Summary. The serum level of free phenylalanine and phenyllactic acid was determined in 18 patients suffering from phenylpyruvic oligophrenia. No significant amounts of the hydroxy acid were found. The concentration range of the amino acid was between 19 and 38 mg per 100 ml of serum with larger inter- than intra-individual variations. No correlation exists between the degree of mental deficiency and the concentration of phenylalanine in serum. Phenylalanine concentration in the spinal fluid varied from 6.1 to 8.2 mg/100 cc.

It is suggested that although the increased amount of phenylalanine is caused by the metabolic error, its blood plasma level is determined by the ability of the kidney tubuli to reabsorb the amino acid.

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Comparison of Influenza B Virus Strains from the 1950 Epidemic with Strains from Earlier Epidemics. (18110)

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Shortly after the discovery of influenza B virus(1,2), antigenic differences between strains of the agent were noted(3,4). Numerous reports have appeared in which serological studies revealed antigenic differences between strains which had been passed in series in the mouse lung or between such strains and strains which had been maintained by passage only in the chick embryo (3-9). In general, new strains have been compared with the Lee strain or with other strains obtained from the same epidemic. It should be emphasized that the Lee strain which was recovered in 1940(1) had been through many mouse lung passages. In the past, when egg strains have been compared with each other(8), they have tended to show closely similar antigenic patterns. If the so-called adaptation of strains of influenza B virus to the mouse lung is associated with unpredictable alterations in antigenic pattern, as occurs with strains of influenza A virus(10,11), then antigenic differences between mouse lung strains of the former virus might be more apparent than real.

During February and March, 1950, there occurred in the New York area, as elsewhere in various parts of the United States, an epidemic of influenza. From some patients studied in the Hospital of the Rockefeller Institute, influenza B virus was recovered; from others, influenza A virus was obtained. Investigations with acute and convalescent sera from the various patients confirmed these findings; certain patients clearly had influenza B, others had influenza A. Because all strains obtained from the 1950 epidemic in this laboratory were recovered after inoculation of chick embryos and because a number of earlier egg strains of influenza B virus were available, an opportunity was provided to compare various strains recovered since 1945, none of which had ever been passed in the mouse lung. The results of serological studies with these strains indicate that egg strains

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of influenza B virus obtained from various epidemics occurring in different years may show marked variations in antigenic pattern. Such antigenic dissimilarities appear to be as wide as those between egg strains of influenza A virus(12-16).

Materials and methods. Viruses. Three strains of influenza B virus recovered in this laboratory from patients in the 1950 New York epidemic were studied: MB(1), 3rd embryo passage, and MB(2), 3rd embryo passage, represent separate recoveries from one patient admitted to this hospital from Brooklyn, N. Y.; IB1, 5th embryo passage, and IB2, 3rd embryo passage, represent separate strains from 2 patients studied at Irvington House, N. Y. The following strains were received from Dr. T. P. Magill, State University Medical Center at New York: Seattle, 5th embryo passage, recovered at Seattle during 1948-1949; Chaddick, 19th embryo passage, recovered at Buckley Field in 1945; Czech, 9th embryo passage, recovered at Camp Edwards in 1945; I-B-45, 14th embryo passage, recovered at Iowa City in 1945. In addition, B1103, 10th embryo passage, recovered in 1947, was obtained from Dr. R. M. Taylor, International Health Division, The Rockefeller Foundation. All strains had been recovered in the chick embryo; none had been passed in the mouse lung. For purposes of comparison the mouse adapted Lee strains as well as the PR8 strain of influenza A virus were included.

Sera. Immune hamster sera were obtained 3 weeks after the intranasal inoculation of a 10^{-2} dilution of allantoic fluid infected with the desired strain. In each instance serum was pooled from 10 or more immune animals. Immune rabbit antisera were prepared as described previously(17). In each case a pool of sera from 2 rabbits was employed. Immune ferret and rabbit sera against B1103 were kindly provided by Dr. R. M. Taylor.

Hemagglutination-inhibition antibody titrations. A final concentration of 4 hemagglutinating units of virus and 2-fold dilutions of serum were employed in all tests. The procedure and the estimation of the end point were identical to those described previously (17). Rabbit and ferret sera were inactivated in a 1:2 dilution in saline at 65°C for 30 minutes; hamster sera at 56°C for 30 minutes. Rabbit antisera were absorbed with packed chicken erythrocytes resuspended in the sera to give a concentration of 20% at 4°C to remove anti-chick RBC agglutinins. In addition, both rabbit and ferret immune sera were treated to eliminate the so-called nonspecific inhibitor in the following manner: equal volumes of serum and PR8 infected allantoic fluid were mixed, held at 42°C for 3 hours, and then heated at 65°C for 30 minutes to eliminate the virus. With the control anti-PR8 serum a similar procedure using Lee virus was employed. Tests with a number of different viruses showed that this procedure was effective in eliminating almost all of the inhibitor.

Neutralizing antibody titrations. A 10^{-4} dilution of allantoic fluid infected with the desired virus and 2-fold dilutions of inactivated serum were employed in all titrations. The method used was identical to that described previously(17). Each mixture was inoculated into a group of 3 chick embryos; the end point was determined on the basis of the occurrence of hemagglutination with allantoic fluids obtained from each of the embryos inoculated.

Experimental. Results of cross hemagglutination-inhibiting antibody titrations with immune sera and various egg strains of influenza B virus recovered in different years are shown in Table I. It appears evident that all of the strains studied differed markedly from the mouse-adapted Lee strain. The extent of the dissimilarity between these strains and the Lee strain was nearly as

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TABLE I. Cross Hemagglutination-Inhibiting Antibody Titrations with Various Strains of Influenza B Virus.

Virus*		Immune serum MB†	B1103‡	Lee†	PR8†
		Serum titer			
MB (1)	1950	*128*	32	0	0
" (2)	"	64		0	0
IB1	"	64		0	0
IB2	"	32		0	0
Seattle	1948-49	16	0	0	0
B1103	1947	64	*1024*	16	
Chaddick	1945	64	128	0	0
Czech	"	64		16	0
I-B-45	"	32		16	0
Lee	1940	0	128	*512*	0
PR8		0	0	0	*2048*
		Rabbit antiserum			
MB (1)	1950	*768*	96	96	0
" (2)	"	256	64	128	0
IB1	"	256	64	32	0
IB2	"	256	64	64	0
Seattle	1948-49	256	32	64	0
B1103	1947	512	*1024*	512	
Chaddick	1945	512	256	512	0
Czech	"	512	128	512	0
I-B-45	"	256	128	512	0
Lee	1940	384	48	*6144*	0
PR8		0	0	0	*4096*

* 4 units final.
† Hamster serum.
‡ Ferret serum.
Lowest serum dilution = 1:8. Rabbit and ferret serum were treated to eliminate inhibitor.

striking when rabbit antiserum was used as when immune hamster or ferret serum was employed. Of more importance is the evidence that there were wide differences in the antigenic composition of various egg strains. This is especially evident when the cross reactions obtained with MB and B1103 as well as those with Seattle are compared. The absence of any reaction between the anti-influenza B virus sera and PR8 provide an indication that the nonspecific inhibitor had been effectively eliminated by the procedure described above.

When the titer ratios, *i.e.*, homologous titer divided by heterologous titer(8), obtained with MB and B1103 are compared, the extent of the antigenic difference between the 2 strains becomes readily apparent. It has been shown(16) that with any 2 strains the degree of antigenic difference can be expressed in a single figure derived from the 2 heterologous titer ratios, r_1 and r_2, by means of the function: $r = \sqrt{r_1 \times r_2}$. With hamster or ferret immune serum, $r = 1/8$, indicative of an antigenic cross relation between MB and B1103 of the order of but 12%; with rabbit antisera, $r = 1/4$, indicating an antigenic relation of approximately only 25%. Although immune sera against Seattle were not available, the reactions with heterologous sera against MB and B1103 provide a clear indication of the extent of the antigenic dissimilarity among these 3 egg strains. It is apparent that relatively little difference was demonstrated among the three 1945 strains. Similarly, marked indications of differences among the three 1950 strains were not found with available sera. However, that the 1947 strain (B1103) possessed an antigenic composition different to either the 1945 or the 1950 strains seems clear. With Lee and MB the hamster and rabbit sera gave r ratios of 1/64 and 1/11, respectively, indicating an antigenic relationship of no more than 1 to 9%.

The results of cross neutralizing antibody titrations *in ovo* between MB and mouse adapted Lee are shown in Table II. The titer ratios obtained by the *in vivo* procedure provide adequate confirmation of the results obtained with the *in vitro* technic and indicate that the Lee strain was antigenically dissimilar to MB. Computations showed that with hamster immune sera the r ratio for these two strains = 1/8 while with rabbit antisera the r ratio = 1/16, indicating a cross relation between MB and Lee of no more than 6 to 12%.

TABLE II. Cross Neutralizing Antibody Titrations *in ovo* with MB and Lee Strains of Influenza B Virus.

Virus*	Serum titer MB	Lee	PR8
	Hamster immune serum†		
MB	*16*	4	0
Lee	0	*32*	0
	Rabbit antiserum†		
MB	*128*	16	0
Lee	16	*512*	0

* 10^{-4} dilution allantoic fluid.
† Lowest serum dilution = 1:4.

Discussion. That there are antigenic differences between various strains of influenza B virus has been clear for a considerable time(3-9). Due to the possibility that so-called adaptation to the mouse lung is associated with alterations in the antigenic pattern of strains of influenza B virus, as appears to be the case with influenza A virus(10,11), it has not been clear that the antigenic differences observed reflected dissimilarities in strains as they were obtained from man. The results of the present study indicate that strains of influenza B virus recovered on inoculation of the chick embryo and maintained but for a few passages in this species may be markedly dissimilar in antigenic composition despite the fact that they were not passed in the mouse lung. Results obtained recently in this laboratory(16) indicate that as many as 12 serial passages of influenza A virus strains in the chick embryo cause no demonstrable alteration in antigenic pattern. It seems probable that similar results would be obtained in comparable experiments with influenza B virus.

That the differences observed were most evident with strains of influenza B virus obtained in different years is consistent with findings relative to strains of influenza A virus(12-16). Moreover, the extent of the dissimilarity in antigenic pattern appears to be comparable to that found among egg strains of influenza A virus. Since 1947 many workers have referred to strains of influenza A virus which differed strikingly from earlier strains as "A prime" strains(18). If the mouse adapted Lee strain is taken as the standard of reference for influenza B virus, as has been done commonly, then it would be consistent with current usage to designate most of the other strains included in this study as influenza B prime strains. This practice seems undesirable and cannot be recommended; it tends to separate strains into artificial antigenic categories and increases markedly the difficulty of classification and relation. Present evidence suggests that among strains identifiable as influenza B virus there is a more or less continuous spectrum of antigenic differences. At this time it appears impossible to define antigenic categories with satisfactory precision.

The bearing of these findings upon the problem of artificial immunization of man against influenza B requires some comment. It may be that the differences found are not sufficiently great to affect significantly the immunity induced with a vaccine prepared with the Lee strain. Evidence obtained in 1945(6) suggested that this was the case. However, strains recovered in 1950 as well as in 1948-1949(9) appeared to be even more dissimilar to Lee than were those obtained in 1945. It seems possible that current strains are sufficiently different to Lee to justify some doubt as to the desirability of continuing to use the Lee strain exclusively for vaccine production.

Summary. Cross serological reactions indicated that egg strains of influenza B virus obtained in different years were dissimilar in antigenic composition. The extent of the differences in antigenic pattern was analyzed. All strains studied were antigenically different from the mouse adapted Lee strain. The implication of these findings on the problem of immunization in man was discussed.

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