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Interpretations of Influenza Antibody Patterns of Man*

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The age distribution of antibodies to A2/Hong Kong/68 influenza virus was ascertained in sera collected before the pandemic of 1968 in order to determine whether Hong Kong-like viruses had previously prevailed in man, and, if so, at what period. The findings indicate that Hong Kong-like viruses were probably involved in outbreaks at or about the turn of the century. The data are interpreted to indicate that antigenic drifting among Asian influenza strains from the A2/Japan/305/57-like variant, believed to be responsible for the pandemic of 1889–90, to the Hong Kong-like variant of 1900, is a phenomenon that has been repeated in recent times. The findings constitute another example of antigenic recycling after a long period of absence, and support the view that the number of antigens of influenza A is finite.

It is now recognized that the major antigens of the strains of first infection of childhood permanently orient the antibody-forming mechanisms so that, on subsequent exposure to related variants of influenza virus, that cohort of the population will respond with marked reinforcement of the primary antibody. Owing to antigenic shifting during the course of time, the primary determinant of the characteristic antibody response of different cohorts is oriented to different families of strains. In consequence, a serological recapitulation of a population's past experiences with influenza viruses may be reconstructed by ascertaining the current age-distribution of specific antibodies reacting with prototype strains (Davenport, Hennessy & Francis, 1953).

Antibody patterns found with swine, A2 and Equi-2 viruses have been used to identify the most probable antigenic composition of the viruses involved in the pandemics of 1918–19 and 1889–90 and in the epidemics which occurred during the decade or more before 1889 (Davenport, Hennessy & Francis, 1953; Davenport et al., 1964; Davenport, Hennessy & Minuse, 1967). The emergence of the Hong Kong variant of 1968 and its striking pandemic behaviour prompted studies designed to disclose whether A2/Hong Kong-like viruses had likewise prevailed during an earlier time period.

Large sets of sera collected in 1958 and 1966 were examined for the presence of haemagglutination-inhibiting (HI) antibody reacting with the A2/Aichi/2/68 strain. Selected samples were tested photometrically for the presence of specifically reacting antibody. The antibody response to monovalent A2/Aichi/2/68 and A/Equi-2/Milford/2/63 vaccines was ascertained in subjects born in the intervals 1928–37, 1888–1901 and 1879–88. The findings are interpreted to indicate that A2/Hong Kong-like viruses have previously prevailed in the human population, most probably in or about 1900.

MATERIALS AND METHODS

Viruses

The strains utilized were selected from the collection of the Virus Laboratory, Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, and are described according to the currently accepted nomenclature.

Vaccines

The vaccines employed were monovalent influenza virus vaccines prepared by two commercial pharma-

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ceutical laboratories. The A/Equi-2/Milford/2/63 vaccine contained 200 chick-cell agglutinating (CCA) units per ml, while the A2/Aichi/2/68 vaccine contained 800 CCA units per ml. The dose of Equi-2 vaccine given was 1 ml subcutaneously. That of Aichi vaccine was 0.5 ml injected by the same route.

Sera

Specimens used to delineate the age distribution of antibodies reacting with A/Equi-2/Milford/2/63, A2/AA/23/57, and A2/Aichi/2/68 were obtained from persons hospitalized in Michigan during inter-epidemic periods in 1957, 1958, 1964, and 1966. The 1964 collection was composed of patients receiving custodial care at 2 hospitals where yearly vaccination against influenza was given. The other collections were random ones from the wards of the University Hospital, Ann Arbor.

Specimens utilized for study of the antibody response to influenza virus vaccines were obtained from persons hospitalized in the spring of 1965 and in the fall of 1968. The A/Equi-2/Milford/2/63 vaccine was given in 1965 to 25 persons with birth years 1928–37 (A0 cohort), 25 persons with birth years 1888–1902 (Hong Kong cohort) and to 24 persons with birth years 1879–88 (A2 pandemic cohort). The A2/Aichi/2/68 vaccine was given in 1968 to 30 persons of the A0 cohort, 29 persons of the Hong Kong cohort and 19 persons of the A2 pandemic cohort. Influenza was not present in Michigan at these times.

Treatment of sera

Sera were inactivated either by heating at 56°C for 30 minutes, by periodate treatment (Communicable Disease Center, 1958) or by overnight treatment at 36°C with 4 volumes of receptor-destroying enzyme (RDE) (Hilleman & Werner, 1953).

Haemagglutination-inhibition (HI) titrations

Determinations were carried out by a standard pattern method with 4 units of virus and 0.5% erythrocytes (Committee on Standard Serologic Procedures . . . , 1950). Photometric titration, the procedure developed for measuring specifically reacting antibodies, was performed exactly as described in the original publication (Drescher, Davenport & Hennessy, 1962). The isotherms used were generously provided by Dr J. Drescher, Institut für Mikrobiologie der Medizinischen Hochschule, Hanover–Kleefeld, Germany.

RESULTS

Age distribution of Hong Kong HI antibody in human sera collected in 1958 and 1966

The frequency of antibody titres of 1 : 20 or greater in these sets of sera and the geometric mean values found in each age-class with the A2/Aichi/2/68 strain are shown in Fig. 1, on which are also indicated the number of specimens examined and the birth dates of the subjects.

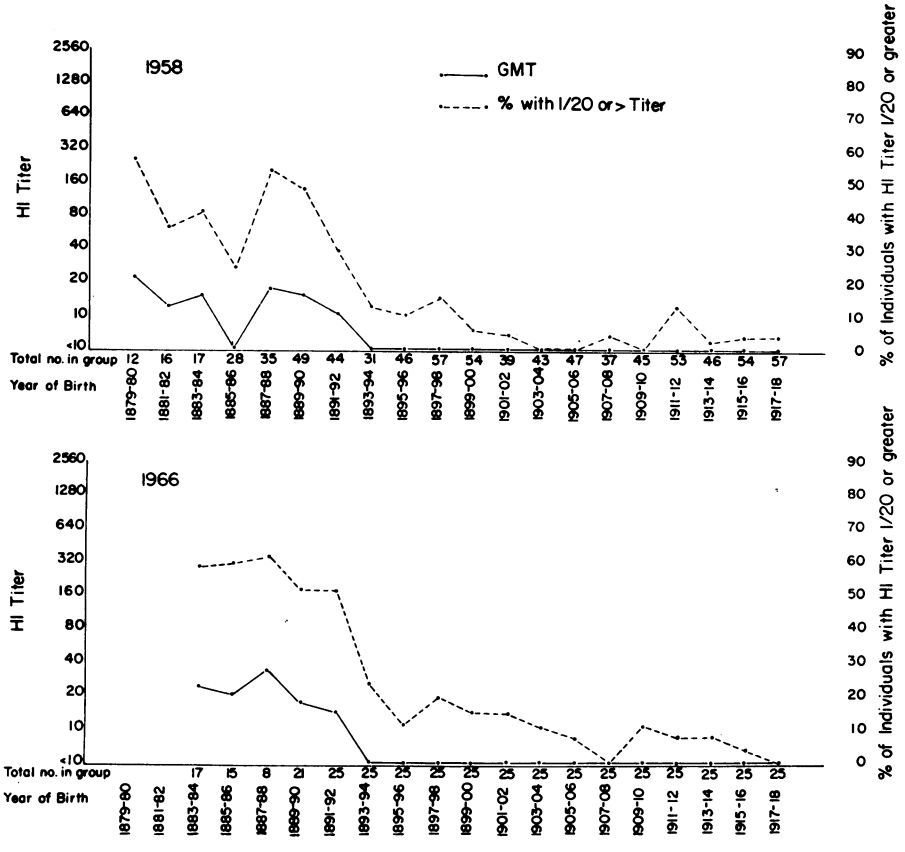
The findings are quite consonant. The major serological involvement with Hong Kong-like antigens is clearly characteristic of persons born in 1893–94 or earlier. Subsequent flurries of antigenic exposure to Aichi-like antigens may have occurred among persons born in 1897–98 and in subjects born between 1909 and 1912. Taken together, the findings imply that Hong Kong-like viruses were involved in at least one epidemic of an earlier epoch and that some of the viruses prevalent thereafter retained and periodically re-emphasized some of the antigenic characteristics of what we now know as the Hong Kong variant. The waxing and waning of antigenic determinants within a family of strains of influenza A is a well recognized phenomenon (Jensen & Peterson, 1957).

With these data an estimate of the time when major exposure to Hong Kong-like antigens ceased to occur can be made. Thus if one assumes, as now, that in former times the attack rate in preschool children is relatively low and therefore adds 6 years to the birth dates of persons first beginning to exhibit the sustained higher Hong Kong antibody frequencies (in 1893–94), arithmetic identifies 1899–1900 as the most likely years that mark the end of the predominant Hong Kong-like exposure. The period 1899–1900 possibly identifies as well the sole or principal experience of this segment of the population with Hong Kong-like virus since in general the antigenic characteristics of each epidemic strain of influenza A are readily distinguishable from those that precede or follow. This estimate is consistent with the record of epidemiological events. Collins (1957) identifies 1899 and 1900 as years in which the excess pneumonia–influenza death rates were the highest observed between 1892 and 1918. For simplicity, the timing of the earlier Hong Kong-like exposure will be referred to as 1900.

Fig. 1 also illustrates one of the most important criteria used to validate the significance of an antibody pattern. Conceivably such an antibody pattern found in a single collection of sera might result from

FIG. 1

DISTRIBUTION OF HONG KONG HI ANTIBODIES IN HUMAN SERA COLLECTED AT 2 DIFFERENT DATES



the accumulation of experience with minor Hong Kong-like antigenic components present in other influenza A strains. If this were the case it would be expected that the age of onset of high frequencies of Aichi antibodies found in the 1966 collection would occur in persons born later than 1893–94 since the recurrence of Asian influenza epidemics after 1958 would have enriched the antigenic exposure of the subjects bled in 1966, and consequently broadened the antibody pattern. The fact that the age of onset of Aichi antibodies remains constant in the two collections refutes the thesis that the patterns observed result from accumulated antigenic experience with minor antigens of other influenza A strains, and lends strong support to the interpretation that these Hong Kong antibody patterns are the result of prior exposure to the major antigens of a Hong Kong-like virus. In addition they provide an accurate running fix on the timing of the period of past prevalence of Hong Kong-like viruses.

Age distribution of Hong Kong antibodies determined photometrically

The photometric test used has been found to provide a sensitive and objective method for distinguishing between homologous and heterologous antigen–antibody reactions in human sera (Davenport et al., 1964; Davenport, Hennessy & Minuse, 1968). To evaluate the strain specificity of the HI antibodies found in human sera and to gain additional information about the timing of the earlier Hong Kong-type epidemic, the test was applied to specimens grouped to represent the cohort born before the 1889–90 pandemic, the cohort born during the next decade, and that born in the early 1900s before the pandemic of 1918–19. The findings are

DISTRIBUTION OF SPECIFIC ANTIBODY TO A2/Aichi/2/68 IN 3 AGE-GROUPS

Birth dates	Positive	ACU [a] titre range	Average ACU [a] titre
1878–1888	31% (8/26)	15–162	22
1889–1900	52% (12/23)	8–27	8
1901–1914	3% (3/100)	28–110	<1

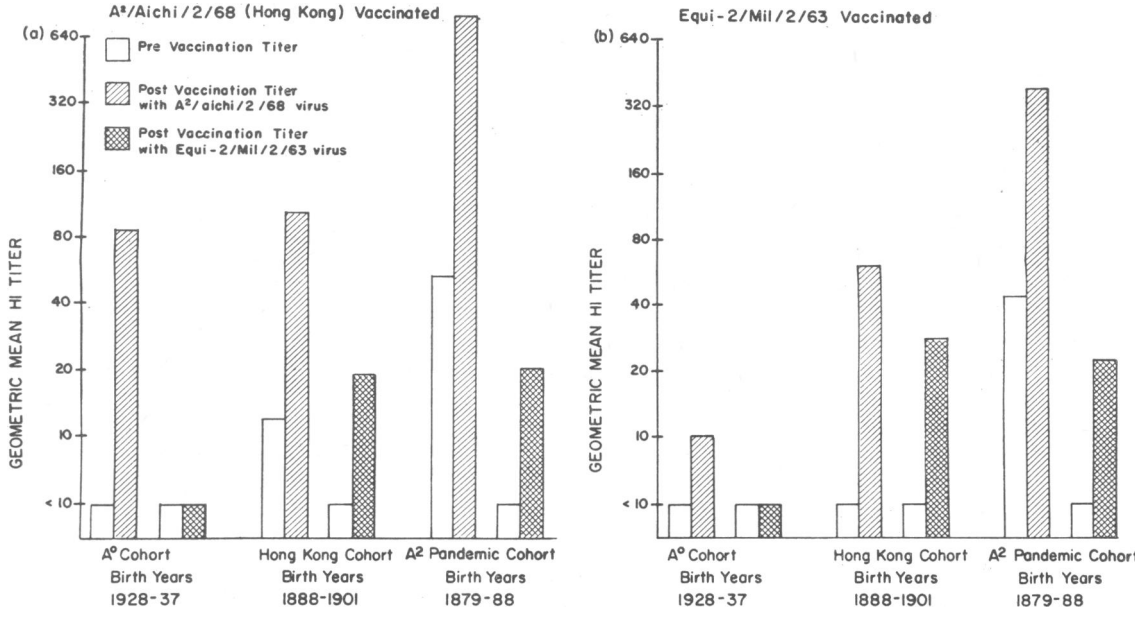
[a] Antibody concentration units (Drescher, Davenport & Hennessy (1962)).

shown in the accompanying table. The highest percentage of specimens exhibiting antibody specifically reacting with the Aichi strain was found in the 1889–1900 cohort. The next highest was found in the 1878–88 cohort and the lowest in specimens collected from persons born in the early 1900s. In all age-classes, titre levels tended to be low, a finding consistent with HI pattern test results. Seven of the 12 specimens found positive in the 1889–1900 age-group were obtained from persons born in 1892 or 1893. While the number of specimens examined photometrically was the smaller, the concordance of birth dates found in the photometric and HI antibody surveys is supportive. The findings confirm the unique antigenic experience of persons born in the early 1890s. They were further substantiated by the demonstration that the antibodies of this age-group neutralized Hong Kong virus *in ovo*.

Antibody response of 3 age-groups to monovalent influenza virus vaccines

The results of earlier studies from this laboratory have shown that information concerning the major antigens of the strains of childhood and subsequent infections can be derived by ascertaining the HI antibody response of persons of different ages to selected monovalent vaccines. In the present study, 3 cohorts were examined for their response to A2/Achi/2/68 and A/Equi-2/Milford/2/63 vaccines. The latter strain was selected because of the demonstration that hyperimmunization of ferrets with Hong Kong virus led to the development of low levels of Equi-2 antibodies. The reverse cross-relationship was seldom found. In addition, it is known that the sera of persons born before 1889 exhibit a uniquely high frequency of Equi-2 antibodies. The cohorts examined were the A0 cohort (birth years 1928–37), the Hong Kong cohort (birth years 1888–1902) and the A2 pandemic cohort (birth years 1879–88). Fig. 2 illustrates the geometric

FIG. 2
GEOMETRIC MEAN HI ANTIBODY RESPONSE OF VACCINATED PERSONS TO A2/Aichi/2/68 AND A/Equi-2/Milford/2/63 ANTIGENS



mean HI antibody titres found before and 2 weeks after administration of each vaccine to each cohort when tested with the homologous and heterologous strains.

Vaccination with 400 CCA units of Aichi vaccine induced a modest Hong Kong antibody rise in the A0 cohort, slightly better post-vaccination levels in the Hong Kong cohort and markedly higher levels in the cohort born before the pandemic of 1889–90. Only the last 2 cohorts exhibited heterologous Equi-2 antibody increase after receiving Aichi vaccine.

When other subjects of the same birth years were given 200 CCA units of Equi-2 vaccine, the A0 cohort failed to develop a detectable mean Equi-2 titre, while the 2 older cohorts responded with Equi-2 antibody levels which were only slightly higher than those found after vaccination with Hong Kong vaccine. In each case the heterologous Aichi antibody response of the cohorts was more striking than the homologous response and it is noteworthy that the post-vaccination mean titres rose progressively in each cohort.

These data obtained with human sera clearly demonstrate the 2-way antigenic cross-relationships of Hong Kong and Equi-2 viruses and support conclusions derived from studies carried out with animal sera (Coleman et al., 1968). In addition, the greater Aichi antibody response of the A2 pandemic cohort after either vaccine is consistent with the interpretation proffered in this and in an earlier study to the effect that the former period of prevalence of the Asian family of strains extended from 1889–90 until about 1902 (Davenport et al., 1964). It would be expected that the A2 pandemic cohort exposed for over a decade to various Asian strains would respond more vigorously than the Hong Kong cohort whose Asian antigenic experience came at the end of the Asian influenza epoch.

Comparison of antibody patterns obtained with Asian influenza strains isolated in 1957 and 1968 in sera collected in 1957 and 1966

On the basis of results of less extensive studies, Marine & Workman (1969) concluded that the virus involved in the 1889–90 pandemic was a Hong Kong-like variant. Masurel (1969) compared antibody patterns found with A2/Hong Kong/1/68 and A2/Japan/305/57 strains in a large set of sera collected in the Netherlands in 1956–57. Because the age of onset of the higher frequencies and level of A2/Japan/305/57 antibodies occurred in persons born much earlier than subjects who first began to exhibit the same phenomenon with the A2/Hong Kong strain, Masurel (1969) concluded that the most probable candidate for identification with the 1889–90 pandemic was an A2/1957-like strain, and that a Hong Kong-like variant became prevalent about 12 years later, i.e., 1902.

To obtain further information, a comparison was made of antibody patterns found with the A2/Aichi/2/68 and the A2/AA/23/57 strains in sets of sera collected in Michigan in 1957 and 1966. The findings are illustrated in Fig. 3. The results shown for the 1957 collection consist of a rearrangement of data previously published since exhaustion of the serum collection did not permit extensive retesting (Davenport & Hennessy, 1958). Nevertheless, the comparison seems reasonable since the methods used for detecting HI antibodies have not been changed in this laboratory during this interval and reproducible antibody patterns have been obtained with other sets of sera on repeated testing. Clearly the age of onset of the sustained higher frequencies of A2/AA/23/57 antibodies occurs in subjects with earlier birth dates (1886–87) than in subjects exhibiting the sustained higher frequencies of Hong Kong antibodies (1893–94). The results of this comparison are compatible with the findings of Masurel (1969) and strengthen the conclusion that the Hong Kong variant emerged from 1889–90 pandemic precursors at a later date, i.e., about 1900.

Comparison of antibody patterns found with Hong Kong and Equi-2 viruses in sera collected in 1964

Because of the demonstration of antigenic relationships between Hong Kong and Equi-2 viruses and because we had previously described an antibody pattern found in human sera with the A/Equi-2/Milford/2/63 strain, it was of interest to repeat the study carried out with specimens collected in 1964 and to include the A2/Aichi/2/68 antigen. The findings in this comparison are shown in Fig. 4.

The age of onset of sustained high frequencies of A2/Aichi/2/68 antibody corresponds to birth dates 1894–95, an observation in close agreement with the data obtained with the 1966 collection of sera. The corresponding date of onset for Equi-2 antibodies is 1890–91. The agreement between these findings and those of Masurel (1969) and Marine & Workman (1969) on the age distribution of Equi-2 and Hong Kong antibodies is striking. They suggested to Marine & Workman (1969) the interpretation that the Equi-2 antibodies found in sera collected in interepidemic periods represent the activity of hete-

FIG. 3

COMPARISON OF ANTIBODY PATTERNS OF HONG KONG AND A2 PANDEMIC COHORTS BY YEAR OF BIRTH

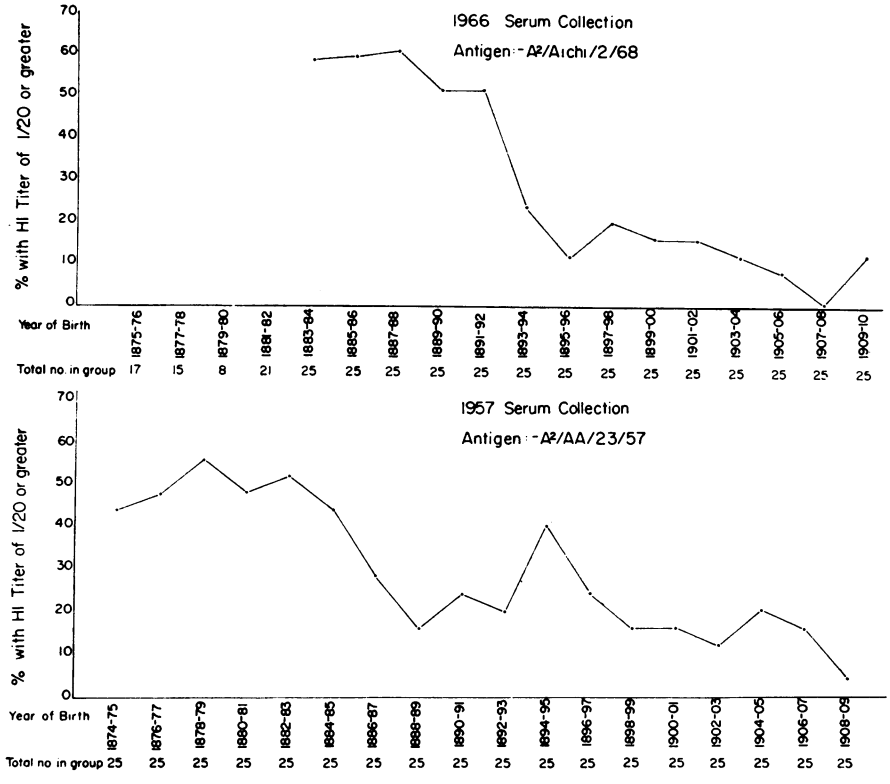
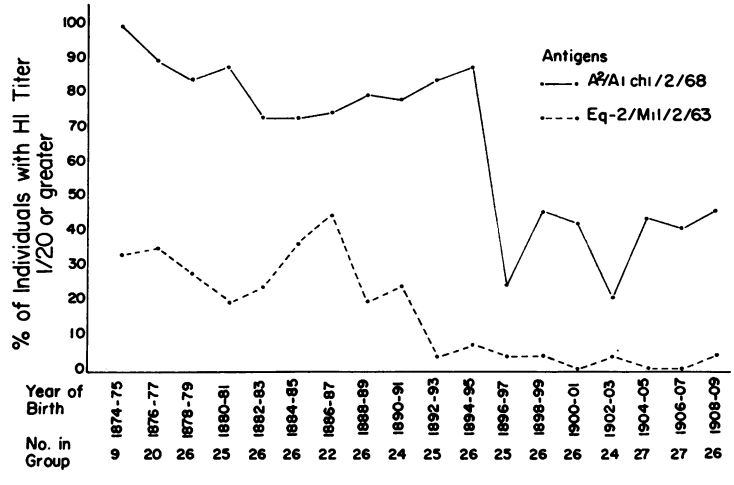


FIG. 4

DISTRIBUTION OF HI ANTIBODIES TO HONG KONG AND EQUI-2 VIRUSES IN HUMAN SERA COLLECTED IN 1964



rologous Hong Kong antibodies, not homologous Equi-2 antibodies. Marine & Workman's (1969) hypothesis was tested by use of the photometric technique of Drescher, Davenport and Hennessy (1962). It will be recalled that the Hong Kong cohort and the A2 pandemic cohort responded uniformly to vaccination with A2/Aichi/2/68 vaccine by boosting Equi-2 antibody levels and that when vaccine A/Equi-2/Milford/2/63 was given, Hong Kong antibody levels were reinforced in all subjects (Fig. 2). Photometric tests of these heterologous antibody increases carried out with individual specimens revealed that while approximately 40% of the Hong Kong antibody increases observed after administration of Equi-2 vaccine reacted specifically with the A2/Aichi/2/68 strain, only 1 of 45 specimens obtained from the groups given Hong Kong vaccine reacted specifically with the A/Equi-2/Milford/2/63 strain. Clearly, as evidenced by the results of this experiment, a high proportion of these subjects had had prior antigenic experience with specific Hong Kong antigens while most did not react as though they had had prior specific Equi-2 exposure. The findings tend to support the hypothesis of Marine et al. (1969). However, this information should not be interpreted to exclude the probability that some persons in these age-groups did in fact experience infection with Equi-2-like viruses in the remote past. It has been shown in other studies of the 1964 collection of sera that 25% or more of samples randomly selected from subjects born in 1903 or earlier contained specific Equi-2 antibody when tested photometrically and that the highest involvement was in the group whose birth dates occurred in the decade before the pandemic of 1889–90 (Davenport, Hennessy & Minuse, 1967). These data are incompatible with the explanation that all of the Equi-2 antibodies detected in human sera by HI are heterologous antibodies. The failure to detect specific Equi-2 antibody increases at a higher frequency in older subjects given A2/Aichi/2/68 vaccine may merely be an indication that the Equi-2 component of the Hong Kong strains is quite dissimilar from that of the virus responsible for the earlier specific Equi 2 exposures of older segments of our population. Masurel & Mulder's (1966) timing of the Equi-2 epoch in man is different from ours. It fails to take into account the age distribution of specific Equi-2 antibodies (Masurel & Mulder, 1966). The questions raised by the different opinions indicate that further studies are needed to clarify the role of Equi-2-like virus in human infections.

DISCUSSION

Basically the findings in the present study and those of Masurel (1969), Fukumi (1969), Marine & Workman (1969), Marine et al. (1969) and Zakstelskaja et al. (1969) are in remarkable agreement. While some opinions may differ concerning the exact timing of the prior event, there is little reason to doubt that in 1968 a revisitation of Hong Kong antigens was experienced. Moreover, the bulk of the evidence is compatible with the interpretation that antigenic shifting of the nature of that seen with the A2/Japan/305/57 and the A2/Aichi/2/68 strains has occurred once before. The data provide another example of the recycling of antigens of strains of former prevalence and support the hypothesis that the possibilities for major rearrangements of the antigenic components of influenza viruses is finite, not infinite. The observations further encourage pursuit to capture all of the essential immunizing antigens needed for compounding an effective inactive vaccine of stable composition (Francis & Maassab, 1965).

The mechanism of antigenic recycling remains obscure. Mulder & Masurel (1958) were attracted by the possibility that the 1957 pandemic might have resulted from the escape of Asian strains from swine in southern China. By analogy, the emergence of the Hong Kong variant could be similarly explained, with the contemporary additive that the animal reservoir may reside in either swine, birds, horses or in an as yet unrecognized subhuman host.

Yet these possibilities are challenged by observations that natural transmission of influenza to mankind has not been observed despite intense and extensive exposures of human subjects to infected birds, horses, or pigs. In contrast, as Shope (1936) suggested, subhuman species may function principally as repositories of strains of former human prevalences. If that is the case, horses may have become involved with Equi-2 viruses in the pre-1889–90 pandemic period when Equi-2-like viruses are thought to have prevailed in man (Davenport, Hennessy & Minuse, 1967). The record of epizootics of influenza in equines in the 1870s is compatible with this speculation. Having established a side-chain of infection in horses, the virus of that period is thought to have evolved in man into the Asian family of strains without losing the potential for expression of the Equi-2 component. The appearance of Hong Kong strains would seem to validate that serological recapitulation (Davenport, Hennessy & Minuse, 1967).

Recombination among strains of human, avian or animal sources is at first glance an attractive explanation for recycling especially since recombinants have been achieved with selected parents in several laboratories.

However, recombination in nature would be expected to be at best a rare event, and to experience the same recombinant in 1900 and in 1968 seems mathematically to be highly improbable. Rather, the similarities found in antigens of strains isolated from different species, or at different times, may well result from a limitation imposed upon the almost infinite number of structures which a protein might assume, as changes in polypeptide sequence occur in response to mutations of the determining ribonucleic acid. The number of protein structures and, hence, antigenic possibilities is restricted by the ability of envelope proteins to undergo assembly with neuraminidases and yet function effectively in a limiting viral membrane. That is to say, the folding of both proteins is presumably determined by bonding at only a few possible sites on the polypeptide chain, and the final association of both as surface elements is a basic limitation to the unrestrained variation of either. Hence, the appearance of the same or similar proteins on different isolates does not necessarily imply recent genetic interaction between related pairs but, since the possibilities are limited, may reflect the repetition of a successful adaptive survival mechanism. The same considerations would also apply to antigenic drifting or rearrangement among strains of one species.

Whatever the source or sources of pandemic strains, the antibody spectrum of the human population, which is remarkably similar throughout the world, acts as a limiting influence on the spread of strains antigenically like their recent predecessors. Large and ordered gaps in the age distribution of antibodies oriented to strains of remote periods of past prevalence favour the spread of " old antigenic acquaintances " once they arise. Hence, given the appropriate variant by genetic limitations, recycling is encouraged.

Questions concerning the frequency, regularity and sequence of recycling are immediately provoked by demonstration of the phenomenon. They cannot be answered without further experience. Nevertheless, the fact that at least 2 examples have been observed in a little more than a decade, the inherent scientific interest generated by the phenomenon, and the potential for practical applications, will stimulate the quest for further data.

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