

## **An antigenic variant of the Hong Kong/68 influenza A 2 virus**

BY MARGUERITE S. PEREIRA

*Virus Reference Laboratory, Central Public Health Laboratory,  
Colindale Avenue, London, NW 9 5HT*

AND G. C. SCHILD

*World Influenza Centre, National Institute for Medical Research,  
Mill Hill, London, N.W. 7*

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### SUMMARY

Both the haemagglutinin and the neuraminidase of influenza A 2 virus have undergone progressive antigenic drift since this subtype first appeared in 1957.

In 1968 a strain showing a much greater antigenic difference was isolated in Hong Kong. This variant has been responsible for epidemics throughout the world in the past 2 years.

In the second year of its prevalence a small proportion of strains isolated showed a small but definite antigenic change, related principally to the haemagglutinin component.

This new variant formed about 4% of all strains investigated and was detected in Britain, Portugal, France and New Zealand.

The influenza A virus undergoes major antigenic changes which occur at intervals of 10 years or more. Such changes result in the appearance of a new virus subtype which shows considerable immunological differences from previously circulating strains in its surface antigens, namely the haemagglutinin and neuraminidase. Such changes are usually easily detected since they are associated with widespread epidemics which occur because the human population is entirely susceptible to the new virus subtype. Besides these major changes, minor changes known as 'antigenic drift' occur during the prevalence of each subtype. These are less easy to detect since the antigenic differences are small. The appearance of variants showing such minor antigenic changes is unlikely to be associated with extensive epidemics because the antigenic differences between the variant and its predecessor are small enough for the two viruses to show cross-immunity.

The gradual but steady drift among influenza A 2 viruses since the first strains were isolated in 1957 was observed in several laboratories (Isaacs, Hart & Law, 1962; Morris *et al.* 1963; Weinberger, Buescher, McCown & Gauld, 1963; Pereira, Pereira & Law, 1964). The usual pattern has been the detection in an epidemic of a proportion of the isolated strains showing a significant difference from the majority. Thus in a single epidemic two easily distinguishable strains may be circulating together. The chances of detecting this antigenic drift depend partly on the total

number of strains available for antigenic analysis. For this reason the existence of many virus laboratories in a country, as in Britain, increases the opportunities for observing it.

Isaacs *et al.* (1962) considered that influenza A1 virus showed more antigenic variability in the first 4 years of its prevalence than influenza A2 virus, which remained antigenically uniform from 1957 until 1961, when the first small but distinct deviation was demonstrable in some strains. The replacement of the original 1957 strains by the variant was not a rapid process and both were circulating concurrently during the epidemics of both 1960-1 and 1962-3, the new variant providing in Britain about one-quarter of the total of 157 strains isolated in the first epidemic and one-quarter of 128 strains isolated in the second. However, the viruses isolated in the winter of 1963-4 showed a further antigenic change. Of the 51 strains tested in England all showed this new development. None were like the original 1957 strains, which were, in fact, never again isolated in Britain. The epidemics of the following years up to 1968 were associated with A2 strains showing only minor differences from the 1964 variant until the winter of 1967-8, when this variant was joined by another one first isolated in Tokyo in 1967 (A2/Tokyo/3/67) and showing a much increased difference. These two variants circulated in the proportion of 3:1 and together were responsible for an epidemic of considerable size. These studies on the antigenic changes in the A2 viruses have been largely based on haemagglutination-inhibition tests and the antigenic changes observed thus reflect changes in the virus haemagglutinin. More recently, comparative studies on the immunological reactions of the neuraminidases of the influenza A2 viruses have been carried out. The neuraminidase is an envelope antigen which is immunologically distinct from the haemagglutinin (Webster & Laver, 1967). Such studies provided evidence that, like the haemagglutinins, the neuraminidases of the A2 viruses have undergone progressive antigenic drift since 1957, when the first A2 strains were isolated. Thus only minor cross-reactions in neuraminidase inhibition tests were detected between the 1957 and the 1967 A2 virus strains (Coleman *et al.* 1968; Schild & Newman, 1969*a*).

The appearance of yet another variant in Hong Kong in 1968 introduced a strain showing a much greater antigenic difference in its haemagglutinin than had been observed previously among A2 variants; however, its neuraminidase was closely related to that of A2/Tokyo/67 (Coleman *et al.* 1968; Schild & Newman, 1969*b*; Schulmann & Kilbourne, 1969). A2/Hong Kong/68 behaved as a new subtype in that it spread rapidly round the world causing epidemics of greater or lesser extent, replacing completely the previous variants in the northern hemisphere, though the A2/Tokyo/67 variant was still isolated for a period in countries in the southern hemisphere.

In Britain in the winter of 1968-9, 900 strains of influenza virus were examined antigenically during the long drawn-out epidemic of influenza A2. All these strains were antigenically identical with the prototype virus A2/Hong Kong/68. In the following winter of 1969-70 over 800 strains were isolated during an epidemic of a very different kind with an explosive onset, widespread morbidity and a sharp rise in the death-rate. The majority of these strains were indistinguishable from the

strains isolated the previous year and were typical Hong Kong/68 variants. However, early in the epidemic a strain was noted (numbered A2/England/878/69) which did not conform exactly. Cross haemagglutination-inhibition tests indicated a definite antigenic change (Table 1). The degree of difference was small and

Table 1. *Cross-reactions of human A2 viruses in haemagglutination-inhibition tests*

(Reciprocal of serum dilution producing 50% inhibition of eight agglutinating doses of virus.)

Virus	Ferret sera				
	A 2/ Singapore/ 1/57	A 2/ England/ 12/64	A 2/ Tokyo/ 3/67	A 2/ Hong Kong/ 1/68	A 2/ England/ 878/69
A 2/Singapore/1/57	<b>5120</b>	1280	10	320	480
A 2/England/12/64	640	<b>5120</b>	320	160	120
A 2/Tokyo/3/67	80	320	<b>640</b>	40	10
A 2/Hong Kong/1/68	120	40	10	<b>5120</b>	640
A 2/England/878/69	120	40	10	1280	<b>2560</b>

Table 2. *Cross-reactions of human A2 viruses in neuraminidase-inhibition tests\**

Source of neuraminidase	Rabbit sera		
	Anti-purified A 2/Singapore/1/57 neuraminidase	Anti-purified A 2/Tokyo/67 virus	Anti-purified A 2/Hong Kong/1/68 virus
A 2/Singapore/1/57	<b>3000†</b>	125	100
A 2/Tokyo/3/67	125	<b>1200</b>	2500
A 2/Hong Kong/1/68	85	1000	<b>3500</b>
A 2/England/878/69	75	850	<b>3500</b>
A0/BEL/42	< 10	< 10	< 10

\* Tests performed as described by Schild & Newman (1969b).

† Reciprocal of serum dilution producing 50% inhibition of 1-2 units of neuraminidase.

could well have been masked if broader-reacting antisera from hyperimmunized animals had been used instead of the highly specific post-infection ferret antisera. Neuraminidase-inhibition tests indicated that the variant contained neuraminidase which was closely related to that of A2/Hong Kong and A2/Tokyo/3/67 but which, like A2/Hong Kong, showed only minor cross-reactions with that of A2/Singapore/1/57 (Table 2). The antigenic changes shown by A2/England/878/69 thus appeared to be restricted to its haemagglutinin.

By the end of the epidemic of 1969-70, 35 strains, amounting to 4% of the total, were found to be similar to this new variant, showing the same antigenic difference from the Hong Kong/68 strain. The first of these variants was isolated from a nurse at a London hospital, ill in December 1969, and the rest appeared irregularly throughout the following weeks. The source of the strains is shown in Table 3.

It can be seen that they form a varying proportion of the strains isolated in any laboratory and their distribution is wide. Several laboratories did not provide any of the variants.

There did not seem to be any association of these strains with the age-incidence of cases or with the severity of illness and it is unlikely that this new variant played any part in causing the greatly increased severity of the second epidemic as compared with the first.

Table 3. *Sources of strains of the new influenza virus variant*

Laboratory	No. strains sent	No. similar to the A 2/England/878/69	
		Adult	Child
London Hospital	3	1	—
Stafford	21	1	1
Leicester	89	1*	1*
Worcester	28	1	2
Bath	75	1	3
Reading	2	—	1
Coventry	11	1	—
Bristol	88	3 (1*)	—
Bedford	10	—	1
Manchester	18	—	2
Cirencester	125	3	—
Nottingham	60	4 (3*)	—
Derby	15	1	—
Brompton Hospital, London	67	3	—
Guildford	6	—	1
Leeds	41	1	1
Norwich	9	1	—
Total	668		35

\* Fatal case.

A total of 1169 strains of the Hong Kong/68 virus isolated between November 1969 and June 1970 were received for identification from 34 countries throughout the world. Of these, 41 resembled the variant strain A 2/England/878/69. They were made up as follows: 35 of a total of 851 from England and Wales; 1 of 53 strains from Scotland; 2 of 25 from France; 2 of 33 from Portugal; and 1 of 4 from New Zealand. It will be of considerable interest to determine which of the viruses A 2/Hong Kong or its variant A 2/England/878/69 becomes the predominant type of A 2 virus in future epidemics of influenza. The degree of antigenic difference shown by the variant is probably not sufficient to suggest it should be included in current vaccines.

We are grateful for the co-operation of the laboratories which submitted strains of influenza virus for identification.

## REFERENCES

- COLEMAN, M. T., DOWDLE, W. R., PEREIRA, H. G., SCHILD, G. C. & CHANG, W. K. (1968). The Hong Kong/68 influenza A2 variant. *Lancet* **ii**, 1384.
- ISAACS, A., HART, R. J. C. & LAW, V. G. (1962). Influenza viruses 1957-60. *Bulletin of the World Health Organization* **26**, 253.
- MORRIS, J. A., HATANO, M., ROBINSON, R. Q., AULISIO, C. G. & SMADEL, J. E. (1963). Antigenic relationship of 1961-1963 A2 influenza viruses to prototype A2 1957 strains. *Proceedings of the Society for Experimental Biology and Medicine* **114**, 406.
- PEREIRA, H. G., PEREIRA, M. S. & LAW, V. G. (1964). Antigenic variants of influenza A2 virus. *Bulletin of the World Health Organization* **31**, 129.
- SCHILD, G. C. & NEWMAN, R. W. (1969*a*). Antibody against influenza A2 neuraminidase in human sera. *Journal of Hygiene* **67**, 353.
- SCHILD, G. C. & NEWMAN, R. W. (1969*b*). Immunological relationships between the neuraminidases of human and animal influenza A viruses. *Bulletin of the World Health Organization* **41**, 437.
- SCHULMAN, J. L. & KILBOURNE, E. D. (1969). The antigenic relationship of the neuraminidase of Hong Kong virus to that of other human strains of influenza A virus. *Bulletin of the World Health Organization* **41**, 425.
- WEBSTER, R. C. & LAVER, W. G. (1967). Preparation and properties of antibody directed specifically against the neuraminidase of influenza virus. *Journal of Immunology* **99**, 49.
- WEINBERGER, H. L., BUESCHER, E. L., MCCOWN, J. & GAULD, J. R. (1963). Antigenic variation of recently isolated A2 influenza viruses. *Proceedings of the Society for Experimental Biology and Medicine* **114**, 413.