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Antigenic variation in current human type A influenzaviruses: Antigenic characteristics of the variants and their geographic distribution

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Outbreaks of influenza due to the virus A/Hong Kong/1/68 (H3N2) began in 1968 and are still occurring. The haemagglutinin of this virus is different from that of the A/Singapore/1/57 virus (the "Asian" strain) but the neuraminidase antigens are the same. Between 1968 and 1971 only minor antigenic "drift" in the haemagglutinin was noted, but in recent months 2 isolates have been identified in which considerable "drift" has occurred in the haemagglutinin and in the neuraminidase antigens. One, A/Hong Kong/5/72 (H3N2), was first detected in outbreaks in Hong Kong between November 1971 and January 1972 and was predominant there and in Korea but did not become widely disseminated. The second strain, A/England/42/72 (H3N2), has been isolated in winter outbreaks in the southern hemisphere and now appears to be the predominant strain in the northern hemisphere. The characteristics of the strains are described.

INTRODUCTION

Following July 1968, recurrent outbreaks of influenza were associated with a new virus subtype, A/Hong Kong/1/68 (H3N2). The Hong Kong/68 virus contained a haemagglutinin antigen (H3) that was distinct from that of the former Asian virus (H2), although the neuraminidase antigens of both viruses were of a common subtype (N2). Up to 1971 only minor antigenic changes in the haemagglutinin antigen of a few isolates, resembling A/England/878/69 (H3N2), of Hong Kong virus were reported (Pereira & Schild, 1971) but such variants appear to have had no epidemiological significance. However, following November 1971, an increasing proportion of isolates showed a greater degree of antigenic "drift" from the 1968 prototype of Hong Kong virus. The antigenic characterization and geographic distribution of these variants is described in this paper. One of the variants described, A/England/42/72, predominated during recent influenza epidemics in Australia, the Far East, and South-East Asia and subsequently spread to Europe and the USA.

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MATERIALS AND METHODS

Haemagglutination-inhibition (HI) tests

These were performed by standard methods (WHO Expert Committee on Influenza, 1953) with postinfection ferret antisera or immune chicken or rabbit antisera to purified haemagglutinin antigens.

Neuraminidase-inhibition (NI) tests

These tests were carried out by a more sensitive modification (Slepushkin et al., 1971) of the method described by Webster & Laver (1967). Immune rabbit antisera to purified neuraminidase antigens were used for antigenic characterization of the neuraminidase antigens of influenzavirus isolates.

Antibody absorption studies using single radial diffusion for antibody absorption

Antibodies to influenza A/Hong Kong/1/68 haemagglutinin (H3) and neuraminidase (N2) were assayed in single-radial-diffusion tests with suspensions of intact purified influenzaviruses (4×10^4 haemagglutinating units (HAU) per ml) in agarose gels (Schild et al., 1972). In this simple 2-component test system antibody potency was expressed as the annulus area (mm^2) of the opalescent zones produced by volumes of 5 μl of test antisera (mean of 5 assays). Statistical studies have indicated that the single-radial-diffusion method affords a high level of reproducibility and accuracy in such antibody assays and enables relative differences of antibody potency as small as 4% to be detected with significance. In contrast, in conventional assays of influenza antibodies based on multicomponent titration systems (HI or complement fixation), only relative differences of potency of 200–400% are significant (Schild & Berryman, unpublished data).

Undiluted immune antisera to purified A/Hong Kong/1/68 haemagglutinin (H3) and neuraminidase (N2) were used. Absorption was carried out by incubating mixtures of antiserum (20 μl) and inactivated purified virus (20 μl) for 3 hours at room temperature. In controls saline was used instead of virus. The residual unbound antibody in the mixtures was determined by the addition of 5-μl volumes of each mixture to wells in single-radial-diffusion immunoplates (5 replicate tests for each mixture) followed by comparison of the areas of opalescent zones produced with those given by control (unabsorbed) sera.

As a preliminary to absorption studies the minimal quantities of homologous A/Hong Kong/1/68 virus required to remove all antibody from the antisera to haemagglutinin and neuraminidase were determined by adding graded concentrations of virus (20 μl) ranging from 10^6 HAU/ml to 10^4 HAU/ml to 20-μl volumes of each antiserum. Throughout the absorption studies the concentrations of homologous virus used were calculated, on the basis of haemagglutinin content, to contain 1.2 times the minimal absorbing dose of homologous virus for the antiserum under test. In comparative studies, the variant strains (A/England/42/72 and A/Hong Kong/5/72) were used at the same haemagglutinin concentration as the homologous virus. The amount of antibody absorbed by the variant strains was calculated as the percentage reduction in the area of opalescence in comparison with control, unabsorbed sera.

Immuno-double-diffusion tests

These tests were performed in agar gels as described by Schild (1972). Purified and concentrated (10^6 HAU/ml) influenzaviruses were used as antigen and a sodium monododecyl sulfate detergent (1% final concentration) was added to the virus to disrupt the particles and permit migration of structural components through the agar.

Preparation of monospecific antisera

Potent antisera to purified haemagglutinin (H3) and neuraminidase (N2) antigens of A/Hong Kong/1/68 and its variants were prepared in chickens and rabbits using Freund's complete adjuvant. For the haemagglutinins, the pure crystalline antigen was prepared by the treatment of purified viruses with the enzyme bromelain (3.4.4.24) (Brand & Skehel, 1972). Antisera to neuraminidase antigens were obtained from recombinants (McCahon & Schild, 1972) containing haemagglutinin derived from A/PR/8/34 H0N1) virus and neuraminidase derived from A/Hong Kong/1/68 virus or its variants. Purified neuraminidase antigens were separated from disrupted virus by electrophoresis on cellulose acetate strips (Laver, 1964).

RESULTS

Antigenic character of variants

Analysis of a large number of type A influenzaviruses isolated from man in many regions of the world during the period from May 1971 to September 1972 revealed a number of strains that showed antigenic variation away from A/Hong Kong/1/68 (H3N2). Antigenic variation was detected in both haemagglutinin and neuraminidase antigens of these viruses. The strains examined could be placed into one of 3 different categories on the basis of the reactions of their haemagglutinin (H) antigens in haemagglutination-inhibition tests with postinfection ferret or chicken antisera and with hyperimmune antisera to isolated haemagglutinin antigens:

(*a*) strains containing H antigens resembling that of prototype A/Hong Kong/1/68 (H3N2) virus; strains of this type predominated from 1968, when the Hong Kong virus first appeared, up to 1972;

(*b*) strains containing H antigens resembling that of A/Hong Kong/5/72;

(*c*) strains containing H antigens resembling that of A/England/42/72.

Table 1. Comparison of A/Hong Kong/1/68 (H3N2) virus and variant strains in haemagglutination inhibition tests

Virus strains	HI titre				
	Postinfection ferret antisera to			Monospecific antisera to purified haemagglutinin (H3)	
	A/Hong Kong/1/68 (H3N2)	A/Hong Kong/5/72	A/England/42/72	A/Hong Kong/1/68 [a]	A/Hong Kong/5/72
A/Hong Kong/1/68 (H3N2)	3 200	3 200	3 200	51 200	400
A/Hong Kong/5/72 [b]	200	25 600	400	3 200	25 600
A/England/42/72	200	400	12 800	6 400	1 200
A/Hong Kong/5/72(H3)-A/PR/8/34(N1) [c]	100	12 800	400	3 200	12 800
A/England/42/72(H3)-A/PR/8/34(N1) [c]	200	200	6 400	3 200	1 600

[a] WHO reference antiserum from the WHO International Influenza Centre for the Americas, Center for Disease Control, Atlanta, Ga., USA.

[b] A/Hong Kong/5/72 is antigenically closely related to A/Hong Kong/107/71.

[c] Recombinant strains (antigenic hybrids) containing haemagglutinin (H3) derived from recent variants and neuraminidase (N1) derived from A/PR/8/34 (H0N1) virus.

Strains giving HI titres that were at least 4 times lower than the homologous titre were regarded as showing significant variation. The results of these tests were in general unequivocal. The results of HI tests to compare the H antigens of representatives of the 3 groups are shown in Table 1. In general, variants characterized as resembling A/Hong Kong/5/72 or A/England/42/72 gave HI titres with antisera to A/Hong Kong/1/68 virus that were 8–16 times lower than those given by the homologous virus. The tests with monospecific antisera to haemagglutinin clearly indicated that the observed antigenic variation involved the haemagglutinin component. Confirmation of this was provided by the HI reactions of recombinant type A influenzaviruses (antigenic hybrids) containing only the haemagglutinin or neuraminidase antigens derived from the variants of Hong Kong virus, the other envelope antigen being derived from A/PR/8/34 (H0N1).

Immunological comparisons of the haemagglutinins of A/Hong Kong/1/68, A/Hong Kong/5/72, and A/England/42/72 were also performed using immuno-double-diffusion tests with monospecific antisera to their haemagglutinins. Antiserum to A/Hong Kong/1/68 haemagglutinin (H3) gave a single, well-defined precipitin line with the homologous virus. The variants A/Hong Kong/5/72 and A/England/42/72 also gave precipitin reactions with this antiserum; however, these showed only partial identity with the line given by the homologous virus. The definite " spur " formation (see Fig. 1A and 1B) indicated that the haemagglutinin of A/Hong Kong/1/68 contained immunological determinants not possessed by the variants. Similar conclusions were obtained in tests with antisera to the haemagglutinins of A/Hong Kong/5/72 and A/England/42/72. These tests thus provided confirmatory evidence of antigenic differences in the haemagglutinins of strains in the 3 categories. However, the existence of cross-reactions in the precipitin tests with antiserum to prototype A/Hong Kong/1/68 haemagglutinin suggests that the haemagglutinins of 2 variants should be regarded as belonging to the same antigenic subtype (H3) as that of prototype A/Hong Kong/1/68 (*Bull. Wld Hlth Org.*, 1971).

Table 2 shows a comparison of the antigenic character of the neuraminidase antigens as indicated by neuraminidase-inhibition tests. Antiserum to the isolated neuraminidase (N2) of A/Hong Kong/1/68 gave titres 50–100 times lower with the variants A/Hong Kong/5/72 and A/England/42/72 than with the homologous neuraminidase. Similarly, antiserum to the neuraminidase of A/Hong Kong/5/72 gave low titres with prototype A/Hong Kong/1/68 virus. For comparison, antiserum to the neuraminidase of an early Asian influenzavirus (A/Singapore/1/57) showed minimal cross-reactivity between the neuraminidases of the 1957 and 1972 isolates. It appears, therefore,

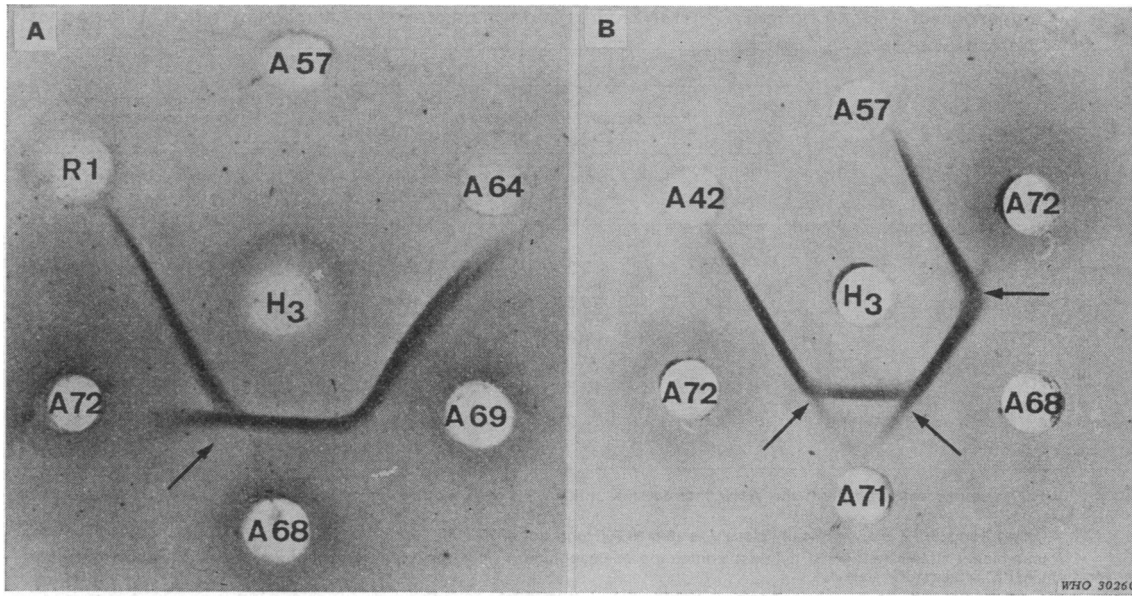

Fig. 1. Immuno-double-diffusion reactions of influenza antigens with immune rabbit antisera to purified haemagglutinin (H3) of A/Hong Kong/1/68 virus. Antisera reacted with A/Hong Kong/1/68 and its variants but not with strains containing H0, H1, or H2 haemagglutinin. Antigenic differences between the haemagglutinins of A/Hong Kong/1/68 and its variants are demonstrated by the appearance of "spurs" (marked by arrows) showing partial sharing of antigenic determinants. The specificity of the precipitin reaction is shown by the failure of R1 to give a reaction. A 42 = A/BEL/42 (N0N1); A 57 = A/Singapore/1/57 (H2N2); A 64 = A/England/12/64 (H2N2); A 67 = A/Tokyo/3/67 (H2N2); A 68 = A/Hong Kong/1/68 (H3N2); A 69 = A/England/878/69 (H3N2); A 71 = A/Hong Kong/107/71 (a strain antigenically identical to A/Hong Kong/5/72); A 72 = A/England/42/72; R1 is a recombinant virus = A/England/42/72 (H0)-A/PR/8/34 (N1).

Table 2. Comparison of neuraminidase antigens from type A influenzaviruses isolated between 1957 and 1972 in neuraminidase-inhibition tests

Source strain of neuraminidase	Rabbit antisera to neuraminidase (N2) of		
	A/Singapore/1/57	A/Hong Kong/1/68 [a]	A/Hong Kong/5/72
A/Singapore/1/57 (H2N2)	5 000 [b]	100	<10
A/England/12/64 (H2N2)	1 000	300	30
A/Tokyo/3/67 (H2N2)	75	1 000	150
A/Hong Kong/1/68 (H3N2)	50	5 000	750
A/Hong Kong/5/72 (H3N2)	20	50	10 000
A/England/42/72 (H3N2)	20	100	10 000
A/England/42/72 (H3)-A/PR/8/34 (N1) [c]	not tested	<10	20
A/PR/8/34 (H0)-A/England/42/72 (N2)	not tested	<10	5 000

[a] WHO reference serum from the WHO International Influenza Centre for the Americas, Center for Disease Control, Atlanta, Ga., USA.

[b] Serum dilution giving 50 % reduction in neuraminidase activity.

[c] Recombinants (reciprocal antigenic hybrids) of A/England/42/72 (H3N2) and A/PR/8/34 (H0N1).

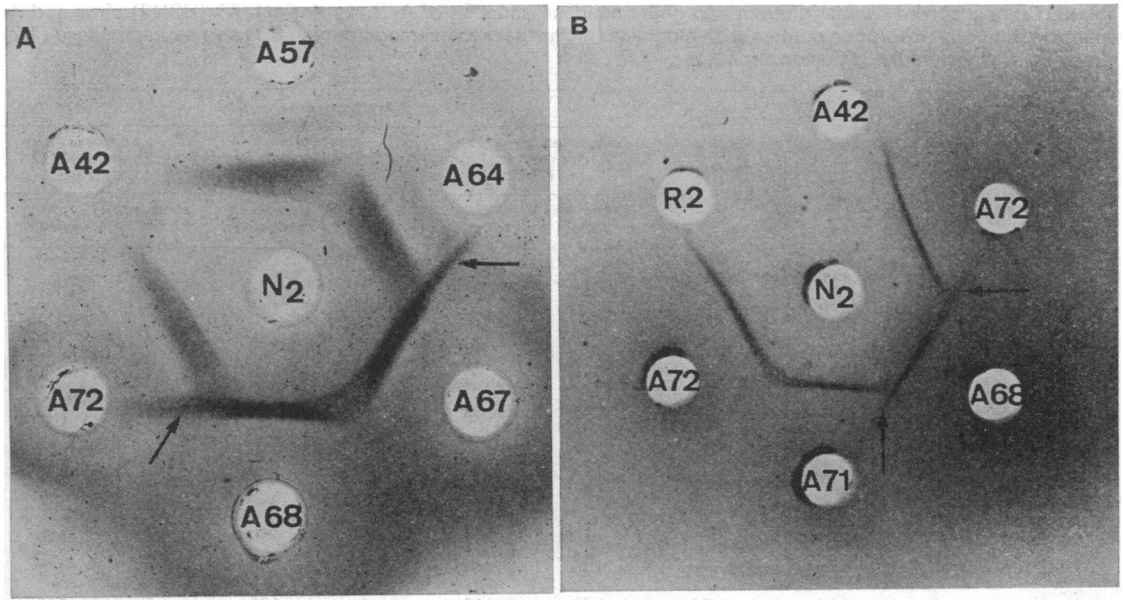

Fig. 2. Immuno-double-diffusion reactions of influenza antigens with immune rabbit antisera to neuraminidase (N2) of A/Hong Kong/1/68 virus, showing antigenic " drift " in the neuraminidase antigen. The neuraminidase of strains isolated from 1957–1967 and in 1971 and 1972 show antigenic differences from that of A/Hong Kong/1/68 demonstrated by " spur " formation (marked by arrows). The specificity of the reaction is indicated by the failure of viruses containing N1 neuraminidase (A 42 and R2) to give reactions. Strains A/Hong Kong/107/71 and A/England/42/72 gave continuous precipitin lines indicating the identity of their neuraminidase antigens. R2 is a recombinant virus = A/PR/8/34 (H0)-A/England/42/72 (N2). See also legend to Fig. 1.

that the neuraminidase antigen (N2) of type A influenzavirus underwent progressive antigenic drift from 1957 to 1972. Evidence of the specificity of the inhibition reactions for the neuraminidase antigen was provided by the use of antigenic hybrids (Table 2).

Despite the very low level of cross-reaction in neuraminidase-inhibition tests, immuno-double-diffusion reactions demonstrated that the neuraminidases of all the isolates examined from 1957 to 1972 belong to a common antigenic subtype (N2). Fig. 2A and 2B show the precipitin reactions of antiserum to the purified neuraminidase of A/Hong Kong/1/68 virus. It is seen that all the test viruses isolated between 1957 and 1972 showed cross-reactions as indicated by the presence of precipitin reactions. Such reactions confirm the existence of shared antigenic determinants among these strains. However, the reactions given by the viruses isolated before 1967 (A/Singapore/1/57 and A/England/12/64 and by the 1972 variant A/England/42/72 were less intense than those given by the homologous virus A/Hong Kong/1/68 and, in addition, showed only partial identity, with marked " spur " formation, indicating the existence of antigenic determinants in A/Hong Kong/1/68 that were not shared by the 1957, 1964, and 1972 isolates. It is of interest that the precipitin tests showed antigenic identity between the neuraminidases of A/Hong Kong/1/68 and A/Tokyo/3/67, confirming previous findings (Coleman et al., 1968). The precipitin studies, like the results of neuraminidase-inhibition tests, suggested that the antigenic character of the neuraminidases of A/Hong Kong/5/72 and A/England/42/72 were closely similar. Both strains gave precipitin lines showing complete continuity in tests with antiserum to the isolated neuraminidase of A/Hong Kong/1/68 or A/Hong Kong/5/72.

The degree of antigenic difference between the surface antigens of A/Hong Kong/1/68 and A/Hong Kong/5/72 has been further examined in antibody absorption studies employing the single-radial-diffusion technique (Schild et al., 1972). The studies were made possible because of the high degree of accuracy achieved in earlier antibody assays carried

Table 3. Comparison of haemagglutinin and neuraminidase antigens of A/Hong Kong/1/68 (H3N2) virus and its variants in antibody absorption studies with monospecific antisera to haemagglutinin (H3) and neuraminidase (N2) employing a single-radial-diffusion technique

Antiserum	Absorbing virus	Antibody potency [a]					
		test virus A/Hong Kong/1/68 (H3N2)		test virus A/Hong Kong/5/72 (H3N2)		test virus A/England/42/72 (H3N2)	
		zone area (mm^2)	% reduction	zone area (mm^2)	% reduction	zone area (mm^2)	% reduction
chicken antiserum to purified A/HongKong/1/68 haemagglutinin (H3)	control (unabsorbed)	21	—	16	—	17	—
	A/Hong Kong/1/68	0	100	0	100	0	100
	A/England/878/69	2	90	0	100	0	100
	A/Hong Kong/5/72	15	28	0	100	13	24
	A/England/42/72	14	33	12	25	0	100
	virus " N " [b]	21	0	16	0	17	0
rabbit antiserum to purified A/Hong Kong/1/68 neuraminidase (N2)	control (unabsorbed)	31	—	25	—	25	—
	A/Hong Kong/1/68	0	100	0	100	0	100
	A/England/878/69	0	100	0	100	0	100
	A/Hong Kong/5/72	25	19	0	100	0	100
	A/England/5/72	27	16	0	100	0	100
	virus " N " [b]	32	0	25	0	25	0

[a] Detected by single radial diffusion before and after absorption with various type A influenzaviruses and expressed as zone annulus area (mm^2) per 5 μl serum.

[b] Influenzavirus A/chicken/Germany " N "/49 (Hav2Neq1) containing envelope antigens unrelated to those of A/Hong Kong/1/68 (H3N2) was used as a control in the absorption studies.

out by means of this technique. Antiserum to the isolated haemagglutinin (H3) of A/Hong Kong/1/68 gave intense and well demarcated zones in single-radial-diffusion tests. This antiserum also reacted with the variants A/England/42/72 and A/Hong Kong/5/72 but gave zones against these strains that were smaller in area than those given by the homologous virus (75% and 80% of homologous area, see Table 3), although of similar intensity. Fig. 3 illustrates the results of studies to assay the dose of virus used in absorption studies. The results of cross-absorption studies on antisera to A/Hong Kong/1/68 haemagglutinin and neuraminidase are shown in Table 3. Absorption of the antiserum to A/Hong Kong/1/68 haemagglutinin by the homologous virus removed all antibody reacting with the homologous virus or its variants. However, absorption with equivalent concentrations of A/England/42/72 or A/Hong Kong/5/72 virus resulted in only a small reduction (28% and 33%, respectively) in area of zones in tests against A/Hong Kong/1/68 virus. Antigenic differences between the haemagglutinins of A/England/42/72 and A/Hong Kong/5/72 were indicated by the findings that absorption of the antiserum with one of these strains only removed a proportion of the antibody (25%) capable of reacting with the other. It is of interest that absorption of the serum with A/England/878/69 virus, which showed only a minor degree of antigenic difference from prototype A/Hong Kong/1/68 (Pereira & Schild, 1971), removed a high proportion (90%) of antibody to A/Hong Kong/1/68. An avian type A influenzavirus, A/chicken/Germany " N "/49, was used as a control since its haemagglutinin and neuraminidase antigens were of a subtype distinct from those of A/Hong Kong/1/68.

Antiserum to the purified neuraminidase (N2) of A/Hong Kong/1/68 virus gave larger but less intense zones than the antiserum to haemagglutinin. As with the antiserum to haemagglutinin, the zones detected in tests with the variants were smaller than those given with the homologous virus. Absorption of the

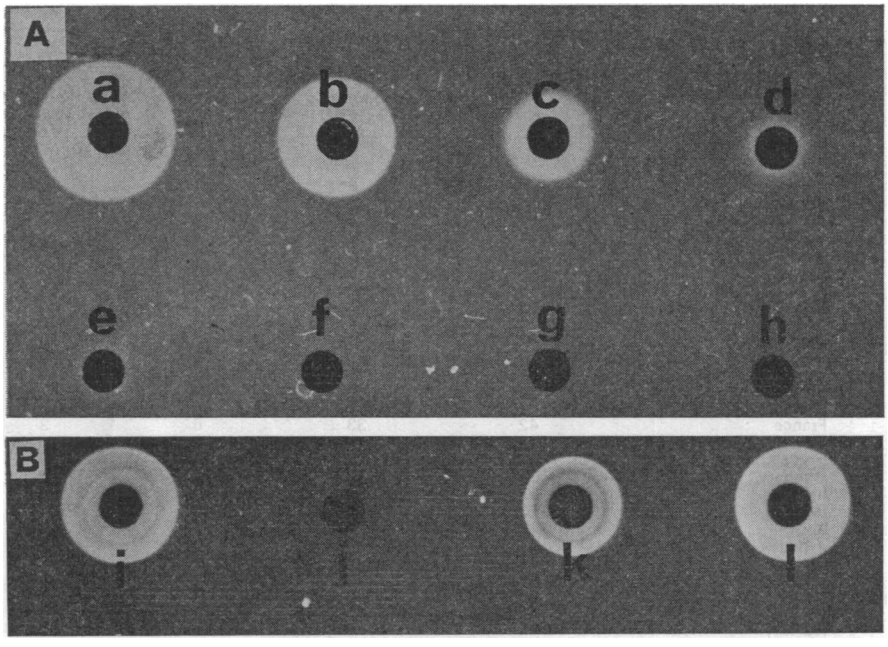

Fig. 3. Single-radial-diffusion reactions of antisera to H3 haemagglutinin in agarose gels containing A/Hong Kong/1/68 (H3N2) virus at a concentration of 2×10^4 HAU/ml. A. Antibody absorption activity of the purified A/Hong Kong/1/68 virus. The wells contained unabsorbed antiserum (a) and antiserum absorbed with (b) 1 : 128, (c) 1 : 64, (d) 1 : 32, (e) 1 : 16, (f) 1 : 8, (g) 1 : 4, and (h) 1 : 2 dilutions of a virus preparation containing 10^6 HAU/ml. The minimal dose required to absorb all the antibody was contained in a 1 : 8 dilution. B. Reactions of antisera before absorption (i) and after absorption with (j) A/Hong Kong/1/68 virus, (k) A/England/42/72 virus, and (l) virus " N ". Absorption with homologous A/Hong Kong/1/68 virus removed all antibody whereas absorption with A/England/42/72 resulted in a relatively small reduction in potency. Absorption with virus " N ", a strain with envelope antigens antigenically distinct from those of A/Hong Kong/1/68, did not reduce potency.

serum by the homologous virus or A/England/878/69 was complete. However, variants A/England/42/72 and A/Hong Kong/5/72 removed only a small amount of antibody (16% and 19%, respectively). Absorption with avian type A influenzavirus did not result in a reduction of antibody potency.

Antigenic analysis of A/Hong Kong/1/68 and its variants, A/Hong Kong/5/72 and A/England/42/72, by immuno-double-diffusion tests also indicated that they shared antigenically identical internal matrix protein antigens (Schild, 1972) and ribonucleoprotein antigens.

Geographical distribution of strains

The classification of the haemagglutinin antigens of isolates from the 2 periods June 1971 to April 1972 and May to September 1972 is shown in Table 4. During the earlier period only a small proportion of isolates fell into categories (*b*) and (*c*).

Viruses resembling the variant A/Hong Kong/5/72 were initially identified in the autumn of 1971 amongst strains isolated in Hong Kong. Further isolations of this type of variant were made in other countries in the winter and spring of 1971–72; from an outbreak in Korea all of 3 isolates were like A/Hong Kong/5/72. In the moderate outbreaks of A-type influenza that occurred in Europe and the Americas in the winter of 1971–72 the majority of isolates were of prototype A/Hong Kong/1/68. Sporadic isolations of variant A/Hong Kong/5/72 were obtained in both Europe and the USA during this period. Amongst European countries from which reasonably large numbers of isolates were examined, Hungary yielded the highest proportion (approximately 30%) of isolates resembling A/Hong Kong/5/72.

A/England/42/72 was first identified in the United Kingdom in January 1972 as one of over 700 isolates

Table 4. Characterization of influenza A isolates from various countries based on HI reactions, with postinfection ferret antisera and monospecific antisera to haemagglutinin (H3), and NI tests

Country or area of origin	No. of isolates examined	Category (a) A/Hong Kong/1/68 (H3N2)[a]	(b) A/Hong Kong/5/72	(c) A/England/42/72
During the period June 1971 to April 1972				
Algeria	1	0	1	0
Argentina	5	5	0	0
Australia	12	12	0	0
Austria	16	16	0	0
Belgium	7	7	0	0
Brazil	6	6	0	0
Bulgaria	10	10	0	0
Canada	3	3	0	0
Chile	4	4	0	0
China (Province of Taiwan only)	5	5	0	0
Colombia	8	8	0	0
Czechoslovakia	13	13	0	0
Federal Republic of Germany	35	30	3	2
Finland	2	2	0	0
France	42	33	6	3
Hawaii	9	7	1	1
Hong Kong	18	13	5	0
Hungary	21	14	7	0
India	12	2	0	10[b]
Israel	3	3	0	0
Italy	14	14	0	0
Jamaica	5	5	0	0
Japan	4	2	2	0
Mongolia	1	0	1	0
Netherlands	6	6	0	0
New Zealand	3	3	0	0
Norway	4	4	0	0
Poland	4	4	0	0
Portugal	20	20	0	0
Puerto Rico	3	3	0	0
Republic of Korea	3	0	3	0
Romania	13	13	0	0
South Africa	7	7	0	0
South America	24	24	0	0
Spain	20	20	0	0
Sri Lanka	6	5	1	0
Sweden	21	21	0	0
Switzerland	18	18	0	0
United Kingdom	753	752	0	1
USA: Continental	366	364	2	0
Alaska	15	15	0	0
Hawaii	9	9	0	0
USSR	11	9	2	0
Venezuela	1	1	0	0
Total	1 563	1 512	34	17
During the period May 1972 to November 1972				
Australia	23	0	0	23[c]
Chile	1	1	0	0
China (Province of Taiwan only)	1	0	0	1
Hong Kong	3	0	1	2
Malaysia	12	0	0	12[c]
New Zealand	3	0	0	3
Papua and New Guinea	1	0	0	1
Republic of Viet-Nam	2	0	0	2
Singapore	4	0	0	4[c]
South Africa	10	10	0	0
South Pacific region: Guam, Yap, and Fiji	21	0	0	21
Thailand	1	0	0	1
United Kingdom	3	0	0	3
USA: Alaska	1	0	1	0
Hawaii	3	0	0	3
Total	89	11	2	76

[a] Strains showing a minor degree of antigenic shift from A/Hong Kong/1/68 (H3N2) and resembling A/England/878/69 (Pereira, M. S. & Schild, G. C., 1971.

[b] Strains from Southern India (Coonoor) isolated between July 1971 and December 1971 represent the earliest isolates of strains resembling A/England/42/72.

[c] Strains isolated from May–June 1972 outbreaks in Singapore and Malaysia and the July–August 1972 outbreaks in Australia were all A/England/42/72.

examined, the remaining strains being like A/Hong Kong/1/68. However, strains of this type were subsequently found to be predominant amongst isolates received from N. Veeraraghavan from an outbreak of influenza in Coonoor, Southern India, between July and December 1971. In addition, sporadic isolates of A/England/42/72 were recognized as a small proportion of strains from France, the Federal Republic of Germany, and the United Kingdom. However, in the epidemics of influenza in South-East Asia, the South Pacific, and Australia between May and September 1972 all isolates were found to be of this type.

The neuraminidase antigens of the isolates described in Table 4 have not all been examined in detail. However, for the 30 strains in haemagglutinin categories (*b*) and (*c*) for which detailed characterization of the neuraminidase antigens was carried out, the neuraminidase was found to be more closely related to that of the variant A/Hong Kong/5/72 than to that of A/Hong Kong/1/68.

It is of interest that retrospective analysis (Henry-Aymard & Schild, unpublished data) of the neuraminidase antigens of type A influenzaviruses isolated since 1968 has revealed evidence of antigenic " drift " away from that of A/Hong Kong/1/68 in a proportion of strains isolated as early as 1969. Such changes, which were detected in a high proportion of isolates obtained in 1970, predate antigenic " drift " in the haemagglutinin antigen.

DISCUSSION

For the Asian virus the earliest evidence of variation in the haemagglutinin antigen (H2) was in 1961 (Isaacs et al., 1962), although in that year such variants were infrequent. The proportion of variants increased progressively until, in 1964, the variant A/England/12/64 (H2N2) completely replaced the prototype virus, A/Singapore/1/57. Thus complete replacement of the Asian virus by its variant took 7 years. In the case of the A/Hong Kong/1/68 virus, minor changes in the haemagglutinin antigen (H3) were detected in the second year of its prevalence (Pereira & Schild, 1971) and the present report describes evidence that a further variant, A/England/42/72 (H3N2), had become predominant in the southern hemisphere by 1972, 4 years after the emergence of the Hong Kong virus. These findings are based essentially on studies of the haemagglutinin antigen of the virus. However, recent studies in our laboratories (Aymard-Henry & Schild, unpublished data) suggest that antigenic changes in the neuraminidase antigen of both the Asian and the Hong Kong virus predate changes in the haemagglutinin antigen.

Immuno-double-diffusion studies carried out with potent monospecific antisera to the haemagglutinin antigen (H3) of A/Hong Kong/1/68 virus provide evidence of shared immunological determinants between the haemagglutinin of the prototype 1968 virus and that of the 1972 variants. These findings, together with the observed cross-reaction in HI tests, suggest that the haemagglutinin antigen of both the variants should be regarded as belonging to the same subtype, H3. Similarly, the neuraminidase antigen of the variants appeared, like that of prototype A/Hong Kong/1/68 virus, to belong to the N2 subtype. The observed antigenic changes are therefore explainable in terms of antigenic " drift ".

Antibody absorption studies employing the single-radial-diffusion technique confirmed and extended the findings available from the other immunological tests in providing evidence for the cross-reactivity of the haemagglutinins of A/Hong Kong/1/68 and its variants and of the neuraminidase. These studies also permitted the conclusions that antibodies to A/Hong Kong/1/68 haemagglutinin and neuraminidase had a low affinity for the variants A/England/42/72 and A/Hong Kong/5/72. The quantitative results suggested that the neuraminidases of the variants were antigenically further from that of prototype A/Hong Kong/1/68 than were their haemagglutinins since the variants absorbed a lower proportion of anti-neuraminidase antibody (16–19%) than of anti-haemagglutinin antibody (28–30%).

As described elsewhere (Pareira et al., 1973), studies on the frequency of HI antibody to A/England/42/72 in human sera collected in the United Kingdom and the USA in the autumn of 1972 indicated that such antibody was infrequent and at a low titre. Of the individuals tested, 37% had titres of 1:40 or greater but in some age groups less than 20% had antibody. In contrast, 73% had titres of 1:40 or greater to A/Hong Kong/1/68 virus. These observations strongly suggested that the population in many areas might be largely unprotected against infection with the new variant A/England/42/72, despite previous exposure to A/Hong Kong/1/68.

Information from WHO influenza centres and other sources indicated that the outbreaks of influenza of moderate severity that occurred between May and September 1972 in South East Asia, the South Pacific, Australia, and New Zealand were associated with the A/England/42/72 virus. Sporadic

influenza A isolates made in the United Kingdom in September and October 1972 were antigenically identical to A/England/42/72.

From late December 1972 to the end of February 1973, outbreaks of influenza occurred in most regions of Europe and in North America. They were in general of moderate severity but were severe in some localities. The prevalent viruses associated with these epidemics were characterized as antigenically close to A/England/42/72 and strains antigenically close to A/Hong Kong/1/68 were not isolated.

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RÉSUMÉ

VARIATION ANTIGÉNIQUE PARMI LES VIRUS GRIPPAUX HUMAINS DE TYPE A EN CIRCULATION: CARACTÉRISTIQUES ANTIGÉNIQUES ET RÉPARTITION GÉOGRAPHIQUE DES VARIANTS

Les virus grippaux A prédominant durant les poussées épidémiques qui se sont produites en Asie du Sud-Est, en Extrême-Orient et en Australie de mai à septembre 1972 présentaient un degré modéré mais indiscutable de variation antigénique par rapport au prototype A/Hong Kong/1/68 (H3N2), principal responsable des épidémies grippales au cours des quatre années précédentes. Les différences antigéniques entre les variants, dont le type est la souche A/England/42/72, et le virus A/Hong Kong/1/68 concernaient les deux antigènes de surface, hémagglutinine et neuraminidase. Cependant les antigènes des virus A/England/42/72 et A/Hong Kong/1/68 appartenaient au même sous-type (H3N2) et donnaient lieu à des réactions croisées. Une comparaison plus poussée des deux virus a montré que les différences antigéniques entre les neuraminidases étaient plus accentuées que celles existant entre les hémagglutinines.

Un autre type de variant, la souche A/Hong Kong/5/72, a fait son apparition pour la première fois à Hong Kong de novembre 1971 à janvier 1972. Ses antigènes de surface sont aussi du sous-type H3N2. Son hémagglutinine diffère de celle du virus A/England/42/72, mais sa neuraminidase est identique à celle de ce dernier. Bien que ce variant ait été le principal virus responsable des épidémies de Hong Kong et de Corée au cours de l'hiver 1971-72, il n'a été identifié que dans une faible proportion des isolats dans d'autres régions, dont l'Europe, et sa propagation est restée limitée.

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