STUDIES ON THE ANTIGENIC COMPOSITION OF INFLUENZA VIRUS B STRAINS

with the aid of
the haemagglutination inhibition technique

L. M. BRANS

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE LEIDEN, OP GEZAG VAN DE RECTOR-MAGNIFICUS Dr J. H. BOEKE, HOOGLERAAR IN DE FACULTEIT DER RECHTSGELEERDHEID, TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER GENEESKUNDE TE VERDEDIGEN OP WOENSDAG 12 MAART 1952 TE 16 UUR

DOOR

LAURENS MARTINUS BRANS
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H. E. STENFERT KROESE N.V. - LEIDEN

Promotor: Professor Dr J. MULDER

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INTRODUCTION

FRANCIS (1940) and MAGILL (1940) were the first to discover influenza virus B strains in the year 1940, and subsequently all workers who have isolated B strains have found their antigenic composition to deviate more or less from the classical strain LEE (1940, U.S.A.). References on this subject are given by TAMM et al. (1950). DUDGEON et al. (1946) state that some pairs of patients' sera, obtained during the influenza B epidemic in the winter of 1945—'46, both on the Continent and in England, showed no rise in antibody titre in the haemagglutination inhibition test against the LEE strain, although a rise was observed against a strain which was isolated during the epidemic (strain CRAWLEY). Other pairs of sera gave a more marked rise against the epidemic strain than they did against LEE. They found three B strains, isolated in 1945—'46, all very similar to one another and also very closely related to the B strains: ELIZ (1945, Austr.) and MIL (1945, Austr.) isolated in Australia. A further strain, isolated in London in 1943 (PADDINGTON (1943, Eng.)) behaved differently. Antisera obtained from ferrets were used. TAMM et al. (1950), employing rabbit sera, compared 9 B strains, isolated in different years, with the LEE strain; these strains proving to deviate greatly from the latter. They also noted slight mutual dissimilarity in antigenic pattern, and this led them to speak of a continuous spectrum of antigenic differences in the influenza B group.

Isolation of influenza B strains in Holland. During the A-prime epidemic in the winter of 1949 we isolated one B strain among 45 A-prime strains. In the spring of 1950 7 B strains were isolated.

Our experiences with the first 6 serum pairs in patients, titrated against the LEE strain, (haemagglutination inhibition test), were identical with those of DUDGEON et al. in 1946. Three out of the 6 serum pairs showed a very slight or no increase at all in titre against this strain, and a distinct increase against a B strain isolated during the epidemic (table 1). As the non-specific

inhibition had been eliminated by treating the sera with a crude filtrate of *Vibrio Cholerae*, these results leave us in no doubt regarding the difference in antigenic composition between the strains IERS (1950, Ned.) and LEE. Since this difference was so great as to interfere with the use of the LEE strain as a reference strain for the serological diagnosis of influenza B, we next compared a number of B strains with regard to their antigenic composition, employing the haemagglutination inhibition technique.

INVESTIGATIONS

Materials and methods. Viruses. Cross tests were first performed with a total of 26 B strains, but later 3 more strains were examined, bringing the final number to 29. The passage formulae are given in the tables. Nearly all strains were inoculated in the amniotic cavity of 13 day old chick embryos, the allantoic route being used for the strains LEE (1940, U.S.A.) and ROHA (1950, Ned.). After two days' incubation the amniotic (or allantoic) fluid was pooled, centrifuged and diluted with physiological saline in the ratio 1 : 4. Merthiolate was added to give a final dilution of 0.01 %. The pooled fluid was stored at 2° C. Not all crossings could be performed with the fluid from one pooling. We aimed constantly at keeping the dissimilarities in passage formulae of antigens and corresponding antisera as small as possible.

Ferret antisera. Anaesthetized ferrets were inoculated intranasally with 1 ml. egg-fluid possessing a minimal C.C.A. titre of 240 (0.5 % final dilution of chicken red cells). The serum was collected after 12 days. All pre- and post-infection sera were checked for the presence of antibodies against strains from the A, A-prime and B groups. The immune sera were stored at 2° without adding preservative. A portion of each serum was freeze-dried.

Elimination of the non-specific inhibition in ferret sera with enzyme of Vibrio Cholerae. (VAN DER VEEN and MULDER (1950)). Enzyme of *Vibrio Cholerae* was prepared according to BURNET and STONE (1947). A good production of enzyme was obtained with the strain 4 Z (kindly sent to us by Prof. F. M. BURNET). As a standard for a good enzyme production we have found that two parts of crude filtrate added to one part of normal ferret serum, after 16 hours' interaction at 37°, should eliminate every non-specific inhibition from this serum, when tested against the strains A (1941, Ned.); A-prime-BARRATT (1947, Eng.); B-Co (1950, Ned.), and B-TODD (1950, Eng.). We have found, that when a filtrate in this dilution eliminates the non-specific inhibition in ferret serum against the strain A-prime BARRATT, it is always sufficiently potent to eliminate the non-specific inhibition against B strains. In our cross tests one part of antiserum was treated with 5 parts of crude filtrate for 16 hours at 37°. After this period the mixture was heated for one hour at 56° to remove the remaining of R.D.E. (BURNET and STONE (1947)). In each experiment a normal, filtrate treated serum, known to contain a large quantity of non-specific inhibitor, was titrated against all the strains used in this experiment. The titre of this normal treated ferret serum was always less than 12 against all B strains tested in the investigations. Any possibility of residual non-specific inhibition, therefore, is excluded in all the experiments.

Haemagglutination inhibition test. This test was performed by means of a micro-method (tiles of porcelain with concavities). The technique used and the estimation of the end-point were identical to those described by VAN DER VEEN and MULDER (1950). All the experiments were performed with great care by ourselves. The standard deviation of the titration method may be estimated at ± 15 % (VAN DER VEEN and MULDER (1950)).

The problem of crossing egg-lines and egg-mouse-egg-lines of influenza virus with the haemagglutination inhibition test. This problem is important in the B group since most strains are pure egg-lines. Ferret-mouse-egg-lines are the strains LEE (1940, U.S.A.), MONTGOMERY (1940, U.S.A.) and TM (1940, U.S.A.). The strain PADDINGTON (1943, Eng.) is a ferret-egg-line. Crossings of B strains, isolated after 1940 with the classical LEE strain are crossings between egg-line and ferret-mouse-egg-line virus.

Results of crossings between egg-lines and egg-mouse-egg-lines of strains of influenza virus of the A and A-prime groups have been published by us elsewhere (MULDER and BRANS (1952)). From this investigation it became clear that we have to make allowance for the fact that antisera against egg-mouse-egg-lines often show low, and even extremely low, titres against homologous and/or heterologous egg-lines from the same subgroup. The same phenomenon may occur in tests with anti-LEE serum against egg-lines of other B strains. This also holds good for the antiserum of the strains MONTGOMERY (1940, U.S.A.), TM (1940, U.S.A.), and PADDINGTON (1943, Eng.).

Cross-tests. Antisera of 10 strains from the period 1940—1948 were crossed, each antiserum being titrated against all the 10 strains in one experiment (table 2). In a second series the antisera of 15 strains, from the period 1949—1950, were crossed in the same way (table 3). From the results of these tests we selected 6 strains (isolated in different years), for a complete cross test with all the other strains. We selected those strains which gave the impression of being serologically separate, and those which showed good homologous and heterologous antititres: LEE (1940, U.S.A.); BON (1943, Austr.); PADDINGTON (1943, Eng.); WARNER (1948, Austr.); BUD 1 (1949, Hung.), and TODD (1950, Eng.). We added the strain CRAWLEY (1946, Eng.), (DUDGEON et al. (1946)), which was not available at the time of the preliminary crossings of tables 2 and 3. This strain happened to show high homologous and heterologous antititres, and fitted with the selected group very well.

Fresh ferret antisera were employed for the crossings with the 7 above mentioned strains. Some antigens had to be made over again. Tables 4 and 5 show the results of the crossings of the 7 strains above mentioned with all the other strains. Tables 6 and 7 show the results of the mutual crossings between 7 and 5 selected strains, carried out in a single experiment.

From tables 4, 5, 6 and 7 the following pecularities of the B strains examined come to light.

Discrepancies in the tests. As the crossings of the selected strains were repeated several times on different days, partly with the original, and partly with fresh antisera, one could expect certain discrepancies, to occur.

A serious discrepancy was found in the crossings of the antiserum of BON (1943, Austr.) with the strain PADDINGTON (1943, Eng.). Serum Bon E_{42} gave high titres with the strain PADDINGTON, while serum E_{47} gave a low titre. Table 8 shows a repetition of the crossings of the two strains and here again the antiserum BON E_{47} yielded a low titre with PADDINGTON: the use of a different anti-BON-serum must account for this discrepancy. The dissimilarities of the heterologous titres of the antisera CRAWLEY E_{15} and E_{14} against the strains WARNER and TODD were probably also due to the use of two different sera. Another discrepancy concerned the homologous titres of the strain GOODLOE, which was determined twice with the same antiserum, yielding widely differing results. The heterologous titres of the antiserum against the strains LEE, BON, PADDINGTON and WARNER on the other hand, agreed very well in the two tests. In this case a titration error might have been present. The strain BERKELEY 1 (1949, U.S.A.) had a low homologous titre in the tests as shown in table 4. In the tests of table 3 the homologous titre was found to be higher, although the same antiserum and the same virus-pool were used. It is just possible that there was a technical error in these titrations too. The same may be said about the tests with the strain BERKELEY 2 (1949, U.S.A.).

Strains with low homologous titres. Some strains are poor antigens, their homologous titres being low. This is particularly true of the strains isolated in 1949 and 1950. Of some strains the homologous titres are very low, and certain heterologous titres higher (BERKELEY 2 (1949, U.S.A.); SLU (1950, Ned.); HES (1950, Ned.)). They may be considered to represent Q-phase strains (VAN DER VEEN and MULDER (1950)). Tests with the

strain BUD 1 (1949, Hung.), however, teach us to use care in considering a strain to be a Q-phase strain, for a second antiserum may show a much higher homologous antititre.

Serological patterns in the B group. Strain Lee (1940, U.S.A.). From the tables 1, 2, 4, 5, 6 and 7 it is quite obvious that the strain LEE stands alone, a fact, which is convincingly proved by the low titres of this strains against heterologous antisera. In order to make sure that this is not due to this strain being a ferret-mouse-egg-line, the strain was crossed with the egg-line and the egg-mouse-egg-line of the strain BON (1943, Austr.) [1]). Tables 9 and 10 show that the mouse-adapted lines of these two strains also show distinct antigenic differences. The only strain of near relation to LEE is the strain MONTGOMERY (1940, U.S.A.), isolated by EATON and BECK (EATON and BECK (1941)) (tables 2, 11 and 14). It is curious that the strain also shows a high titre with the serum CRAWLEY (1946, Eng.) and reacts fairly strongly to the serum $F_xE_xE_3$ PADDINGTON (1943, Eng.). The strain, therefore, shows a greater polyvalence against heterologous antisera than LEE.

The strain Paddington (1943, Eng.). This strain, isolated by Dr F. HIMMELWEIT in London (HIMMELWEIT (1943)), seems to have a separate place in the series. The high heterologous titre of this strain with one of the antisera BON (1943, Austr.) is striking.

The group Bon (1943, Austr.). It is an important feature that the remaining strains, isolated after 1943, may all be reasonably considered to be strongly related to the strain BON (1943, Austr.). The strain CRAWLEY (1946, Eng.) behaves almost identical to BON, except in the behaviour of its antiserum against the strain MONTGOMERY.

The strain TM (1940, U.S.A.). This strain, isolated by Dr T. P. MAGILL (MAGILL (1940)), and only examined at a later date, has been proven to be an independent (tables 12, 13 and 14).

Identification of B strains isolated in 1951 in Holland. These strains were isolated in the spring of 1951 by Prof. Dr J.D. VERLINDE in Amersfoort (Holland), when the investigation mentioned above had already been completed. Both strains may be incorporated in the subgroup BON-CRAWLEY (tables 15 and 16).

[1]) DR E. HERTZBERGER (Weesp) kindly sent us the mouse adapted line of the strain Bon.

DISCUSSION

One surprising result of the present investigation is the fact that, among 29 B strains, we have found only one related to the classical strain LEE (MONTGOMERY (1940, U.S.A.)). It is moreover noteworthy that a third strain isolated in 1940 in America (TM) stands alone as does a strain isolated in England in 1943 (PADDINGTON). The remaining number of the strains investigated (25), may, with a reasonable degree of certainty, be classed together in a single group, the first representative of which would seem to be the strain BON, isolated by BEVERIDGE, BURNET and WILLIAMS in 1943 in Melbourne (BEVERIDGE et al. (1944)). This strain is a good antigen, and it gives high titres with heterologous antisera from the same subgroup. The strain is therefore very suitable as a reference strain for this group. The chance that (for instance) the strain TM may diverge from LEE because both underwent many animal passages, is, in our opinion, very slight. In the case of A and A-prime strains, mouse passage tends to make those strains serologically more homogeneous (MULDER and BRANS (1952)); moreover the ferret-mouse-egg-line of the strain MONTGOMERY (1940, U.S.A.) is very closely related to LEE. Although the total of the serological B variants found does not differ from that of the A strains (among which we distinguish nowadays 4 subgroups, viz. A-swine, A-WS, A-PR_8, and A-prime), yet the B group has the pecularity that more than one subgroup was isolated in a single year (1940 and 1943), which does not seem to have been proven for the human A-group so far (ISAACS and ANDREWES (1951)) and that no other representatives of the strains TM and PADDINGTON were found. Since 1943, however, the antigenic pattern of the B strains seems to have undergone little or no change.

With regard to the problem of vaccination against influenza B, we would be inclined at present to replace the strain LEE by some more recently isolated strain; or perhaps the strain BON,

or CRAWLEY. It should be remembered that good results were reported by FRANCIS et al. (1946) on vaccination with the strain LEE in an influenza B epidemic (1945). This could be explained by the assumption that vaccination with the strain LEE stimulated residual antibodies to the BON group which produced a titre high enough to prevent this type of strain from causing infection.

SUMMARY

Twenty nine B strains isolated in the period 1940—1951 were examined with regard to their antigenic composition with the aid of the haemagglutination inhibition technique, ferret antisera being employed. Four more or less separate patterns of subgroups were found, viz. LEE (1940, U.S.A.), TM (1940, U.S.A.), PADDINGTON (1943, Eng.) and BON (1943, Austr.). Only one strain was closely related to LEE (MONTGOMERY (1940, U.S.A.)). No other representatives were found of the strains TM and PADDINGTON.

Twenty five strains could reasonably be considered as belonging to a single subgroup of strains, of which the strain BON (1943, Austr.) seems to have been the first representative discovered. Q-phase strains were infrequent among the 29 strains that were examined.

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GENERAL INDEX TO THE TABLES

The nomenclature for each strain is that used by the Centre from which the strain was obtained. The year of isolation and the country of origin are added. Austr. = Australia; Czech. = Czechosl. = Czechoslovakia; Eng. = England; Hung. = Hungary; Ned. = The Netherlands; U.S.A. = United States of America.

In the passage formulae the letter F, M or E indicate the number of ferret-, mouse- or egg-passages. So, $F_8M_{137}F_{160}$ implies that the strain had 8 ferret-, 137 mouse- and 160 egg-passages. F_x, M_x or E_x implies that the number of previous ferret-, mouse- or egg-passages is unknown.

The inhibition titres were calculated theoretically, and are expressed as reciprocals of the final serum dilution which produced a partial agglutination (50 %) when 3 A.U. of virus were used.

TABLE 1

Haemagglutination inhibition tests with 6 pairs of human sera (1950) against strains of influenza virus (one experiment)

PAIRS OF SERA (Human)	STRAINS			
	A (mouse adapted) PR_8 (1934, U.S.A.)	A-prime (egg) Heer (1949, Ned.)	B (mouse adapted) Lee (1940, U.S.A.)	B (egg) Iers (1950, Ned.)
Iers	<12/<12	96/96	<12/18	<12/108
Wou	168/168	72/72	<12/20	<12/256
Slu	<12/<12	<12/<12	<12/<12	<12/1024
Liesh	80/80	160/144	<12/1792	<12/1792
Pugf	320/320	<12/<12	<12/746	21/1194
Hesk	72/72	21/21	<12/1024	<12/384

TABLE 2

Cross haemagglutination inhibition tests with strains of

FERRET ANTISERA	STRAINS			
	Lee (1940, U.S.A.) $F_8M_{137}E_{160}M_{10}E_3$	Montgomery (1940, U.S.A.) $F_{11}M_{25}E_6$	Bon (1943, Austr.) E_{44}	Allen (1943, U.S.A.) E_{25}
Lee (1940, U.S.A.) $F_8M_{137}E_{160}$	**13653**	4267	128	<12
Montgomery (1940, U.S.A.) $F_{11}M_{25}E_6$	4608	**10240**	426	<12
Bon (1943, Austr.) E_{42}	768	640	**9557**	1536
Allen (1945, U.S.A.) E_{25}	40	<12	672	**1152**
Paddington (1943, Eng.) $F_xE_xE_1$	160	448	120	<12
Mil (1945, Austr.) E_7	373	512	1536	1344
Chaddick (1945, U.S.A.) E_{18}	93	160	1536	672
Goodloe (1945, U.S.A.) E_{12}	<12	24	320	746
Czech (1945, U.S.A.) E_xE_7	224	853	1344	480
Warner (1948, Austr.) E_xE_4	<12	<12	1152	1610

influenza virus B, isolated in the period 1940—1948

STRAINS						Expt.
Paddington (1943, Eng.) $F_xE_xE_2$	Mil (1945, Austr.) E_8	Chaddick (1945, U.S.A.) E_{20}	Goodloe (1945, U.S.A.) E_{13}	Czech (1945, U.S.A.) E_xE_6	Warner (1948, Austr.) E_xE_5	
1380	768	54	<12	214	<12	1
1380	448	336	86	144	<12	2
13653	2304	5120	2560	5120	1610	3
<12	576	768	1024	320	896	
16384	112	134	30	75	<12	4
108	**1920**	2986	2560	1152	2304	5
108	960	**5376**	1024	1380	576	
18	597	480	**3840**	672	2048	6
336	1194	2688	1024	**1344**	320	7
<12	896	960	2133	746	**2304**	8

TABLE 3

Cross haemagglutination inhibition tests with 15 strains of influenza virus B, isolated in the period 1949—1950

FERRET ANTISERA	STRAINS															
	Bud 1 (1949, Hung.) E_xE_5	Bud 2 (1949, Hung.) E_xE_4	Bud 3 (1949, Hung.) E_xE_6	Czechosl (1949, Czech.) E_xE_7	London 5 (1949, Eng.) E_xE_{15}	Berkeley 1 (1949, U.S.A.) E_{15}	Berkeley 2 (1949, U.S.A.) E_{10}	Sweden (1949, Sweden) E_{56}	Seattle (1949, U.S.A.) E_{18}	Roha (1949, Ned.) E_{22}	Slu (1950, Ned.) E_6	Co (1950, Ned.) E_6	Hes (1950, Ned.) E_5	Todd (1950, Eng.) E_9	Cowing (1950, Eng.) E_{11}	Expt.
Bud 1 (1949, Hung.) E_xE_2	**373**	373	320	192	128	56	<12	36	28	106	14	672	54	576	320	1
Bud 2 (1949, Hung.) E_xE_2	682	**597**	512	384	768	21	<12	24	56	56	21	1380	198	1024	512	2
Bud 3 (1949, Hung.) E_xE_2	298	384	**373**	214	106	40	<12	60	16	14	<12	160	54	160	373	3
Czechosl (1949, Czech.) E_xE_2	336	336	298	**298**	160	106	128	96	108	160	134	384	192	384	320	4
London 5 (1949, Eng.) E_xE_6	320	373	373	256	**672**	144	96	96	144	213	144	533	160	576	320	5
Berkeley 1 (1949 U.S.A.) E_{12}	214	384	597	320	168	**213**	40	160	168	106	93	112	112	192	448	6
Berkeley 2 (1949, U.S.A.) E_8	168	288	256	160	96	168	**80**	96	214	93	64	126	86	108	112	7
Sweden (1949, Sweden) E_{52}	288	480	533	597	160	336	40	**336**	192	106	106	106	144	112	320	8
Seattle (1949, U.S.A.) E_{19}	96	79	72	14	12	23	27	28	**224**	93	23	96	<12	72	42	9
Roha (1949, Ned.) E_{20}	47	47	47	54	84	56	32	48	126	**373** [1]	28	160	27	160	93	10
Slu (1950, Ned.) E_5	171	171	205	224	160	298	112	373	597	224	**128**	384	128	320	373	11
Co (1950, Ned.) E_7	533	512	533	480	192	256	56	171	320	384	120	**853**	126	853	768	12
Hes (1950, Ned.) E_6	427	384	427	267	108	213	36	128	108	144	90	320	**160**	320	512	13
Todd (1950, Eng.) E_{10} (a)	427	448	448	384	320	28	<12	21	112	1365	18	2389	298	**2389**	597	14
Cowing (1950, Eng.) E_{12}	192	224	198	198	224	24	<12	18	<12	48	36	198	126	160	**198**	15

[1]) The titres obtained with the B strains isolated in the Netherlands in 1949 and 1950 have been indicated separately.

TABLE 4

Cross haemagglutination inhibition tests with 26 anti influenza virus B sera against 7 selected strains of influenza virus B, isolated in the period 1940—1950

FERRET ANTISERA	STRAINS								Expt.
	Lee (1940, U.S.A.) $F_8M_{137}E_{160}M_{10}E_9$	Bon (1943, Austr.) E_{47}	Paddington (1943, Eng.) $F_XE_XE_3$	Crawley (1946, Eng.) E_{14}	Warner (1948, Austr.) E_XE_7	Bud 1 (1949, Hung.) E_XE_5	Todd (1950, Eng.) E_{10}	Homologous strains	
Lee (1940, U.S.A.) $F_8M_{137}E_{160}$	**10752**	84	576	512	24	384	24	**10752**	
Montgomery (1940, U.S.A.) $F_{11}M_{25}E_6$	2688	224	768	576	12	256	12	**9557**	1
Bon (1943, Austr.) E_{42}	336	**7168**	9216	2560	1536	768	1536	**7168**	
Allen (1943, U.S.A.) E_{25}	12	512	<12	640	1536	448	384	**672**	2
Paddington (1943, Eng.) $F_XE_XE_1$	192	96	**8192**	48	<24	<24	<24	**8192**	
Mil (1945, Austr.) E_7	256	1344	80	1920	2048	2688	1536	**2560**	
Chaddick (1945, U.S.A.) E_{18}	80	2688	160	640	512	672	640	**5376**	3
Goodloe (1945, U.S.A.) E_{12}	<12	336	24	480	1152	672	640	**896**	
Czech (1945, U.S.A.) E_XE_7	384	1536	427	1380	640	640	576	**2389**	
Crawley (1946, Eng.) E_{15}	640	3072	480	**5120**	2304	2133	2048	**5120**	4
Warner (1948, Austr.) E_XE_4	<12	1152	<12	2304	**2560**	1920	1152	**2560**	

Bud 1 (1949, Hung.) E_xE_2	<12	576	12	427	1152	**298**	533	**298**	
Bud 2 (1949, Hung.) E_xE_2	26	1152	48	640	2304	597	1707	**576**	5
Bud 3 (1949, Hung.) E_xE_2	<12	320	<12	533	576	224	320	**320**	
Czechosl. (1949, Czech.) E_xE_2	<12	480	<12	512	512	160	320	**336**	6
London 5 (1949, Eng.) E_xE_6	20	533	40	384	768	256	768	**384**	
Berkeley 1 (1949, U.S.A.) E_{12}	<12	288	17	288	384	96	198	**93**	7
Berkeley 2 (1949, U.S.A.) E_8	<12	72	<12	96	144	36	93	**24**	
Sweden (1949, Sweden) E_{52}	<12	373	144	746	198	384	192	**267**	8
Seattle (1949, U.S.A.) E_{19}	<12	126	36	224	298	214	168	**533**	
Roha (1949, Ned.) E_{20}	32	56	64	47	168	27	93	**448**	
Slu (1950, Ned.) E_5	<12	112	24	224	224	106	112	**93**	9
Co (1950, Ned.) E_7	<12	336	<12	672	1792	512	896	**1344**	
Hes (1950, Ned.) E_6	36	192	96	213	597	198	224	**108**	
Todd (1950, Eng.) E_{10} (a)	<12	640	96	853	2389	896	**1610**	**1610**	10
Cowing (1950, Eng.) E_{12}	24	384	32	427	298	298	298	**448**	

TABLE 5

Cross haemagglutination inhibition tests with 7 selected anti influenza virus B sera against 26 influenza virus B strains isolated in the period 1940—1950

FERRET ANTISERA [1]	STRAINS																									Expt.
	Lee (1940, U.S.A.) $F_8M_{137}E_{160}M_{10}E_{12}$	Montgomery (1940, U.S.A.) $F_{11}M_{25}E_7$	Bon (1943, Austr.) E_{47}	Allen (1945, U.S.A.) E_{49}	Paddington (1943, Eng.) $F_xE_xE_3$	Mil (1945, Austr.) E_9	Chaddick (1945, U.S.A.) E_{21}	Goodloe (1945, U.S.A.) E_{16}	Czech (1945, U.S.A.) E_xE_{10}	Crawley (1946, Eng.) E_{14}	Warner (1948, Austr.) E_xE_7	Bud 1 (1949, Hung.) E_xE_5	Bud 2 (1949, Hung.) E_xE_5	Bud 3 (1949, Hung.) E_xE_6	Czechosl. (1949, Czech.) E_xE_7	London 5 (1949, Eng.) E_xE_{15}	Berkeley 1 (1949, U.S.A.) E_{15}	Berkeley 2 (1949, U.S.A.) E_{16}	Sweden (1949, Sweden) E_{56}	Seattle (1949, U.S.A.) E_{18}	Roha (1949, Ned.) E_{22}	Slu (1950, Ned.) E_6	Co (1950, Ned.) E_6	Hes (1950, Ned.) E_6	Todd (1950, Eng.) E_{10}	Cowing (1950, Eng.) E_{19}	
Lee (1940, U.S.A.) $F_8M_{137}E_{171}$	**4137**	1380	86	<12	134	308	20	<12	168	108	<12	16	84	128	40	<12	18	<12	64	<12	<12	<12	<12	18	<12	75	1
Bon (1943, Austr.) E_{47}	98	198	**5376**	448	267	1840	2730	768	4096	2560	672	1536	2389	3072	1610	768	426	126	672	128	168	160	597	1365	746	2730	2
Paddington (1943, Eng.) $F_xE_xE_3$	640	2304	576	<12	**18432**	320	256	60	896	320	<12	90	336	480	298	160	80	<12	288	<12	<12	54	<12	144	<12	298	3
Crawley (1946, Eng.) E_{14}	229	2560	2133	576	320	2304	960	480	4778	**2560**	448	1536	1610	2986	1792	640	640	267	1920	267	134	267	576	1380	533	2048	4
Warner (1948, Austr.) E_xE_7	<12	<12	960	896	<12	853	427	640	1920	1920	**1536**	1150	533	853	480	640	213	108	533	298	1194	112	3072	512	1920	1380	5
Bud 1 (1949, Hung.) E_xE_5	80	672	2304	2688	240	1920	1150	640	3413	2560	2304	**2730**	2304	2688	1610	1150	480	672	1380	746	640	320	2688	1150	2304	2133	6
Todd (1950, Eng.) E_{10} (b)	64	126	1610	1194	108	853	672	320	1840	2048	2688	1194	853	1024	768	672	198	288	672	373	640	112	2986	672	**4608**	1536	7

[1] The antisera employed in these tests are different from those of the tables 2, 3 and 4.

TABLE 6

*Cross haemagglutination inhibition tests with 7 selected strains of influenza virus B, isolated in the period **1940—1950** (one experiment)*

FERRET ANTISERA	STRAINS						
	Lee (1940, U.S.A.) $F_8M_{137}E_{182}$	Bon (1943, Austr.) E_{59}	Paddington (1943, Eng.) $F_XE_XE_4$	Crawley (1946, Eng.) E_{16}	Warner (1948, Austr.) E_XE_5	Bud 1 (1949, Hung.) E_XE_6	Todd (1950, Eng.) E_{13}
Lee (1940, U.S.A.) $F_8M_{137}E_{171}$	**4516**	76	152	161	<12	90	17
Bon (1943, Austr.) E_{47}	178	**3870**	271	2896	356	1024	633
Paddington (1943, Eng.) $F_XE_XE_3$	317	271	**17378**	256	<12	90	<12
Crawley (1946, Eng.) E_{14}	158	2172	271	**2896**	317	1290	711
Warner (1948, Austr.) E_XE_7	20	968	19	813	**1267**	362	896
Bud 1 (1949, Hung.) E_XE_5	79	2172	152	2896	1742	**2048**	1422
Todd (1950, Eng.) E_{10} (b)	40	1422	79	1267	2896	896	**2172**

TABLE 7

Cross haemagglutination inhibition tests with 5 selected strains of influenza virus B isolated in the period 1940—1950 (one experiment)

FERRET ANTISERA	STRAINS				
	Lee (1940, U.S.A.) $F_8M_{137}E_{182}$	Bon (1943, Austr.) E_{59}	Crawley (1946, Eng.) E_{16}	Warner (1948, Austr.) E_XE_5	Todd (1950, Eng.) E_{13}
Lee (1940, U.S.A.) $F_8M_{137}E_{171}$	**4096**	112	121	<12	19
Bon (1943, Austr.) E_{47}	181	**4516**	2172	362	711
Crawley (1946, Eng.) E_{14}	203	1742	**2172**	203	1129
Warner (1948, Austr.) E_XE_7	25	896	968	**1448**	1129
Todd (1950, Eng.) E_{10} (b)	40	1267	1086	2896	**2845**

TABLE 8

Cross haemagglutination inhibition tests with the strains Bon (1943, Austr.) and Paddington (1943, Eng.*) (one experiment)*

FERRET ANTISERA	STRAINS			
	Bon (1943, Austr.) E_{48} (8/4-'50)	Bon (1943, Austr.) E_{47} (12/6-'50)	Paddington (1943, Eng.) $F_xE_xE_3$ (8/14-'50)	Paddington (1943, Eng.) $F_xE_xE_3$ (12/6-'50)
Bon (1943, Austr.) E_{42}	**4608**	**3840**	4608	4608
Bon (1943, Austr.) E_{47}	**3072**	**2133**	288	288
Paddington (1943, Eng.) $F_xE_xE_1$	24	17	**8192**	**5120**
Paddington (1943, Eng.) $F_xE_xE_3$	512	267	**16384**	**10240**

TABLE 9

Cross haemagglutination inhibition tests with the antisera of 7 selected influenza virus B strains against the egg- and the mouse adapted egg-line of the strain Bon (1943, Austr.) (one experiment)

FERRET ANTISERA	STRAINS		
	Egg-line Bon (1943, Austr.) E_{55}	Egg-mouse-line Bon (1943, Austr.) $E_{57}M_{26}E_5$	Homologous strain
Lee (1940, U.S.A.) $F_8M_{137}E_{172}$	81	242	**2845**
Bon (1943, Austr.) E_{51}	**2048**	6144	**2048**
Paddington (1943, Eng.) $F_xE_xE_3$	203	271	**11585**
Crawley (1946, Eng.) E_{14}	2896	4344	**2258**
Warner (1948, Austr.) E_xE_7	512	1219	**711**
Bud 1 (1949, Hung.) E_xE_5	1448	4344	**1267**
Todd (1950, Eng.) E_{10} (b)	724	2172	**1742**

TABLE 10

Cross haemagglutination inhibition tests with the antisera of the strain Bon (1943, Austr.) (both egg- and mouse-adapted egg-line) against 6 selected influenza virus B strains and the two lines of the strain Bon (one experiment)

FERRET ANTISERA	STRAINS							
	Egg-line Bon (1943, Austr.) E_{55}	Egg-mouse-line Bon (1943, Austr.) $E_{57}M_{26}E_5$	Lee (1940, U.S.A.) $F_8M_{137}E_{160}M_{10}E_8$	Paddington (1943, Eng.) $F_xE_xE_3$	Crawley (1946, Eng.) E_{13}	Warner (1948, Austr.) E_xE_7	Bud 1 (1949, Hung.) E_xE_5	Todd (1950, Eng.) E_{11}
Bon (1943, Austr.) E_{51} Egg-line	**1625**	4876	362	406	1129	356	633	633
Bon (1943, Austr.) $E_{57}M_{26}E_3$ Egg-mouse-line	2580	**8689**	813	645	711	711	633	711

TABLE 11

Cross haemagglutination inhibition tests with 7 selected anti influenza virus B sera against the B strains Lee[1]) *and Montgomery (one experiment)*

FERRET ANTISERA	STRAINS			
	Lee (1940, U.S.A.) $F_8M_{137}E_{160}M_{10}E_{12}$	Lee (1940, U.S.A.) $F_8M_{137}E_{172}$	Montgomery (1940, U.S.A.) $F_{11}M_{25}E_7$	Homologous strains
Lee (1940, U.S.A.) $F_8M_{137}E_{171}$	**4608**	**3413**	1610	**3413**
Bon (1943, Austr.) E_{47}	108	213	298	**3840**
Paddington (1943, Eng.) $F_XE_XE_3$	640	480	1194	**9216**
Crawley (1946, Eng.) E_{14}	192	134	2389	**2048**
Warner (1948, Austr.) E_XE_7	<12	<12	<12	**1380**
Bud 1 (1949, Hung.) E_XE_5	12	12	336	**1610**
Todd (1950, Eng.) E_{10} (b)	16	12	37	**1792**

[1]) Two different lines of the strain Lee are used in this experiment.

TABLE 12

Haemagglutination inhibition tests with 7 selected anti influenza virus B sera against the strain TM (1940, U.S.A.) (one experiment)

FERRET ANTISERA	STRAINS	
	TM (1940, U.S.A.) $F_XM_XE_5$	Homologous strains
Lee (1940, U.S.A.) $F_8M_{137}E_{172}$	136	**2845**
Bon (1943, Austr.) E_{51}	<12	**2048**
Paddington (1943, Eng.) $F_XE_XE_3$	38	**11585**
Crawley (1946, Eng.) E_{14}	<12	**2258**
Warner (1948, Austr.) E_XE_7	<12	**711**
Bud 1 (1949, Hung.) E_XE_5	<12	**1267**
Todd (1950, Eng.) E_{10} (b)	<12	**1742**

TABLE 13

Haemagglutination inhibition tests with the antiserum TM against 7 selected strains of influenza virus B and the homologous strain (one experiment)

FERRET ANTISERUM	STRAINS							
	TM (1940, U.S.A.) $F_XM_XE_5$	Lee (1940, U.S.A.) $F_8M_{137}E_{160}M_{10}E_8$	Bon (1943, Austr.) E_{55}	Paddington (1943, Eng.) $F_XE_XE_3$	Crawley (1946, Eng.) E_{13}	Warner (1948, Austr.) E_XE_7	Bud 1 (1949, Hung.) E_XE_5	Todd (1950, Eng.) E_{11}
TM (1940, U.S.A.) $F_XM_XE_4$	**4876**	<12	<12	19	<12	<12	<12	<12

TABLE 14

Cross haemagglutination inhibition tests with different strains of influenza virus B (one experiment)

FERRET ANTISERA	STRAINS			
	Lee (1940, U.S.A.) $F_8M_{137}E_{160}M_{10}E_8$	TM (1940, U.S.A.) $F_XM_XE_5$	Montgomery (1940, U.S.A.) $F_{11}M_{25}E_7$	Paddington (1943, Eng.) $F_XE_XE_3$
Lee (1940, U.S.A.) $F_8M_{137}E_{172}$	**5792**	242	2258	305
TM (1940, U.S.A.) $F_XM_XE_4$	<12	**17378**	14	12
Montgomery (1940, U.S.A.) $F_{11}M_{25}E_6$	4096	968	**22757**	968
Paddington (1943, Eng.) $F_XE_XE_3$	322	24	1129	**10187**

TABLE 15

Haemagglutination inhibition tests with 7 selected anti influenza virus B sera against two new strains of influenza virus B, isolated in 1951 (one experiment)

FERRET ANTISERA	STRAINS		
	Hokru (1951, Ned.) E_8	Huve (1951, Ned.) E_8	Homologous strains
Lee (1940, U.S.A.) $F_8M_{137}E_{172}$	81	<12	**2438**
Bon (1943, Austr.) E_{51}	512	224	**7168**
Paddington (1943, Eng.) $F_XE_XE_3$	102	<12	**8192**
Crawley (1946, Eng.) E_{14}	1448	141	**6144**
Warner (1948, Austr.) E_XE_7	90	<12	**2172**
Bud 1 (1949, Hung.) E_XE_5	645	158	**4344**
Todd (1950, Eng.) E_{10} (b)	<12	51	**1267**

TABLE 16

Haemagglutination inhibition tests with the antisera of two influenza virus B strains isolated in 1951 against 7 selected strains of influenza virus B and the homologous strains (one experiment)

FERRET ANTISERA	STRAINS								
	Hokru (1951, Ned.) E_8	Huve (1951, Ned.) E_8	Lee (1940, U.S.A.) $F_8M_{137}E_{175}$	Bon (1943, Austr.) E_{51}	Paddington (1943, Eng.) $F_XE_XE_3$	Crawley (1946, Eng.) E_{13}	Warner (1948, Austr.) E_XE_6	Bud 1 (1949, Hung.) E_XE_4	Todd (1950, Eng.) E_{10}
Hokru (1951, Ned.) E_8	**2845**	1024	102	3548	161	4344	610	1422	161
Huve (1951, Ned.) E_8	2258	**2896**	51	2258	51	1536	1219	711	645

STELLINGEN

STELLINGEN

I.

De antigene structuur van de influenza virus B stammen is waarschijnlijk sinds 1943 weinig of niet veranderd.

II.

Bij vaccinatie tegen influenza B dient de in 1940 geïsoleerde stam Lee vervangen te worden door een later geïsoleerde stam, daar het huidige stamtype duidelijk afwijkt van Lee.

III.

Gedurende een influenza epidemie verdient het aanbeveling om, indien mogelijk, artsen, verpleegsters, staphylococcus dragers, lijders aan chronische infectieziekten der luchtwegen en zwangeren te vaccineren.

IV.

Het is gebleken, dat laboratoriumbesmetting bij poging tot isolatie van nieuwe influenza virus stammen nog veelvuldig voorkomt.

V.

Voor de neutralisatie van de aspecifieke remming die mensen- en frettensera in de haemagglutinatie inhibitie test met influenza virussen vertonen, zijn tenminste 2 enzymen nodig (mucinase en R.D.E.). Beide enzymen worden zowel door de Vibrio Cholerae als door de Vibrio El Tor geproduceerd.

VI.

Het is niet onmogelijk, dat de enzymen geproduceerd door de Vibrio Cholerae in gezuiverde vorm therapeutische of diagnostische waarde zullen hebben.

VII.

Pepton, vooraf getest op toxiditeit voor Brucella, kan na verloop van tijd toch toxisch worden voor deze bacteriën. Dit berust mogelijk op een oxydatie-product van cystine.

Ook kan pepton na verloop van tijd de productie van mucinase door de Vibrio Cholerae remmen.

VIII.

De verstrekking van gehoorapparaten dient dringend onder verplicht medisch-technische controle te komen.

IX.

Bij de beoordeling van de cholesterol stofwisseling bij de mens dient rekening gehouden te worden met het feit dat cholesterol snel endogeen, uit eenvoudige bestanddelen, gevormd kan worden.

X.

De mening van NELEMANS, dat het coronair venen bloed een stof zou bevatten die de longvaten kan verwijden, is onvoldoende gefundeerd.

(NELEMANS, F. A.: 1951, Acta Phys. Pharm. Neerl., 2, 19).

XI.

De argumenten van LUMSDEN ten gunste van intrinsieke factoren als beslissend voor de oriëntatie van neurietenuitgroei in vitro zijn niet steekhoudend.

(LUMSDEN, C. E.: 1951, Anat. Rec., C X, 145).

XII.

De anatomie van de para-sympathische vagusinnervatie van de tractus digestivus dient nader onderzocht te worden.