PAPERS AND ORIGINALS

Antigenic Variants of Influenza B Virus

G. C. SCHILD, MARGUERITE S. PEREIRA, PRATIMA CHAKRAVERTY, MARION T. COLEMAN
W. R. DOWDLE, W. K. CHANG

British Medical Journal, 1973, 4, 127-131

Summary

From 1967 to 1971 little antigenic variation was detected in the prevalent influenza B viruses but in December 1972 a new antigenic variant of influenza B was isolated in Hong Kong from sporadic cases of influenza. The new variant, B/Hong Kong/5/72, possessed a haemagglutinin antigen which showed considerable antigenic differences from that of former influenza B strains while its neuraminidase antigen was closely related to that of the earlier, 1967-71, isolates. The B/Hong Kong/5/72 variant became the predominant influenza B strain in the United Kingdom by early summer 1973, and in both the United Kingdom and Japan this strain was associated with outbreaks of influenza. Strains antigenically intermediate between the 1967-71 isolates and B/Hong Kong/5/72 were isolated in a number of countries, often concurrently with B/Hong Kong/5/72-like strains.

Serological studies indicated that antibody to B/Hong Kong/5/72 was infrequent in the populations in the United Kingdom and the U.S.A., suggesting the possibility of future epidemic activity associated with the new variant. In addition, conventional inactivated influenza vaccines containing the older influenza B isolates given to volunteers stimulated poor antibody responses to B/Hong

Kong/5/72 virus. These studies indicate the desirability of immunizing high risk patients against B/Hong Kong/5/72-like viruses before the coming influenza season. Vaccine containing the new variant influenza B strain are currently available in the United Kingdom.

Introduction

Antigenic variation in its surface antigens (haemagglutinin and neuraminidase) is one of the most important characteristics of the influenza virus and a property which produces many problems for the control of influenza by vaccination. Antigenic variation in influenza A viruses may be in the form of progressive minor modification of the haemagglutinin or neuraminidase antigen, or both of these, when it is given the name "antigenic drift." Such changes take place irregularly at intervals of two or more years and involve changes within a given antigenic subtype of haemagglutinin or neuraminidase. From time to time, at longer intervals, major antigenic changes occur which result in the appearance of influenza A viruses bearing haemagglutinin and/or neuraminidase antigens of new subtypes. These changes are indicated in the nomenclature of influenza viruses (W.H.O., 1971) by H0, H1, H2, and H3 for different subtypes of haemagglutinin and N1 and N2 for different subtypes of neuraminidase. The antigens of influenza B viruses on the other hand have not been shown to undergo such major changes of subtype. Though progressive "antigenic drift" has occurred since 1940, when the earliest strains were isolated, the antigens are related and are not regarded (W.H.O., 1971) as sufficiently different to designate subtypes as is the case with influenza A viruses.

The recent appearance of antigenic variants of influenza B virus which show noticeable differences in their haemagglutinin antigens when compared with earlier isolates has stimulated awareness of the possibility of future epidemics associated with influenza B. From 1967 up to 1972 the influenza B isolates examined at the W.H.O. influenza centres in London and Atlanta showed only minor evidence of "antigenic drift." However, in December 1972 a sporadic influenza isolate from Hong Kong, B/Hong Kong/5/72, showed major antigenic differences from the isolates prevalent from 1967 to 1972. Subsequently B/Hong Kong/5/72-like strains were isolated in other areas. Isolates recovered in early 1973 in Germany,

W.H.O. World Influenza Centre, National Institute for Medical Research, Mill Hill, London NW7 1AA

G. C. SCHILD, B.SC., PH.D., Director of the World Influenza Centre

Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

MARGUERITE S. PEREIRA, M.D., Director of Virus Reference Laboratory

PRATIMA CHAKRAVERTY, B.SC., M.PHIL., Member of Scientific Staff

W.H.O. International Influenza Centre for the Americas, Centre for Disease Control, Atlanta, Georgia, U.S.A.

MARION T. COLEMAN, PH.D., Member of Scientific Staff

W. R. DOWDLE, PH.D., Member of Scientific Staff

Medical and Health Department, Virus Unit, Queen Mary Hospital, Hong Kong

W. K. CHANG, M.D., Director of Virus Unit

the U.K., the U.S.A., and Japan were found to be antigenically intermediate between previously prevalent isolates and B/Hong Kong/5/72. The antigenic differences from older strains appear to be sufficiently great to indicate that immunity derived from previous infections with influenza B virus might not give adequate cross-protection and that the 1967-71 influenza B strains at present incorporated in vaccines might not protect against the new variants.

The antigenic character and geographical distribution of strains so far isolated, the prevalence of antibody to them, and the antibody response to the new variants generated by the current influenza vaccine are described below.

Materials and Methods

Haemagglutination Inhibition tests were performed by standard methods (W.H.O. Expert Committee on Influenza, 1953). Sera were treated with receptor destroying enzyme (*Vibrio cholera* filtrate) before use.

Neuraminidase Inhibition tests were carried out as described by Aymard-Henry *et al.* (1973). The source of neuraminidase was purified influenza virus particles disrupted by detergent (1% Nonidet NP40) to liberate neuraminidase, thus reducing the possibility of the non-specific inhibition of neuraminidase activity by anti-haemagglutinin antibody.

Immuno-double-diffusion tests in agar gels were as described by Schild (1972). Purified, concentrated influenza virus preparations containing 10-15 mg/ml viral protein were used as antigen after disruption with detergent (sodium sarkosyl sulphate, Geigy NL97—1% final concentration) to liberate diffusible antigens.

Preparation of Antisera to Purified Haemagglutinin and Neuraminidase Antigens.—Purified haemagglutinin was obtained by treatment of purified influenza virus with a proteolytic enzyme, bromelain, to remove haemagglutinin which was then purified by centrifugation on sucrose gradients (Brand and Skehel, 1972). Purified neuraminidase antigens were separated from purified influenza virus particles disrupted by 1% sodium dodecyl sulphate at pH 8.9 by electrophoresis on cellulose acetate strips (Laver, 1964). Antisera were prepared in rabbits by using a single dose of purified antigen together with Freund's complete adjuvant. Sera were collected after four weeks.

Results

ANTIGENIC CHARACTERIZATION OF NEW INFLUENZA B VARIANTS

The antigenic relation between representative influenza B viruses isolated from 1940 to 1973 were studied in haemagglutination inhibition tests with postinfection ferret sera (table I). The results indicated that progressive "antigenic drift" occurred among the haemagglutinin antigens of the strains prevalent from 1940-71 but did not enable clear subdivisions to be made between these isolates. These findings are similar to those reported previously by Chakraverty (1971) and others. However, B/Hong Kong/5/72 virus showed considerable antigenic differences from the 1967-71 isolates. With the exception of B/Taiwan/2/62 virus, the antigenic differences were reciprocal in that antisera to B/Hong Kong/5/72-like strains failed to react with any of the viruses isolated from 1940-71 and antisera to the older isolates failed to react with B/Hong Kong/5/72. It was noteworthy that B/Taiwan/2/62, a virus which shows antigenic differences from other influenza B viruses isolated between 1940 and 1971, showed moderate but clear cross-reactions with B/Hong Kong/5/72 virus. On the basis of their reactions in haemagglutination inhibition tests a number of 1973 isolates, typified by B/England/847/73, were designated "intermediate" strains since they reacted with antisera to the older 1967-71 isolates as well as to B/Hong Kong/5/72 antiserum.

Antiserum prepared in rabbits against the purified haemagglutinin antigen of B/Hong Kong/5/73 was also used in haemagglu-

ination inhibition tests. This serum gave high titres with the homologous virus, somewhat lower titres with "intermediate" strains, and produced little or no inhibition of strains, except B/Taiwan/62, isolated between 1940 and 1971. These tests thus provided confirmation of the antigenic differences between the haemagglutinin of the various strains as indicated in the tests with ferret sera, and also confirmed the relation of B/Taiwan/62 to the new variant.

In order to establish whether B/Hong Kong/5/72 represented an influenza B virus with completely new haemagglutinin antigen subtype or antigenic "drift" within a single subtype, the antigenic relation between the haemagglutinins of representative influenza B strains (B/Lee/40, B/Victoria/98926/70, and B/Hong Kong/8/73*) were further examined in immuno-double-diffusion tests using rabbit antisera prepared against the purified haemagglutinin antigens of each of these strains. Schild (1970) and Schild *et al.* (1973) showed that cross-reactions between influenza virus haemagglutinin antigens can be demonstrated in such tests in that the formation of continuous precipitin lines between two strains indicates complete antigenic identity of the haemagglutinins while precipitin lines showing only partial identity for two strains (with spur formation) indicate some degree of antigenic difference (antigenic drift) between haemagglutinins within a single antigen subtype (W.H.O., 1971). Complete failure to cross-react indicates that the antigens are of different subtypes.

The precipitin reactions of antiserum to purified B/Hong Kong/5/72 haemagglutinin are shown in fig. 1. The homologous virus gave a single well defined precipitin line while B/Victoria/70 and B/Lee/40 gave weaker reactions. Spurs between the adjacent viruses are clearly seen and indicate antigenic differences between the haemagglutinin of the three strains. These findings support the conclusion that B/Hong Kong/5/72 was probably derived from formerly prevalent influenza B viruses by antigenic "drift" and that B/Lee/40, B/Victoria/70, and B/Hong Kong/72 represent members of a single haemagglutinin subtype. Precipitin tests carried out with antisera to the purified haemagglutinins of B/Lee/40 virus and B/Victoria/98926/70 showed that these antisera also reacted with each of the test strains and thus gave results which supported the conclusion that all the influenza B viruses were of a single subtype. There is at present no evidence for the existence of influenza B viruses with haemagglutinins of a different subtype as in the case for influenza A viruses.

FIG. 1—Immuno-double-diffusion reactions of antiserum to purified haemagglutinin of B/Hong Kong/5/72 virus. Antiserum is placed in centre well. Wells marked B/LEE, B/VIC, and B/HK contain detergent-disrupted B/Lee/40, B/Victoria/98926/70, and B/Hong Kong/5/72 viruses. Wells marked A/BEL and A/HK contain detergent-disrupted influenza viruses A/BEL/42(HON/1) and A/Hong Kong/1/68(H3N2) viruses. Antiserum reacted with each of influenza B strains, indicating that they belong to a single haemagglutinin subtype, but not with influenza A strains. Spurs between precipitin lines given by adjacent influenza B strains indicate antigenic differences ("drift") between their haemagglutinins.

*Antigenically identical to B/Hong Kong/5/72.

The results described here for influenza B virus, like those on previous studies involving influenza A (Schild, 1970; Schild *et al.*, 1973), indicate that immuno-double-diffusion tests with potent antisera against purified haemagglutinin antigens are more sensitive than haemagglutination inhibition tests in detecting antigenic relation between the haemagglutinin antigens of different strains. This is presumably because the haemagglutination inhibition test shows antigenic relation only on those portions of the haemagglutinin subunit which are involved in haemagglutination, while in the diffusion test antigenic determinants on the entire haemagglutinin subunit are examined.

The antigenic relation between the neuraminidase antigens of a number of influenza B strains were studied by neuraminidase inhibition tests with antisera to purified neuraminidase (B/Lee/40 virus) or to purified virus. The results (table II) indicated that the neuraminidase antigens of all the test strains were antigenically related. The tests indicated minor antigenic differences between strains suggesting that some "antigenic drift" in the neuraminidase occurred from 1940 to 1970. However, no clear antigenic differences were detected between the enzymes of B/Victoria/70 and B/Hong Kong/5/72 viruses. Since they were not performed with neuraminidase-specific antisera these tests should be regarded as preliminary. Preparation of antisera to purified neuraminidase antigens is in progress.

Paired sera from five cases of laboratory confirmed influenza B infection apparently associated with virus strains related to

B/Victoria/70 were tested by haemagglutination inhibition and neuraminidase inhibition tests for antibody response to B/Victoria/98926/70 and B/Hong Kong/5/72 antigens (table III). No antibody to B/Hong Kong/5/72 was detected in haemagglutination inhibition tests on these sera. The antibody responses to the neuraminidase of B/Victoria and B/Hong Kong/5/72 were comparable in every case. These results confirm the previously found antigenic differences between the haemagglutinins of B/Hong Kong/5/72 and of former influenza B isolates and the similarity of the B/Victoria/70 and B/Hong Kong/5/72 neuraminidase antigens.

GEOGRAPHICAL DISTRIBUTION OF INFLUENZA B STRAINS 1972-73

The antigenic character of isolates submitted to the influenza centres in London and Atlanta are summarized in table IV. Up to December 1972 all isolates examined were found to be antigenically closely related to those which had circulated from 1967 onwards. The earliest strain showing antigenic differences from the older isolates was obtained from a sporadic case of influenza in Hong Kong in December 1972, and in the following month a further three sporadic isolates identical to this were obtained there. However, no general outbreak of disease was associated with this strain in Hong Kong. Strains identified as resembling B/Hong Kong/5/72 were subsequently isolated in

TABLE I—Cross-reaction of Influenza B Viruses in Haemagglutination Inhibition Tests

Virus Strains	Postinfection Ferret Sera										Rabbit Serum Anti-purified B/HK/5/72 Haemagglutinin
	B/Lee/40	B/JHB/58	B/Taiwan/62	B/Sing/64	B/Roma/67	B/Vict/70	B/HK/1/72	B/Eng/847/73	B/Hann/3/73	B/HK/5/72	
Previously prevalent strains:											
B/Lee/40	960	40	20	20	20	20	<10	<10	<10	<10	<20
B/JHB/33/58 .. .	80	160	20	320	80	20	20	<10	<10	<10	<20
B/Taiwan/2/62 ..	10	10	1,920	20	20	<10	<10	<10	<10	60	160
B/Sing/3/64 .. .	40	40	40	1,920	320	640	320	<10	<10	<10	<20
B/Roma/1/67 .. .	<10	<10	<10	160	640	640	480	20	960	<10	20
B/Vict/98926/70*	<10	<10	<10	320	320	640	480	20	640	<10	30
B/Mass/1/71 .. .	<10	<10	<10	160	240	320	480	30	640	<10	30
B/HK/1/72	<10	<10	<10	80	240	320	480	40	640	<10	30
Intermediate strains:											
B/Eng/847/73 .. .	<10	<10	<10	20	80	40	120	320	80	240	960
B/Hann/3/73 .. .	<10	<10	<10	40	320	240	240	640	1,920	120	80
B/Hawaii/3/73 ..	<10	<10	<10	N.T.	320	240	N.T.	640	640	80	N.T.
B/Japan/1/73† ..	<10	<10	<10	N.T.	320	240	N.T.	640	1,280	320	N.T.
B/HK/5/72-like strains:											
B/HK/5/72	<10	<10	240	<10	<10	10	<10	120	60	80	1,280
B/Vict/102/72 ..	<10	<10	N.T.	<10	<10	<10	<10	N.T.	N.T.	320	1,280
B/HK/5/73	<10	<10	160	<10	<10	<10	<10	80	15	320	1,280
B/HK/8/73	<10	<10	120	<10	<10	<10	<10	40	80	160	960
B/Eng/901/73 ..	<10	<10	N.T.	<10	<10	<10	<10	80	40	80	1,280
B/Tokyo/1/73 ..	<10	<10	N.T.	<10	10	20	<10	240	120	320	1,280

*Victoria, Australia. †Japan, Kanagawa. N.T. = Not tested.

TABLE II—Comparison of Influenza B Isolates by Neuraminidase Inhibition Tests

Source of Neuraminidase*	Rabbit Antisera against				
	Purified B/Lee/40 Neuraminidase	B/Lee/40 Virus	B/England/5/66 Virus	B/Victoria/98926/70 Virus	B/Hong Kong/5/72 Virus
B/Lee/40	50†	75	300	75	20
B/Eng/5/66	15	30	250	150	35
B/Vict/70	15	15	150	300	75
B/HK/5/72	10	15	150	300	150

*Purified viruses were disrupted with 1% (final concentration) Nonidet NP40 before use in order to liberate envelope proteins. This procedure eliminates the non-specific (steric) inhibition of neuraminidase by antibody to haemagglutinin.
†NI50 titres.

TABLE III—Haemagglutination Inhibition and Neuraminidase Inhibition Titres of Human Acute/Convalescent Serum Samples from Influenza B Cases in 1973

Case No.	Results of Haemagglutination Inhibition Tests				Results of Neuraminidase Inhibition Tests			
	B/Victoria/98926/70		B/Hong Kong/5/72		B/Victoria/98926/70		B/Hong Kong/5/72	
	Acute	Convalescent	Acute	Convalescent	Acute	Convalescent	Acute	Convalescent
1	<1/10	1/20	<1/10	<1/10	<1/10	1/320	<1/10	1/708
2	1/10	1/40	<1/10	<1/10	1/158	1/178	1/795	1/630
3	<1/10	1/160	<1/10	<1/10	<1/10	1/563	1/14	1/1,000
4	<1/10	1/160	<1/10	<1/10	1/45	>1/1,000	<1/10	1/630
5	<1/10	1/640	<1/10	<1/10	<1/10	>1/1,000	<1/10	>1/1,000

TABLE IV—Antigenic Characteristics of Influenza B Viruses Isolated from June 1972 to August 1973

Country of Origin	Total Isolates Examined	Strains Resembling Previously Prevalent, 1967-71 Isolates.	"Intermediate" Strains	Strains Resembling B/Hong Kong/5/72
Australia	7	1	5	1
B.D.R. (W. Germany)	5	2	3	0
D.D.R. (E. Germany)	1	0	1	0
Greece	1	1	0	0
Hong Kong	4	0	0	4
Japan	3	0	1	2
South Africa	5	4	1	0
Senegal	2	0	2	0
United Kingdom	86	56	20	10
U.S.A.	27	15	12	0
All areas	141	79	45	17

Australia and in the U.K. This strain in the U.K. was associated with localized outbreaks and became the prevalent influenza B variant (see below). Strains characterized as "intermediate" were first recovered in West Germany in January 1973 and subsequently were isolated in several areas including the U.K. and Japan. During the preparation of this paper influenza outbreaks occurred in Japan (W.H.O., 1973) and several influenza B isolates were obtained.* Two of these isolates examined at the World Influenza Centre were characterized as being antigenically close to B/Hong Kong/5/72 and one was an intermediate strain (see table IV). The Japanese outbreaks were reported as severe, the peak of the epidemic being in May and June 1973, and schoolchildren in particular were affected.

In Britain where an extensive network of virus laboratories ensures a large number of isolates whenever influenza virus is prevalent, an unusual pattern was noted in that three different variants were circulating during the same period in the winter of 1972-3. The antigenic character of the influenza B isolates obtained from January to the end of June 1973 are indicated in fig. 2. During the first weeks of the year the strains isolated were all antigenically similar to 1967-72 strains. These were obtained from both sporadic cases and from outbreaks in schools. In the middle of March, however, an extensive outbreak of influenza in a residential school yielded strains showing antigenic differences from the 1967-72 strains and which were characterized as "intermediate" strains. During the next six weeks both "intermediate" and "old" strains were isolated in about equal proportions. However, in May an isolate was obtained which was antigenically indistinguishable from B/Hong Kong/5/72 virus. At this point there were all three antigenic varieties circulating at the same time. In the following weeks the "old" and later the "intermediate" strains became infrequent, leaving B/Hong Kong/5/72-like strains as the prevalent influenza B viruses.

ANTIBODY IN HUMAN SERA AND RESPONSE TO VACCINATION

The incidence of haemagglutination inhibiting antibody to representative strains of influenza B virus in human serum samples collected from normal adults in the U.S.A. and the U.K. in May 1973 is shown in table V. The test viruses were selected to be representative of the previously prevalent 1967-71 variant (B/Victoria/70), "intermediate" strains (B/Hawaii/2/73 and B/England/847/73), and the new variant B/Hong Kong/5/72. Fifty-two per cent. of sera from the U.S.A. and 64% from the U.K. contained detectable antibody to B/Victoria/70. In contrast, only 2% from the U.S.A. and 4% from the U.K. contained antibody to B/Hong Kong/5/72. In sera from the U.S.A. the frequency of antibody to B/Hawaii/3/73 was about the same as for B/Victoria/70. However, with the "intermediate" strain B/England/847/73 19% of sera from the U.K. contained antibody, and this result was in keeping with the "intermediate" antigenic character of this virus detected in tests with postinfection ferret sera.

The haemagglutination inhibiting antibody responses in adult volunteers who received inactivated bivalent influenza vaccine containing 200 IU of B/Victoria/98926/70 are shown in table VI. It was noteworthy that in this group of 112 adults the prevaccination serum samples obtained in October 1972 contained a much lower incidence of antibody to B/England/1/71 virus than that detected to the closely related B/Victoria/70 virus in the sera collected in May 1973 in the U.K. and the U.S.A. Because of differences in test strains and geographical locations of the populations it is not possible to derive definite conclusions from this finding. However, the frequency of antibody to B/Hong Kong/5/72 and B/England/847/73 was closely similar in the sera collected in May and October. Fourteen days after administration of B/Victoria/70 vaccine 65% of the subjects showed a fourfold or greater increase in antibody titre to B/England/71 virus and the proportion of sera without detectable antibody fell from 78 to 22%. The antibody response to the "intermediate" strain B/England/847/73 was less noticeable with only 43% showing fourfold rises and the proportion of subjects with no detectable antibody falling to 42%. The antibody response to B/Hong Kong/5/72 was poor; only 18% showed fourfold rises and the proportion of subjects without antibody fell from 94% to only 74%.

In a further study carried out in the U.S.A. a bivalent inactivated vaccine containing 300 CCA* units of B/Massachusetts/1/71 was administered to 20 adult human volunteers. Fifty per cent. of postvaccination sera contained antibody to the homologous strain at titres of 1/40 or greater while none of the subjects developed detectable antibody to B/Hong Kong/5/72.

Discussion and Conclusions

Though the influenza A virus is by far the more important cause of epidemic influenza it is well recognized that influenza B may on occasion cause widespread outbreaks, as in 1962 in the U.K., which are associated with increased morbidity and mortality. There appears to be general agreement (Perkins and Regamy, 1973) that annual vaccination of high risk groups against influenza B, as well as influenza A, is highly desirable. It is thus important that the influenza B strains used for vaccine manufacture should be updated as and when new variants appear.

Evidence is presented in the present paper of considerable "antigenic drift" in the haemagglutinin antigens of recently isolated influenza B viruses, though a corresponding antigenic change was not found for the neuraminidase antigen. Despite the lack of cross-reaction of the B/Hong Kong/72 variant with earlier influenza B isolates, precipitin tests nevertheless indicated that both the earlier and the recent variants contain haemagglutinin of a single antigenic subtype. For influenza B there is thus

*Since different units are used in the U.K. (international units) and the U.S.A. (CCA units) to define antigenic content of inactivated vaccines, it is not possible to make an exact comparison of the vaccine potencies in the two studies.

FIG. 2—Diagrammatic representation of changing prevalence of influenza B variants in U.K. from January to June 1973.

*These strains were kindly submitted for examination by Dr. H. Fukumi, National Institute of Health, Tokyo, Japan.

TABLE V—Incidence and Titre of Haemagglutination Inhibiting Antibody to Various Influenza B Viruses in Recently Collected Human Sera in U.S.A. and U.K.

	Haemagglutination Inhibition Titre	Proportion of Sera with Haemagglutination-inhibiting Antibody at Stated Titre to Virus:					
		B/Victoria/98926/70		"Intermediate" B Strain		B/Hong Kong/5/72	
		No.	%	No.	%	No.	%
105 sera from adults, May 1973 Atlanta, U.S.A.	<1/10	50	48	45	43*	103	98
	1/10-1/20	41	39	15	43	2	2
	>1/40	14	13	15	14	0	0
150 sera from adults, May 1973 London, U.K.	<1/10	54	36	122	81**	143	96
	1/10-1/20	45	30	15	10	5	1
	>1/40	51	34	13	9	2	1

*Tests carried out at Centre for Disease Control, Atlanta, with B/Hawaii/3/73 virus.

**Tests carried out in London with B/England/847/73 virus.

TABLE VI—Distribution of Haemagglutination Inhibition Antibody in 112 Adult Human Volunteers before and 14 days after Administration of Polyvalent Inactivated Influenza Vaccine Containing 200 IU B/Victoria/98926/70 Virus

Serum Samples	Haemagglutination Inhibition Titre	B/England/1/71		B/England/847/73		B/Hong Kong/5/72	
		No.	%	No.	%	No.	%
Prevaccine	<10	87	78	98	88	105	94
	10-20	17	15	6	5	6	5
	>40	8	7	8	7	1	1
14 days post vaccine	<10	25	22	47	42	83	74
	10-20	19	17	20	18	11	10
	>40	68	61	45	40	18	16
Fourfold rises in antibody		73	65	48	43	20	18

no evidence of the existence of distinct subtypes of haemagglutinin and neuraminidase. These findings are in agreement with those of Hennessy *et al.* (1965) and Chakraverty (1971, 1972). Though other workers (Tumova *et al.*, 1963; Francis and Maassab, 1965) have suggested the existence of clearcut subtypes, the general view of a W.H.O. Expert Committee on Influenza Nomenclature (W.H.O., 1971) was that there were insufficient grounds for the separation of influenza B viruses into distinct subtypes. The apparent antigenic relation between the haemagglutinins of B/Hong Kong/5/72 and B/Taiwan/2/62 was unexpected. B/Taiwan is an antigenic variant which produced local outbreaks in Taiwan but despite its antigenic differences from contemporary strains failed to spread to other areas. Whether the present findings indicate an epidemiological relation between the two strains is not clear.

It is clear that a high proportion of the population in the U.K. and the U.S.A. lack antibody to B/Hong Kong/5/72-like viruses and are thus potentially susceptible to infection; a rather smaller proportion lack antibody to "intermediate" strains such as B/England/847/73. In addition, inactivated influenza vaccines containing the previously prevalent influenza B strains (1970 or 1971 isolates) stimulated haemagglutination-inhibiting antibody to B/Hong Kong/72 virus in only a small proportion (18% of vaccines) and would thus be expected to provide little protection against this variant. There was a somewhat better antibody response to "intermediate" strains (43% responded) and 65% produced antibody to the homologous strain. Though B/Hong Kong/72-like strains or "intermediate" strains appear to be becoming prevalent in some areas it cannot yet be predicted whether they or the older strains will become the prevalent influenza B virus in all areas. For this reason it seems reasonable that persons at high risk during the coming influenza season should receive vaccines containing both B/Victoria/70-like and B/Hong Kong/5/72-like strains. This may be achieved by incorporating a B/Hong Kong/5/72-like strain into a trivalent vaccine including A/England/42/72(H3N2) and a B/Victoria/70-

like antigen. Alternatively, the B/Hong Kong/72-like strain may be prepared as a monovalent formulation to supplement a conventional bivalent vaccine containing A/England/42/72 and B/Victoria/70-like strains. In areas such as the U.K., where the new influenza B variants have already been shown to be the prevalent strains, continued inclusion of the older influenza B strains in addition to a B/Hong Kong/72-like strain in the vaccine would seem optional.

We wish to thank the many laboratories who collaborated in this study by submitting influenza viruses for examination, and the excellent technical help of Miss R. Gonsalves, Mr. V. Law, Mr. R. Newman, and Mr. E. P. Peacock. Dr. C. M. Brand, of the Wellcome Research Laboratories, Beckenham, Kent, kindly supplied specific antisera to influenza B/Lee neuraminidase.

References

- Aymard-Henry, M., Coleman, M. T., Dowdle, W. R., Schild, G. C., and Webster, R. W. (1973). *Bulletin of the World Health Organization*, **48**, 199.
- Brand, C. M., and Skehel, J. J. (1972). *Nature*, **238**, 145.
- Chakraverty, P. (1971). *Bulletin of the World Health Organization*, **45**, 755.
- Chakraverty, P. (1972). *Bulletin of the World Health Organization*, **46**, 473.
- Francis, T., and Maassab, H. F. (1965). In *Viral and Rickettsial Infections of Man*, ed. F. L. Horsfall and I. Tamm, 4th edn., p. 689. Philadelphia, Lippincott.
- Hennessy, A. V., Minuse, E., and Daveport, F. M. (1965). *Journal of Immunology*, **94**, 301.
- Laver, W. G. (1964). *Journal of Molecular Biology*, **9**, 109.
- Perkins, F. T., and Regamy, R. H. (editors) (1973). *Symposia Series in Immunobiological Standardization*, **20**, 151.
- Schild, G. C. (1970). *Journal of General Virology*, **9**, 191.
- Schild, G. C. (1972). *Journal of General Virology*, **15**, 99.
- Schild, G. C., Aymard-Henry, M., Pereira, M. S., Chakraverty, P., Coleman, M. T., and Dowdle, W. R. (1973). *Bulletin of the World Health Organization*. In press.
- Tumova, B., Fedova, D., Boskova, D., Volenikova, J., Prochaskova, V., and Ludvik, J. (1963). *Acta Virologica*, **7**, 156.
- World Health Organization Expert Committee on Influenza (1953). *World Health Organization. Technical Report Series*, No. 64.
- World Health Organization Expert Committee on Influenza Nomenclature (1971). *Bulletin of the World Health Organization*, **45**, 119.
- World Health Organization (1973). *Weekly Epidemiological Record*, No. 31.