, although most of the latest sera tested were from people in the south-east of England, it is likely that the picture would be similar for the rest of the country. The age distribution and titres of antibody to the Hong Kong/68 variant and to three other A2 strains are shown in Fig. 3. It can be seen that antibody to the original A2/57 virus was present not only in a high proportion of all groups except the under-5-year-olds, but was also present at titres of 1/80 or higher. Antibody to the two strains prevalent in the 1967–8 epidemic represented by A2/England/68/68 and A2/Tokyo/3/67, although present in a high proportion of sera, was found at lower titres compared with the A2/Singapore/1/57 strain. The contrast between these results and those obtained with the A2/Hong Kong/1/68 strain is striking. With this virus the proportion of persons with no detectable antibody is 60% or more in all adults and in those under 5 years of age, and the number with titres

https://doi.org/10.1017/S0022172400041991 Published online by Cambridge University Press

above 1/80 is very small. It is noticeable that in the younger age groups, school-age children and young adults, more than half had antibody at titres of 1/10 or higher. The more frequent detection of antibody to the two recent variants in the younger age groups suggests that these young people (since many of them were not alive at the time of the biggest epidemics due to the original A 2 virus) have been infected more recently with variants antigenically closer to the new strains.

DISCUSSION

Predictions of the amount of illness due to influenza virus during any winter are greatly affected by the antigenic composition of the viruses which will circulate in the population, and the amount of the appropriate antibody available in the population. With a new sub-type the prediction is simple, since the population is not immune and spread of the virus is almost certain. In successive years, as antibody becomes more generally acquired, epidemics, although they occur, become smaller in size and duration and this situation may persist over several winters. The appearance of variants changes the pattern and allows the virus to attack those who have already been infected by the same sub-type but whose antibody has perhaps fallen to unprotective titres. Exactly what these titres have to be is one of the points on which firm evidence is scanty. Meiklejohn, Kempe, Thalman & Lennette (1952) were able to make some estimate in a study where antibody titres in military personnel were related to attack rates in a subsequent epidemic due to Influenza A 1 virus. The attack rate when the haemagglutination-inhibition antibody titre was $< 1/8$ was estimated at 18·3 %, at 1/8 the rate fell to 7·6 % and at 1/16 to only 1·5 %. In the epidemic of 1967–8 the lack of antibody to the Tokyo/67 variant may have allowed this virus to infect many who had antibody to the early A 2 strains and this could well have contributed to the somewhat unexpected size of the epidemic.

The position with the Hong Kong/68 variant is similar in that antibody is absent in a large proportion of the population. However, if as has been suggested by Meiklejohn, low antibody titres do afford protection, the presence of antibody found among the younger age groups in this country could well have a modifying effect on an epidemic, particularly since these groups are suspected of playing a significant part in the dissemination of infection.

SUMMARY

The antigenic variation of influenza A 2 virus and the antibody present in the population to different variants are described and related to the occurrence of epidemics of influenza in England since 1957.

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