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Antigenic Relationship between Influenza B Viruses*

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The object of this study was to determine whether antigenic groupings exist among influenza B viruses. Altogether, 22 influenza type B strains isolated during the years 1940–68 were examined by reciprocal haemagglutination-inhibition, strain-specific complement-fixation, and serum neutralization tests with sera produced in ferrets and guinea-pigs. It was found that the strain-specific complement-fixation test was superior for separating influenza B viruses into groups whereas the haemagglutination-inhibition and serum neutralization tests were better for demonstrating similarities. The results obtained with these three immunological techniques confirmed that antigenic variation exists among influenza B viruses, although it is not as clearcut as among influenza A viruses.

The results were subjected to numerical taxonomic analysis. Dendrograms and minimum-spanning trees were constructed, using methods based on cluster analysis of similarity coefficients. Four main groups of influenza B viruses were established, although they were all interlinked. The results of this study do not justify the separation of influenza B viruses into subtypes similar to those of influenza A viruses.

The changing antigenic composition of influenza viruses is one of their most important characteristics and plays a large part in determining their pathogenic potential and epidemiological behaviour. Antigenic variation among influenza A viruses is well established but although variation certainly occurs among influenza B viruses, it is less marked in degree and all strains so far isolated appear to be directly or indirectly related to each other. It has been suggested that clearcut subtypes exist (Tumova et al., 1963; Francis & Maassab, 1965), but Hennessy, Minuse & Davenport (1965) have suggested that antigenic relationships form a continuous spectrum. These antigenic relationships may not be obvious when only a few strains are compared, and may also vary with the technique employed.

The present study was designed to examine these points and to attempt to determine whether antigenic groupings could be demonstrated among strains of influenza B virus collected from a wide geographic area and over as long a period as possible.

The antigenic pattern of influenza viruses revealed by haemagglutination-inhibition tests alone is difficult to interpret because of the wide differences in strain avidity. It therefore seemed possible that other tests, such as serum neutralization and the strain-specific complement-fixation test (Lief & Henle, 1959), would provide more information on the differences between strains. Altogether, 22 influenza B viruses isolated since 1940 were included in this study. Representative strains from outbreaks in Britain, and a number of strains isolated in other places, were examined by means of the three methods. The results were analysed by computer.

MATERIALS AND METHODS

The sources of the influenza B virus strains included in this study are shown in Table 1. All strains were propagated in chick embryos and the infected allantoic fluids were stored at 4°C or –70°C.

Haemagglutination-inhibition (HI) test

For each strain, one ferret was inoculated intranasally with 1 ml of a 10^{-2} dilution of freshly harvested infectious allantoic fluid. The animal was kept under strict isolation and was exsanguinated 10–12 days after inoculation. Serum was separated and stored at –20°C.

The complete cross HI test was carried out in 1 day, using exactly the same reagents throughout the test, which was performed as described by Pereira, Pereira & Law (1964), except that the unit volume was 0.025 ml. Sera were treated before the test with a

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Table 1. Strains of influenza B virus used in the study

Code no.	Strain	Country and date of isolation	Laboratory history when received [a]	Obtained from: [b]
V1	B/Lee/1940	USA, 1940	? egg	VRL
V2	B/Bon/1943	Australia, 1943	? egg	WIC
V3	B/Crawley/1946	England, 1946	? egg	VRL
V4	B/England/10/1954	England, 1954	? egg	VRL
V5	B/England/28/55	England, 1955	? egg	VRL
V6	B/Johannesburg/33/58	South Africa, 1958	? egg	WIC
V7	B/England/939/59	England, 1959	Mk2, M2, L1	VRL
V8	B/England/159/61	England, 1961	Mk2, M2, L3	VRL
V9	B/England/61/62	England, 1962	Mk2, M1, L1	VRL
V10	B/Taiwan/2/62	Taiwan, 1962	? egg	WIC
V11	B/England/4/64	England, 1964	L3	VRL
V12	B/India/363/64	India, 1964	Mk6, M3	WIC
V13	B/Singapore/3/64	Singapore, 1964	? egg	WIC
V14	B/Hong Kong/2/64	Hong Kong, 1964	? egg	WIC
V15	B/Amakusa/1/64	Japan, 1964	? egg	WIC
V16	B/Colorado/2/65	USA, 1965	? egg	WIC
V17	B/England/2/65	England, 1965	? egg	VRL
V18	B/England/5/66	England, 1966	Mk1, M2, L2	VRL
V19	B/England/2/67	England, 1967	Mk2, L2	VRL
V20	B/Roma/1/67	Italy, 1967	Mk1, L2	WIC
V21	B/Switzerland/265/67	Switzerland, 1967	Mk5, egg 1	WIC
V22	B/England/21/68	England, 1968	Mk2, L3	VRL

[a] Mk = rhesus monkey kidney culture; M = amniotic culture; L = allantoic culture. The number indicates the number of passages.
[b] VRL = Virus Reference Laboratory, London; WIC = World Influenza Centre, London.

filtrate of cholera vibrios of known potency. The standard dose of each antigen was freshly prepared and checked before the test.

Strain-specific complement-fixation (CF) test

Both S and V antigens and antisera were prepared as described by Lief & Henle (1959). Tests were performed by testing guinea-pig V antisera to all strains against one antigen at a time. Infected allantoic fluids purified by one cycle of adsorption to, and elution from, fowl cells were used as antigens. Anti-V sera were carefully checked before use by a chessboard titration for freedom from anti-S. Several attempts were needed to prepare anti-V sera with some strains that grew poorly, and finally sera were obtained with all strains except one—namely, B/Hong Kong/2/64, which never grew well. Sera were stored at −30°C and inactivated at 56°C for 30 minutes before use. The method used for complement fixation tests was as described by Pereira, Pereira & Law (1964).

Serum neutralization (SN) test

The technique employed in this study was that described by Pereira (1958) with certain modifications, e.g., rhesus monkey kidney cultures were incubated at 33°C and serum–virus mixtures were incubated at room temperature (22°C) for 1 hour before inoculation. A single virus was tested against all sera on each occasion. The sera were those used in the HI

tests. The neutralization titre was taken as the reciprocal of the highest final serum dilution inhibiting 50–100 haemadsorption doses of virus.

Computation

For their clarification, the results obtained by the HI, CF, and SN tests were subjected both individually and in combination to numerical taxonomic analysis. A computer was used to construct dendrograms and minimum-spanning trees by numerical taxonomic methods based on cluster analysis of similarity coefficients. This method was first employed by Lee (1968) with a limited number of influenza B viruses, and later by Lee & Tauraso (1968) and Dowdle et al. (1969).

The minimum-spanning tree provides a second means of identifying clusters of virus strains and is a very useful way of representing the similarities. It reveals the relationships between clusters, the links that join the clusters, and the internal composition of each cluster (Gower & Ross, 1969).

Differential shading of the similarity matrix (Robinson, 1951) is another technique for recognizing at a glance the groups among the taxonomic units with a similarity coefficient matrix. The method consists of adopting a system for grouping the similarities into evenly spaced classes arrayed by order of magnitude and representing each of these classes by different shading in the squares of a half-matrix. Generally, the highest value is shown by the darkest shading and the lowest value by the lightest shading.

RESULTS

All strains were first examined by complement fixation tests with influenza A and B S antisera to confirm that they were indeed strains of influenza B viruses. The results of the HI tests are shown in Table 2. Some cross-reactions were observed between all strains. The antiserum to the first strain (V1) contained antibody to every strain, but the virus itself was inhibited by antisera to two other strains only; it was the only strain to show this degree of difference. Cross-reactions with the other strains were two-way. It is clear that no sharp division can be made among the strains on the basis of these results.

Table 3 shows the results of the cross-reactions in the strain-specific CF tests. The reactivity of the first strain (V1) in these tests reveals differences from the results of the HI tests; only two other strains reacted with the antisera and only 8 sera reacted with the strain itself. In the CF tests, differences were more apparent than in the HI tests and similarities were fewer.

The results of the cross SN tests are shown in Table 4; again, the V1 strain shows poor reactivity with all strains except V2 and V3.

The variation observed in the strains had little relation to the year of isolation, except that the three earliest strains showed greater similarity. Further comparison of the strains from inspection of the data in Tables 2–4 did not make the situation any clearer. To obtain a better perspective of the antigenic relationships, dendrograms and minimum-spanning trees were constructed with the help of a computer, using numerical taxonomic methods based on cluster analysis of similarity coefficient by single linkage technique (Sokal & Sneath, 1963). The results of such an analysis of the HI data presented in Table 2 are shown in Fig. 1 and 2. In Fig. 2, by drawing lines across the dendrogram at the 75% similarity level, the strains can be divided into two main groups. The first consists of all the strains isolated in 1946 or before, i.e., influenza B/Lee/1940, B/Bon/1943, and B/Crawley/1946 in one group and all the strains isolated since that time in the other. This division is probably the only one that is significant. A phenon line drawn at the next major level divides the strains roughly into six groups. Fig. 2 shows the results of analysing the HI data as a minimum-spanning tree. By this method also, strains can be divided into two main groups, but the internal divisions within a group are more obvious than in Fig. 1.

The data obtained from the CF and SN tests was analysed in the same way as the HI data. Fig. 3 and 4 show the dendrogram and minimum-spanning tree, respectively, obtained by the analysis of the CF tests. Grouping here was somewhat more clearcut than in HI tests, and only a few strains stand alone except for a link with a single strain in a group. By the CF tests, strains were subdivided into four groups plus six individual strains.

Fig. 5 and 6, a dendrogram and a minimum-spanning tree, are based on an analysis of the data obtained by SN tests. The separation of the strains is similar to that given by the HI tests, but the position of the strains in the formation of a group varies considerably.

The formation of group 1 is similar in all three tests. Thus it is possible to divide all influenza B strains into two groups. Group 1 consists of only three strains isolated during 1940–46. The rest of the strains vary substantially in their position according to individual tests. To simplify this problem, the

Table 2. Cross haemagglutination-inhibition test with influenza B strains isolated between 1940 and 1968. The values are the reciprocals of serum dilutions estimated to cause 50 % inhibition of 6–8 virus haemagglutinating doses. Homologous titres are underlined.

Antigen	Code	ANTISERUM																					
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22
B/Lee/40	V1	1280	10	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B/Bon/43	V2	320	160	320	80	20	-	-	-	20	10	-	40	-	-	40	-	-	10	-	10	-	10
B/Crawley/46	V3	160	160	640	20	20	-	-	-	10	-	-	40	10	-	-	-	10	10	-	10	10	10
B/Eng/10/54	V4	40	20	10	320	320	40	40	40	80	10	40	10	80	80	160	40	10	40	80	160	10	160
B/Eng/28/55	V5	20	20	20	40	640	20	40	80	160	20	20	20	40	40	80	40	20	40	40	80	20	40
B/Jhb/33/58	V6	40	20	10	160	320	1280	1280	2560	1280	40	320	40	320	160	640	160	160	160	160	320	80	160
B/Eng/939/59	V7	40	10	10	80	320	1280	2560	2560	1280	40	320	40	640	320	640	160	80	160	320	320	80	160
B/Eng/159/61	V8	40	-	-	40	80	640	640	640	640	20	320	10	320	80	320	160	80	20	40	160	40	80
B/Eng/61/62	V9	20	-	-	40	40	80	160	160	2560	20	320	10	320	20	320	40	20	20	20	80	20	40
B/Taiwan/2/62	V10	10	-	-	10	10	10	10	10	40	80	-	-	20	40	40	80	10	10	40	20	10	40
B/Eng/4/64	V11	40	-	-	80	160	160	80	160	320	20	1280	10	80	160	1280	160	40	80	40	320	160	80
B/Ind/363/64	V12	160	40	40	160	40	40	80	80	320	160	40	160	160	40	40	20	80	40	80	320	40	160
B/Sing/3/64	V13	40	-	-	40	80	80	160	160	640	40	80	20	1280	160	160	160	160	20	320	320	169	320
B/Hong Kong/2/64	V14	10	-	-	20	10	20	20	10	40	20	20	-	20	80	20	80	10	10	40	40	40	40
B/Ama/1/64	V15	10	-	-	40	80	80	40	80	320	20	320	10	40	40	640	40	20	40	20	320	40	80
B/Colo/2/65	V16	40	-	10	40	160	160	80	80	160	40	80	20	160	320	160	640	80	40	320	320	640	320
B/Eng/2/65	V17	40	10	10	40	80	80	80	80	320	40	80	80	320	160	160	40	320	40	320	320	320	320
B/Eng/5/66	V18	20	10	-	80	80	80	20	40	40	20	40	-	40	40	80	40	10	80	20	80	-	10
B/Eng/2/67	V19	20	-	-	40	40	40	20	20	80	20	20	20	40	40	40	40	40	10	320	160	320	160
B/Roma/1/67	V20	40	20	20	80	40	20	40	40	160	40	40	40	40	40	80	40	40	20	320	160	160	320
B/Switz/265/67	V21	20	-	10	40	20	20	20	20	80	40	20	20	40	40	80	40	20	10	160	80	320	160
B/Eng/21/68	V22	20	-	-	20	20	40	20	-	40	20	20	10	20	20	40	40	40	10	160	160	320	160

Table 3. Strain-specific complement-fixation test with influenza B strains isolated between 1940 and 1968. The values are the reciprocals of serum dilutions estimated to cause 50 % fixation of complement. Homologous titres are underlined. Values of < 20 are indicated by dashes.

Antigen	Code	ANTISERUM																					
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22
B/Lee/40	V1	1280	80	320	20	-	20	-	-	20	320	-	160	-		-	-	20	-	-	-	-	-
B/Bon/43	V2	80	160	640	20	-	40	-	-	20	-	20	80	-		-	-	20	40	-	-	-	-
B/Crawley/46	V3	160	640	1280	40	-	80	-	-	20	-	-	-	-		-	-	-	-	-	-	-	-
B/Eng/10/54	V4	-	40	20	320	40	640	320	40	40	40	320	640	160		80	160	640	1280	-	20	-	-
B/Eng/28/55	V5	-	-	20	80	80	640	160	20	320	-	40	320	20		-	80	40	80	-	40	-	-
B/Jhb/33/58	V6	-	40	40	40	-	1280	1280	40	320	80	640	1280	1280		160	320	640	1280	80	40	-	640
B/Eng/939/59	V7	-	40	40	40	-	640	640	160	160	320	320	640	640		80	320	320	640	-	-	-	80
B/Eng/159/61	V8	-	40	20	40	-	640	1280	320	320	80	320	640	1280		160	160	160	640	80	40	-	160
B/Eng/61/62	V9	-	80	-	40	-	640	640	320	640	20	640	640	1280		320	-	320	1280	-	20	-	160
B/Taiwan/2/62	V10	-	20	-	40	-	1280	160	40	20	1280	40	1280	160	NOT AVAILABLE	-	320	80	160	-	-	-	-
B/Eng/4/64	V11	-	20	20	80	20	1280	320	160	1280	80	1280	1280	1280		320	640	320	1280	40	160	-	320
B/Ind/363/64	V12	-	-	-	20	20	320	-	160	20	-	-	1280	20		-	-	80	-	-	20	-	-
B/Sing/3/64	V13	-	40	-	20	-	640	320	320	640	80	160	1280	1280		-	160	1280	1280	-	320	-	320
B/HongKong/2/64	V14					NOT DONE											NOT DONE						
B/Ama/1/64	V15	-	20	-	80	-	1280	640	320	1280	40	640	1280	640		160	320	160	1280	-	80	40	80
B/Colo/2/65	V16	-	40	-	20	20	640	320	20	20	160	-	320	160		-	2560	40	40	-	-	-	-
B/Eng/2/65	V17	-	20	-	-	-	320	320	80	1280	40	80	1280	1280		-	160	640	160	160	320	160	320
B/Eng/5/66	V18	-	20	20	80	-	640	320	80	640	20	640	320	160		160	320	160	2560	-	80	-	80
B/Eng/2/67	V19	-	40	20	40	-	1280	80	160	160	40	80	1280	640		-	320	640	160	320	640	160	640
B/Roma/1/67	V20	-	20	-	20	20	20	-	40	160	-	-	1280	320		-	160	320	-	80	320	-	320
B/Switz/265/67	V21	-	40	-	-	-	160	-	-	20	20	80	640	640		-	160	320	160	40	320	320	640
B/Eng/21/68	V22	-	80	40	80	-	160	80	40	160	80	320	1280	1280		-	640	1280	1280	320	320	1280	1280

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Table 4. Cross serum neutralization test in monkey kidney tissue cultures with influenza B strains isolated between 1940 and 1968. The values are the reciprocals of serum dilutions estimated to cause 50 % neutralization of 50–100 TCD_{50} doses of virus. Homologous titres are underlined. Values of < 40 are indicated by dashes.

Antigen	Code	ANTISERUM																					
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22
B/L33/40	V1	2560	80	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B/Bon/43	V2	80	640	640	160	320	-	-	-	40	-	-	-	-	-	80	-	-	80	-	-	-	-
B/Crawley/46	V3	160	160	1280	160	320	-	-	-	-	-	-	-	-	-	40	40	-	160	-	-	-	40
B/Eng/10/54	V4	-	40	80	2560	2560	80	160	40	80	40	40	80	80	80	160	80	80	160	160	160	40	160
B/Eng/28/55	V5	-	-	40	320	1280	40	80	40	40	-	40	40	40	40	40	-	-	80	40	160	-	-
B/Jhb/33/58	V6[A]	-	-	-	80	640	1280	1280	1280	320	-	80	40	320	80	160	40	80	80	80	320	40	80
B/Eng/939/59	V7	-	-	-	80	640	640	640	1280	320	-	80	40	320	80	160	40	80	160	80	320	80	160
B/Eng/159/61	V8	-	-	-	-	160	320	320	640	320	-	80	-	80	40	160	40	80	160	80	320	-	160
B/Eng/61/62	V9	-	-	-	80	160	320	640	1280	10240	40	640	80	1280	160	320	80	160	160	80	640	160	320
B/Taiwan/2/62	V10	-	-	-	40	80	40	80	40	40	160	80	80	80	160	80	160	80	80	80	80	80	80
B/Eng/4/64	V11	-	-	-	-	320	160	80	160	160	-	2560	-	80	160	1280	80	80	160	80	320	80	160
B/Ind/363/64	V12	160	160	160	640	160	160	160	160	640	640	640	1280	320	320	320	160	640	640	640	640	320	1280
B/Sing/3/64	V13	-	-	-	40	320	160	160	160	160	40	160	80	2560	160	160	80	640	80	640	320	160	320
B/Hong Kong/2/64	V14	40	-	40	40	160	80	40	40	40	40	80	80	160	1280	80	320	80	80	1280	320	640	320
B/Ama/1/64	V15	-	-	-	80	320	80	160	80	160	-	1280	40	160	80	1280	40	160	160	160	1280	320	320
B/Colo/2/65	V16	40	-	40	40	320	320	40	80	40	40	160	40	160	1280	160	1280	160	80	5120	2560	5120	320
B/Eng/2/65	V17	-	-	-	40	160	160	80	80	80	40	80	80	320	320	160	80	640	160	640	640	640	640
B/Eng/5/66	V18	-	-	-	80	640	80	80	80	40	-	80	-	40	80	320	40	80	640	40	160	40	80
B/Eng/2/67	V19	-	-	40	320	640	160	160	80	160	160	160	320	320	320	160	640	640	160	2560	2560	5120	5120
B/Rome/1/67	V20	-	-	-	40	160	40	40	40	40	-	40	80	80	80	80	80	160	40	640	640	640	1280
B/Switz/265/67	V21	-	-	-	40	160	40	80	40	80	40	80	40	80	160	80	80	160	40	1280	640	1280	640
B/Eng/21/68	V22	-	-	-	40	160	40	80	40	40	40	80	80	160	160	160	160	320	80	1280	640	1280	1280

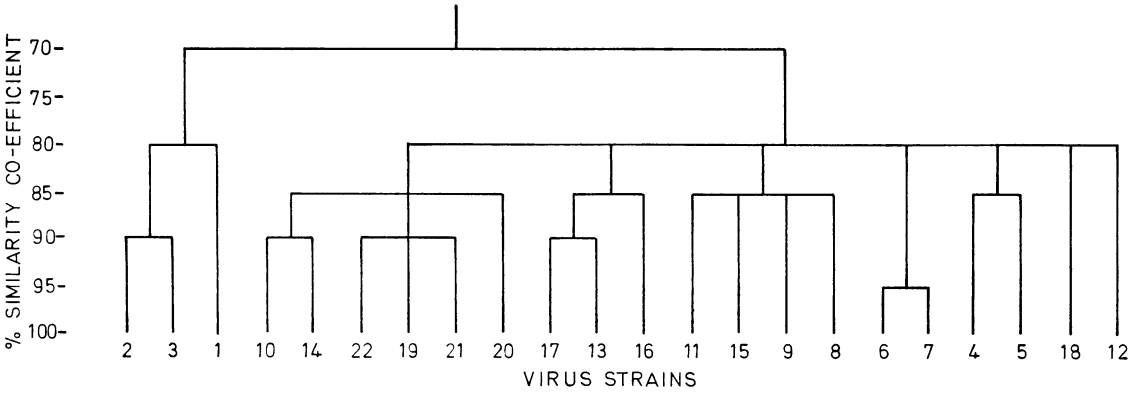

Fig. 1. Dendrogram of relationships between strains of influenza B virus based on an analysis of similarities in Table 2 (HI test) by the single linkage cluster procedure of Sokal & Sneath (1963).

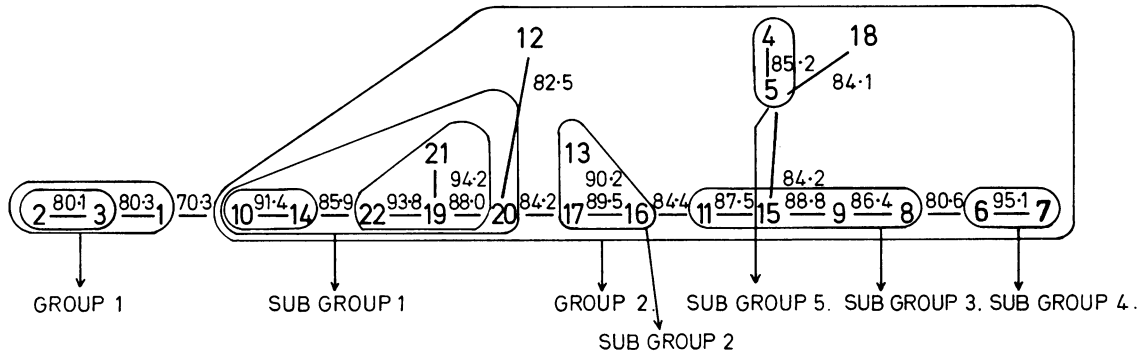

Fig. 2. Minimum-spanning tree based on the analysis of HI data from Table 2 by the single linkage cluster technique. Similarity links are indicated thus: $\overset{90\cdot1}{\text{—}}$, etc., where the number is the percentage similarity coefficient. Groups and subgroups are indicated by fine outlines; numbers 1, 2, 3, etc., are the virus strains.

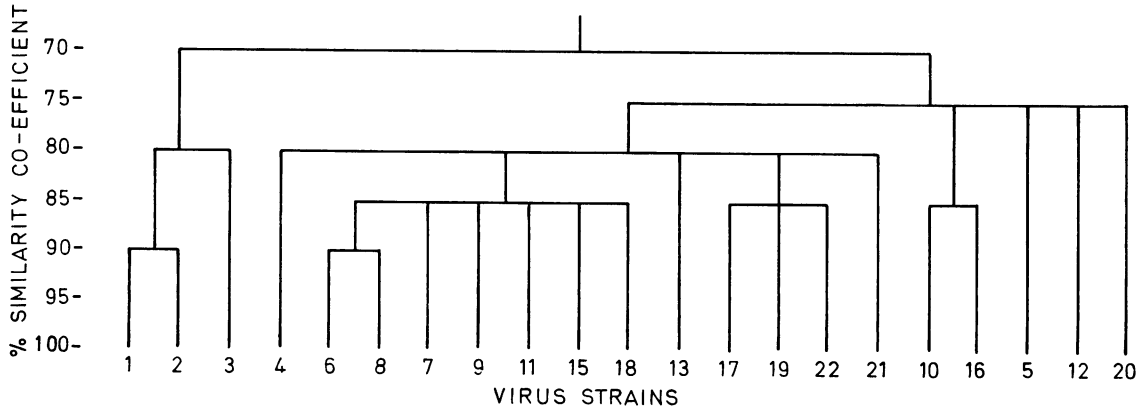

Fig. 3. Dendrogram of relationships between strains of influenza B virus based on an analysis of similarities in Table 5 (CF test) by the single linkage cluster procedure.

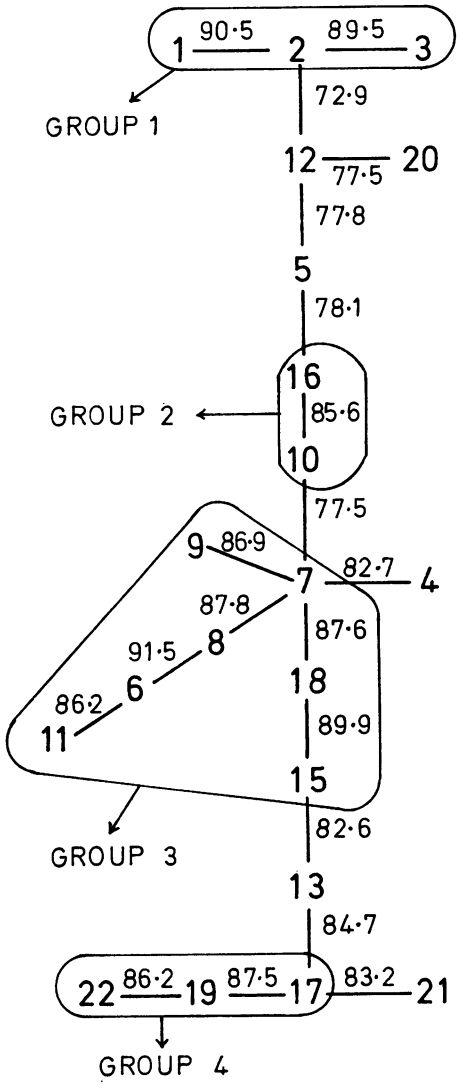

Fig. 4. Minimum-spanning tree based on the analysis of CF data from Table 5 by the single linkage cluster technique. Similarity links are indicated thus : $\overset{90.5}{—}$, etc., where the number is the percentage similarity coefficient. Groups and subgroups are indicated by fine outlines; numbers 1, 2, 3, etc., are the virus strains.

mean titres from all the tests were analysed again using the same procedures that were used in individual tests (Fig. 7 and 8).

Four main groups were obtained in this way, leaving a few strains on their own. Fig. 9 illustrates the similarity matrix by differential shading. By use of this technique, the strains can be divided into three main groups and a few individual strains. Strain V12 is not related to any of these groups; strains V4 and V5 form the bridge between group 1 and group 2.

DISCUSSION

Antigenic variation is an important factor in the development of epidemic disease. In various studies, investigators have found that the B/Lee/1940 influenza virus strain is only distantly related to later isolates. Concerning the relationships of strains isolated since 1940, three different views have been offered. According to Bozzo (1952), antigenic differences between post-1940 B strains are so slight that all may be considered as essentially identical. He emphasized the possible source of error in the antigenic comparison of influenza B viruses by HI tests depending on the use of different fowl cells and the presence of non-specific haemagglutination inhibitors in the sera used in the test.

Hilleman, Mason & Buescher (1950), Soloviev, Orlova & Tatarinova (1962), Robinson et al. (1963), and Tumova et al. (1963) have divided the strains of influenza B virus into either two or three groups. The inclusion of a strain in a particular group proposed by any of these authors has varied considerably. Tamm, Kilbourne & Horsfall (1950), Jordon & Gaylin (1953), and Hennessy, Minuse & Davenport (1965) have suggested that antigenic relationships comprise a continuous spectrum.

The results obtained with three different immunological techniques confirmed that antigenic variation is present among influenza B viruses and that these changes occur gradually, and not abruptly as in influenza A strains. A considerable degree of cross-reactivity was observed between all three tests. From experimental data, such as those in Tables 2–4, it was not easy to see immunological relationships between several virus strains, and for clarification these results were subjected to numerical taxonomic analysis. The method was found valuable for dividing strains into subgroups and offered a means to simplify the visualization of serological test results. The number of divisions among influenza B viruses again depends on the selection of the different techniques used to represent the cluster analysis. By constructing dendrograms and minimum-spanning trees it is possible to divide all influenza B strains included in this study into four groups plus a few transitional strains, whereas by using differential

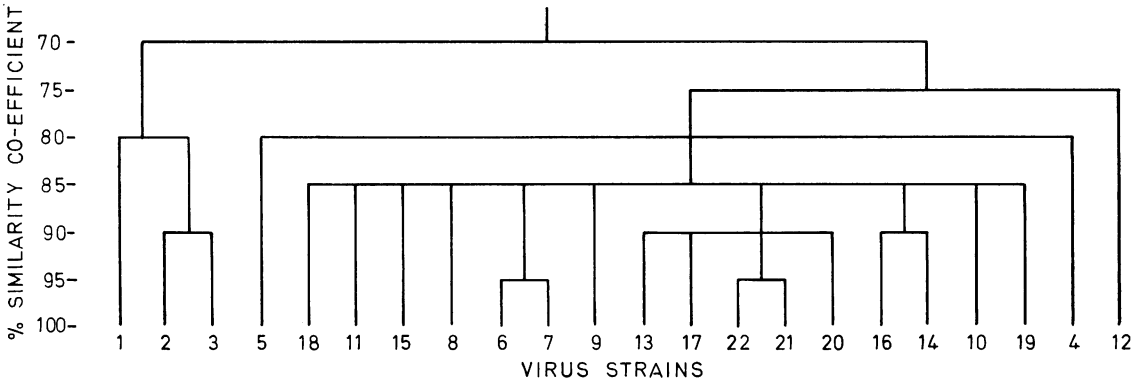

Fig. 5. Dendrogram of relationships between strains of influenza B virus based on an analysis of similarities in Table 8 (SN tests) by the single linkage cluster procedure.

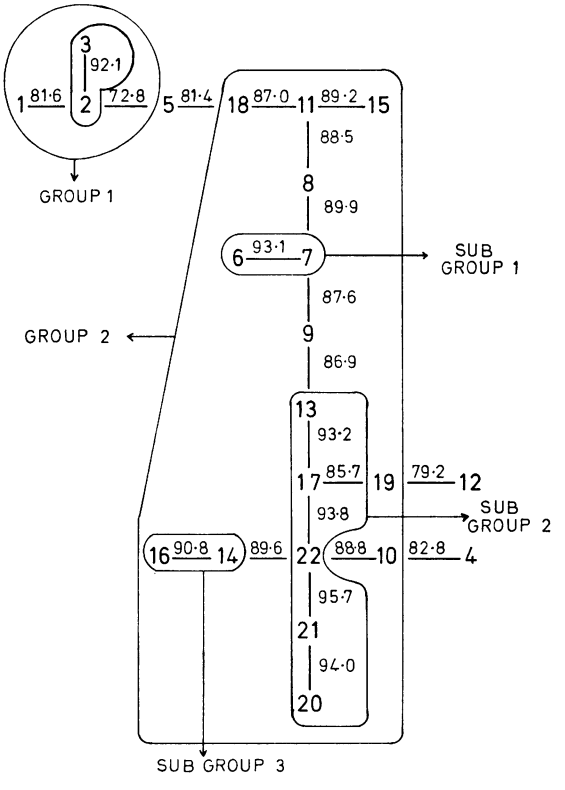

Fig. 6. Minimum-spanning tree based on the analysis of SN data from Table 8 by the single linkage cluster technique. Similarity links are indicated thus: $\overset{81.6}{—}$, etc., where the number is the percentage similarity coefficient. Groups and subgroups are indicated by fine lines; numbers 1, 2, 3, etc., are the virus strains.

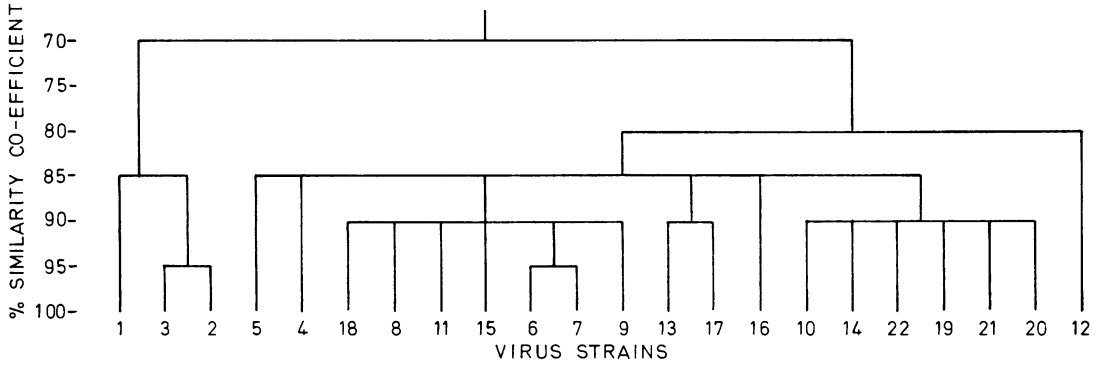

Fig. 7. Dendrogram of relationships between strains of influenza B virus based on an analysis of similarities of all tests combined by the single linkage cluster procedure.

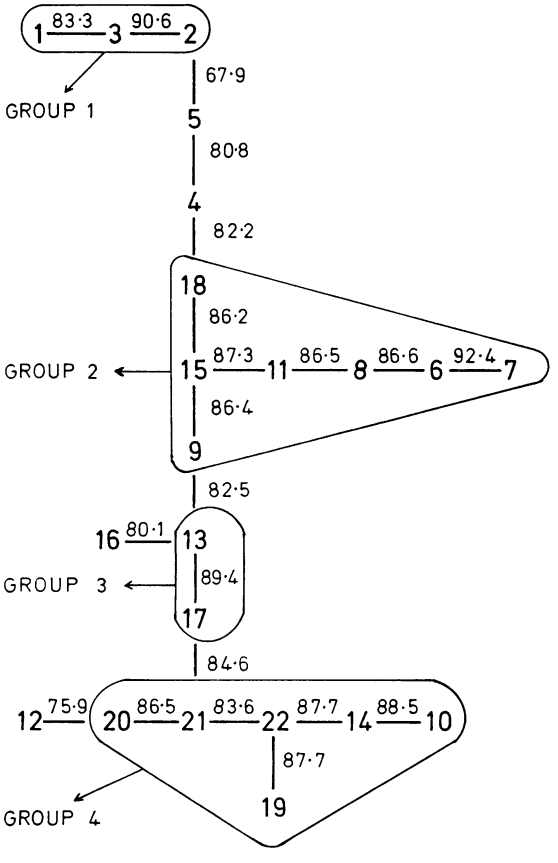

Fig. 8. Minimum-spanning tree based on the analysis of all tests combined by the single linkage cluster technique. Similarity links are indicated thus: $\overset{83.3}{\text{——}}$, etc., where the number is the percentage similarity coefficient. Groups and subgroups are indicated by fine lines; numbers 1, 2, 3, etc., are the virus strains.

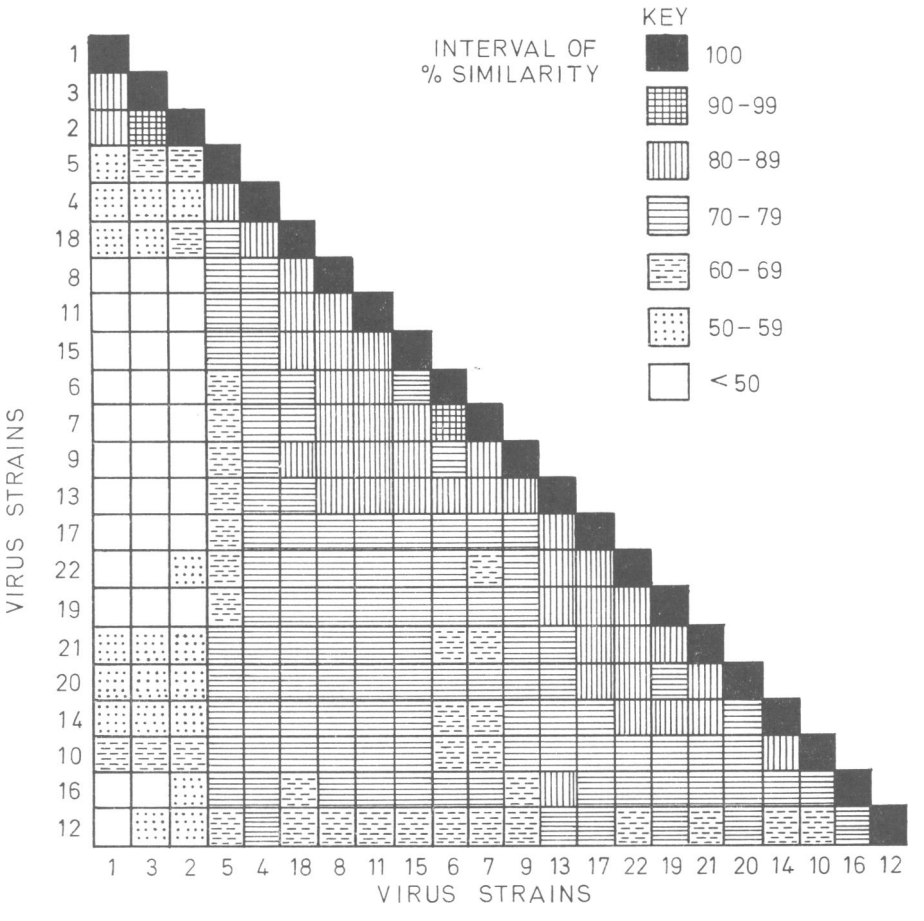

Fig. 9. Shaded similarity matrix for influenza B virus.

shading with exactly the same data, strains can be divided into three groups plus three transitional strains.

It is clear from the results of this study that all influenza B strains belong to one antigenic range and that all share different parts of it. They are undergoing a definite, gradual shift in antigenic structure but do not appear to vary antigenically with time or place of isolation. This conclusion is emphasized by the lack of sudden worldwide spreading and, in comparison with influenza A strains, by the longer intervals between outbreaks. Effective vaccination of human subjects against influenza B has been little affected by strain differences since the cross-relationships between type B strains are so extensive that immunity is of considerable duration. This in turn modifies the age incidence of disease, and influenza B epidemics are characteristically detected in the younger age groups.

It is possible that a comparison of the neuraminidases of influenza B viruses could demonstrate the existence of other relationships.

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RÉSUMÉ

RELATIONS ANTIGÉNIQUES ENTRE VIRUS GRIPPAUX B

Le but de la présente recherche était d'étudier les rapports antigéniques existant entre des virus grippaux B isolés dans le plus grand nombre de régions et durant le plus long laps de temps possibles. Les résultats des épreuves d'inhibition de l'hémagglutination (IH) étant malaisés à interpréter en raison des fortes différences d'affinité des souches, on a eu également recours à l'épreuve de fixation du complément (FC) spécifique de souche et à l'épreuve de séroneutralisation (SN), pratiquées à l'aide de sérums de furets et de cobayes.

Au total, 22 virus grippaux B isolés depuis 1940 en Grande-Bretagne ou dans d'autres pays ont été soumis aux trois tests. Les résultats relatifs à chaque virus ou groupes de virus ont fait l'objet d'une analyse taxonomique chiffrée, fondée sur le rapprochement par grappes des coefficients de corrélation. Des dendrogrammes ont été construits à l'aide d'un ordinateur.

L'épreuve FC spécifique de souche s'est révélée la plus apte à définir des groupes au sein des virus grippaux B tandis que les épreuves IH et SN faisaient mieux ressortir les ressemblances antigéniques entre les souches. Les résultats fournis par ces trois techniques immunologiques confirment l'existence de variations antigéniques parmi les virus grippaux B, moins évidentes cependant que celles qui caractérisent les virus A. Les virus B ne semblent pas soumis à des variations antigéniques en fonction de l'époque et du lieu d'isolement, comme le démontrent l'absence de toute pandémie soudaine et l'ampleur des intervalles entre les épidémies.

L'analyse des données amène à définir au sein des virus grippaux B quatre grands groupes, présentant néanmoins certaines affinités antigéniques, mais rien ne justifie leur répartition en sous-types analogues à ceux des virus A.

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