drops are very similar to Okuno's in that both clinical and immunologic reactions were indistinguishable from those obtained by parenteral route.

Rates of complication and death following natural measles are influenced by age of the child when infected, nutritional and health status, and medical facilities of the population. In most well-developed countries, mortality from measles is so low that acceptability of live attenuated measles vaccination has been questioned because of side reaction of high fever. The situation in Taiwan is different. Data from the Provincial Public Health Administration of Taiwan reveals that about the same number of deaths per year (500) could be attributed to measles in Taiwan as in the entire United States with 20 times the population. Prevention of measles infection as a cause of death is more important in Taiwan than in western countries. Consequently, we do not believe that side reactions of attenuated measles vaccine will discourage use of this vaccine on Taiwan.

Large scale field studies of protection of vaccinated children in measles epidemics are needed to verify advantages of live attenuated vaccine administered by the nasal route.

Summary. Edmonston strain measles virus attenuated by repeated passages in chick fibroblast cells was inoculated by nasal drops into 49 infants and children. No signs of immediate reaction to vaccine were observed. Five to 13 days after vaccination 78% of the tested children developed fever. Mild skin eruptions occurred in 29% with onset 9-14 days after inoculation. Sickness or restricted activity was rare even during periods of fever. Both high fever and skin eruption occurred more frequently in infants under 20 months of age. All 13 children tested who did not have antibody in their acute serum developed neutralizing antibody after vaccine inoculation.

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Antigenic Relationship of 1961-1962 Type B Influenza Viruses to Earlier Type B Strains. (28132)

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The changing antigenic constitution of influenza viruses is under continual surveillance to meet the practical problems which arise when viruses become prevalent which differ from predecessors of the same type. The evolution of a new strain of virus presents prob-

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TABLE I. Cross HI Test Using Type B Influenza Viruses and Ferret Antisera.

Ferret antiserum	Influenza virus antigen				
	B/Lee/40	B/Allen/45	B/GL/1739/54	B/Md/1/59	B/Ariz/1/61
B/Lee/40	*320*	0*	0	0	0
B/Allen/45	30	*240*	20	0	0
B/GL/1739/54	20	80	*80*	20	20
B/Maryland/1/59	20	20	320	*1280*	1280
B/Arizona/1/61	30	20	160	1280	*1280*

* 0 = less than 1:10, the lowest serum dilution used.

TABLE II. Cross HI Tests Using Type B Influenza Viruses and Chicken Antisera.

Chicken antiserum	Influenza virus antigen					
	B/Lee/40	B/Allen/45	B/GL/ 1739/54	B/AA/55	B/Md/1/59	B/Ariz/1/61
B/Lee/40	*320*	20	40	40	40	40
B/Allen/45	0*	*160*	10	0	20	20
B/GL/1739/54	10	20	*320*	80	40	40
B/Ann Arbor/55	20	160	320	*320*	20	40
B/Maryland/1/59	20	20	20	20	*160*	160
B/Arizona/1/61	20	10	20	10	120	*120*

* 0 = less than 1:10, the lowest serum dilution used.

lems in diagnosis and control. While the problem of diagnosis need not be serious in the light of present knowledge, considerable information is required for adequate justification for revision of vaccine formulas. This is particularly true with type B influenza strains since antigenic dissimilarities are not nearly as clear cut as among type A strains. It has been suggested that type B viruses be divided into 2 subgroups or sets, B_1 and B_2, by Francis(1). Type B influenza viruses isolated in 1961 and 1962 are closely related to strains isolated since 1956 but show a distinct antigenic difference from strains isolated prior to that time.

Materials and methods. Virus strains. All virus strains used in this study are those maintained at the World Health Organization International Influenza Center for the Americas, Communicable Disease Center, Atlanta, Ga. Strains used in the major portion of this study are infected egg allantoic fluids prepared with B/Lee/40, B/Allen/45, B/Great Lakes/1739/54, B/Maryland/1/59, and B/Arizona/1/61. Other current virus strains used for comparative purposes are indicated below, giving their geographic origin. *Antisera.* All ferret antisera were prepared by intranasal instillation of 2.0 ml of virus diluted to a hemagglutinin titer not exceeding 1:200 as measured by the technic listed below. Ferrets were bled prior to infection and after an interval of 2 weeks. Chicken antisera were prepared by inoculation of 2.5 ml intravenously and 5.0 ml intraperitoneally of virus diluted to a hemagglutinin titer not exceeding 1:200. Serum was obtained prior to immunization and after an interval of 2 weeks.

Hemagglutination (HA) and hemagglutination inhibition (HI) tests. The standard Salk pattern test(2) was used for determining HA titers and performing HI tests. Ferret sera were treated with trypsin and heated at 56°C for 30 minutes, and chicken sera were heated at 56°C for 30 minutes prior to use (3). Serum titers were taken as the highest initial serum dilution showing complete inhibition of hemagglutination or, in some cases, where partial agglutination was observed, titers were determined by interpolation.

Results. Strains representative of virus isolations over a period of years, together with their respective chicken or ferret antisera, were used in the HI test to determine 2-way antigenic relationships of the viruses under study. While only one set of results is presented in Tables I and II, repeated testing has revealed the same antigenic relationships. Although results obtained with chicken antisera are generally less specific, the same pattern

TABLE III. Identification of Several Type B Influenza Viruses by Hemagglutination Inhibition Test.

Virus antigen	Geographic origin	Ferret antiserum B/Lee/40	Ferret antiserum B/GL/1739/54	Ferret antiserum B/Ariz/1/61
B/Lee/40		*640*	40	80
B/Great Lakes/1739/54		0*	*320*	320
B/Arizona/1/61	Arizona	0	20	*1280*
B/Miami/1/61	Florida	0	40	640
B/Canada/380/61	Canada	0	80	640
B/Belfast/1/61	Scotland	0	80	1280
B/Missouri/1/61	Missouri	0	80	1280
B/Georgia/1/61	Georgia	0	160	1280
B/UC/1/62	Illinois	0	160	1280
B/Poland/4/62	Poland	0	80	1280

* 0 = less than 1:10, the lowest serum dilution used.

of reactivity is evident. It is clear, using ferret antisera, that the current strain of virus is antigenically different from strains isolated prior to 1959, although a relationship does exist. When chicken antisera are used, the differences are less striking, although the various strains may be differentiated readily. The B/Ann Arbor/55 strain, related to the B/GL/1739/54 strain, is included to establish the relationship of B/Allen/45 to B/GL/1739/54 since the B/Allen/45 virus did not react to a significantly high titer with the particular B/GL/1739/54 chicken antiserum used in this test.

To eliminate the possibility that a single antigenically different virus might have been selected for study, several viruses isolated in varying geographic areas were used in the HI test with key ferret antisera to establish the fact that the viruses isolated during the current season were an homogenous group. Results are presented in Table III.

It is also possible to demonstrate antigenic differences among influenza viruses on the basis of antibody response following natural infection. Table IV presents 68 cases of current type B influenza virus infection in children of varying ages. The antibody response, approximately 3 weeks following onset, is measured using the B/Great Lakes/1739/54 and the B/Arizona/1/61 antigens. The older children show a good antibody response to both strains in most cases, while the younger children show predominantly only a poor antibody response to the B/Arizona/1/61 antigen. This is presumably due to the younger children not having had prior experience with influenza B by vaccination or infection. The lack of antibody production against the B/Great Lakes/1739/54 antigen in young children may be explained on the basis of the dissimilarities in antigenic constitution described above. Since it is not possible to determine prior antigenic experience in individual cases, a clear cut separation according to age is not evident although there is a preponderance of cases without antibody response to the B/Great Lakes/1739/54 antigen, at age 10 and below. The year of birth of these children is consistent with a time when they may conceivably have escaped initial type B virus infection.

Discussion. It is evident from the foregoing results that the type B influenza viruses isolated since 1959, and more particularly during the 1961-1962 epidemics, are anti-

TABLE IV. HI Antibody Response to Type B Influenza Virus Following Natural Infection.

Age of subject	No. of cases with 4-fold or greater rise/total No. of cases using: B/GL/1739/54 antigen	B/Ariz/1/61 antigen
3	1/2	2/2
5	1/5	5/5
6	3/4	4/4
7	2/4	4/4
8	2/5	5/5
9	7/9	9/9
10	9/13	13/13
11	3/6	5/6
12	3/5	5/5
13	3/3	3/3
14	5/6	6/6
15	4/4	4/4
16	1/1	1/1
17	1/1	1/1
Total	45/68	67/68

genically different from those isolated in previous years. Whether or not these viruses first became prevalent in 1959 is not clear. Pereira(4) at the World Influenza Center, London, indicates that the 1961-1962 viruses are related to a strain isolated as early as 1956. It appears that since the original isolation of type B influenza virus in 1940, there have been at least 2 distinct, but related, groups of agents. Francis(1) has suggested division of earlier type B viruses into 2 subgroups—B_1 and B_2. The B_1 subgroup is represented by the B/Lee/40 and related strains, while the B/Great Lakes/1739/54 and related strains represent the B_2 subgroup.

The strains isolated since 1959 and studied in this report are antigenically different from the B_1 and B_2 subgroups on the basis of results obtained in the HI test. While heterologous titers of the new strains indicate a relationship to earlier strains, the cross reactions are such that the newer viruses may be differentiated easily from the earlier subgroups. That the newer strains are indeed different is also demonstrable using paired sera collected from current cases of type B influenza. Results obtained by such tests are in keeping with the "Doctrine of Original Antigenic Sin"(5), in that antibody responses in older children are measurable by both the infecting virus and an earlier strain. This is presumably due to the patients' prior experience with type B influenza virus. In the younger children, where the likelihood of prior infection is reduced, antibody response to current infection is measurable only with a virus similar or identical to the infecting strain in a significant number of cases.

It appears that the viruses isolated during the 1961-1962 epidemics constitute an homogenous group of viruses since strains isolated in several geographic areas behave similarly in the HI test and the same pattern of antigenic difference is demonstrable with any current virus used as an antigen.

On the basis of these results there is evidence for consideration of further subdivision of the type B influenza viruses. While these results should be supported by data from other laboratories, the newer viruses might be designated as "B_3" since, in this laboratory, there is found as much antigenic difference between the current strains and either the B_1 or B_2 subgroup as there is between the B_1 and B_2 subgroup. However, in keeping with the system of nomenclature officially recommended by the World Health Organization Expert Committee on Respiratory Virus Diseases for type A viruses(6), the newer viruses might be designated as "B_2" if the Committee recognizes the B_2 subgroup of Francis as "B_1".

Summary. Evidence is presented that there has been considerable antigenic change in type B influenza viruses. This evidence is obtained on the basis of results in the hemagglutination inhibition test using sera prepared in animals, and in sera collected from patients recovering from current cases of influenza virus infection. That the viruses constitute an homogenous subgroup is shown by demonstrating that viruses isolated in different geographic areas behave similarly in serologic tests. Consideration should be given to separation of type B influenza viruses into subgroups in an officially adopted system of nomenclature.

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