to 50°C. This observation was further investigated to determine if the second component of the biphasic curve was due to a heterogenous phage population consisting of a relatively small proportion of thermally resistant particles(15). A single plaque obtained by plating phage exposed to 45°C and 50°C for 60 minutes was cored and the phage eluted in broth. Each stock was adjusted to $5 \times 10^5$ PFU/ml and again exposed to 45°C and 50°C. Essentially the same biphasic curves and rates resulted, thus indicating that the survival curves were due to a homogenous phage population.

Thermal inactivation for the basic series of staphylococcus typing phages has not been studied in sufficient detail to warrant a valid comparison of the inactivation results obtained with phage UC-18 and those of the other typing phages. However, Rountree(10) has reported a 5-10% survivor rate for the group B phages exposed to 49°C for 60 minutes. A 10% survivor rate was observed with phage UC-18 under similar test conditions and indicates a degree of similarity between phage UC-18 and those group B phages studied by Rountree(10).

*Summary.* Phage UC-18 was shown to have a latent period of 35 minutes and a burst size of 7 particles per cell. The phage had an average buoyant density of 1.508 g/cm$^3$ and on electron microscopic examination the head was found to have a regular hexagonal silhouette (60 m$\mu$ in diameter) with a tail (165 m$\mu$ in length) terminating in a knob-like baseplate with appendages. Antigenically phage UC-18 appears most closely related to the group B staphylococcal phages. Additionally, the phage was markedly sensitive to heat at 45° to 55°C. Comparison of the properties of phage UC-18 with those reported for the basic series of typing phages suggests that UC-18 should be included within the group B phages.

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## Influenza B in the Spring of 1965. (31647)

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A controlled field study(1) was carried out by our group during the respiratory disease season of 1964-65 to evaluate the protective efficacy of a heptavalent respiratory agent vaccine which contained respiratory syncytial, parainfluenza 1, 2 and 3, and influenza A2 and B viral antigens and *Mycoplasma pneumoniae*. The investigation was carried out in 407

kindergarten and nursery school children in the Havertown-Springfield community, a suburb of Philadelphia. During the course of surveillance and diagnostic testing of cases of respiratory illnesses among the group, a total of 20 strains of influenza B and a single strain of influenza A2 virus were isolated. Four additional strains of influenza B were isolated from children with respiratory illness who were not included in the Havertown-Springfield study. One of the strains of influenza B virus and the influenza A2 virus were isolated from cases which occurred during March, 1965. The remaining influenza viruses were recovered from cases which occurred in April of that year.

The hemagglutination-inhibiting (HI) antibody response in the children to the homologous influenza B/Maryland/1/59 antigen was considered to be excellent(1). Yet the vaccine afforded less than expected reduction in infection with the influenza B viruses which were prevalent among the school children in the vaccination study group in 1965. This appeared to be related to an antigenic difference between the influenza B viruses prevalent in the population and the standard B/Maryland/1/59 vaccine strain which was used in the heptavalent vaccine.

The present report summarizes the findings in a study to compare the antigenic pattern of the influenza B viruses isolated from the children in the 1965 study with that of prototype strains of previous eras. Additionally, the antibody responses to the vaccine in children are presented and the findings in a study to characterize the 1965 B viruses with respect to sensitivity to nonspecific inhibitors are given. The influenza A2 strain was found to be typical of A2 viruses of the contemporary period and is not discussed further.

*Materials and methods. 1965 influenza B viruses.* The details relating to the isolation of the influenza viruses were described previously(1) and a list of the strains is given in Table I. *Prototype influenza B viruses.* The passage histories and sources of both the prototype and 1965 influenza B viruses are summarized in Table II. *Hemagglutinating antigens.* These were allantoic fluids from

TABLE I. Viruses Isolated from Influenza B Cases, 1965.

| Strain | Patient: Vaccination status | Patient: Illness | Patient: Date of illness |
|---|---|---|---|
| Vaccine study group* | | | |
| B/Phil/151/65 | Yes | Mild | April 8 |
| " " 248 " | " | " | " 12 |
| " " 254 " | " | " | " 20 |
| " " 379 " | " | " | " 1 |
| " " 453 " | " | " | " 20 |
| " " 495 " | " | " | " 19 |
| " " 503 " | " | " | " 12 |
| " " 537 " | " | Severe | " 22 |
| " " 245 " | No | Mild | " 13 |
| " " 454 " | " | " | " 6 |
| " " 507 " | " | " | " 15 |
| " " 575 " | " | " | " 27 |
| " " 593 " | " | " | " 6 |
| " " 607 " | " | " | " 12 |
| " " 54 " | " | Severe | March 4 |
| " " 371 " | " | " | April 19 |
| " " 378 " | " | " | " " |
| " " 394 " | " | " | " 15 |
| " " 487 " | " | " | " " |
| " " 591 " | " | " | " 27 |
| Other | | | |
| B/Phil/295/65 | Yes | Mild | April 13 |
| " " 311 " | " | " | " 30 |
| " " 300 " | " | Severe | " 20 |
| " " 301 " | No | Mild | " " |

* There were 199 vaccinated and 208 unvaccinated children in the study groups.

embryonated hens' eggs infected with influenza B viruses in the passages shown in Table II. *Chicken antisera.* Adult White Leghorn roosters were inoculated intravenously with 3 ml and intraperitoneally with 5 ml of the appropriate virus-infected allantoic fluid followed by a second 5 ml of the same virus given intraperitoneally 5 to 6 weeks later. The passage histories of the viruses used to immunize chickens are shown in Table II. The chickens were bled prior to the first dose of virus and 1 week following the second inoculation. In certain instances a third intraperitoneal dose of virus followed by bleeding 1 week later was required to achieve a sufficiently high antibody titer. *Vaccine.* The vaccine(1,2) was a heptavalent alum formulation which contained formalin-killed respiratory syncytial, parainfluenza 1, 2 and 3, influenza A2 and B viruses and *Mycoplasma pneumoniae.* The influenza B strain (B/Maryland/1/59) was the standard vaccine virus(3). The experimental vaccine

TABLE II. Source and Passage History of Influenza B Viruses Used to Prepare Typing Antisera and Hemagglutinating Antigens.

| Strain | Passage history of virus — Chicken immunization | Passage history of virus — HI tests | Source |
|---|---|---|---|
| 1965 B viruses | | | |
| B/Phil/537/65 | GMK-2/E-1 and 2* | GMK-2/E-2 | Present study |
| B/Phil/593/65 | GMK-1/E-2 and 3 | GMK-1/E-3 | " " |
| B/Phil/607/65 | GMK-1/E-3 | GMK-1/E-3 | " " |
| B/Phil/301/65 | GMK-1/E-1 and 2 | GMK-1/E-2 | " " |
| B/Phil/295/65 | GMK-1/E-2 | GMK-1/E-2 | " " |
| Other 1965 strains | — | GMK-1/E-2 or 3 | " " |
| Prototype viruses | | | |
| B/Taiwan/2/62 | E-8 and 9 | E-9 | N.I.H.† |
| B/Miami/2/61 | E-8 | E-8 | " |
| B/Canada/380/61 | E-10 | E-10 | " |
| B/Md/1/59 | MK-3/E-7 | MK-3/E-9 | " |
| B/Va/301/55 | E-8 | E-7 | E. Buescher |
| B/IBl/50 | E-5 and 6 | E-6 | F. Horsfall |
| B/Lee/40 | F-8/M-310/E-8 and 9 | F-8/M-310/E-9 | T. Francis |

* GMK = Grivet monkey kidney cell culture; E = Embryonated hens' eggs; F = Ferret; Numbers = Passage number.

† Division of Biologics Standards, National Institutes of Health.

contained 125 CCA units of influenza B antigen per dose in contrast to the ordinary vaccine which contains 200 CCA units per dose. However, the experimental vaccine was given in 3 rather than 1 or 2 doses ordinarily employed for immunization of human beings against influenza. *Human sera.* The children (1) received 3 one ml doses of the killed heptavalent virus vaccine given 1 month apart during the period from September 24 to December 17, 1964. The sera included in the present tests were taken immediately prior to vaccination and 1 month following the third vaccine dose. All sera were stored frozen at —20°C up to nine months prior to testing. The serum titers were expressed as initial dilution in the tests prior to adding the other reagents.

*Hemagglutination-inhibition tests.* The tests were performed by the usual Salk(4) procedure. The sera were treated with trypsin-periodate, or trypsin or periodate alone to attempt removal of nonspecific inhibitors. These procedures were consistent with the methods described by Jensen *et al*(5). Periodate treatment was unsatisfactory and trypsin followed by heating was used routinely unless otherwise stated. Heating was at 56°C for 30 minutes.

*Results. Isolation of influenza viruses.* Table I summarizes the isolation of influenza B viruses from throat specimens collected from the children who exhibited signs and symptoms of respiratory illness. Severe respiratory illness was separated from mild illness based on clinical findings and on whether the maximum recorded temperature was above or below 100°F (0), respectively (1). As described earlier(1), 8 of the 199 vaccinated children and 12 of the 208 controls who were ill with respiratory disease excreted influenza B virus in the time periods shown in the Table. Isolation of influenza B from a child on more than 1 occasion in serial specimens was counted only once. The infection rates among the controls and vaccinees were 5.8/100 and 4.0/100, respectively. The difference in rates was —1.8/100 or —31%; this difference was not significant statistically. The illness in the vaccinated persons was less severe than in the controls; ⅛ (13%) of vaccinees who excreted influenza B virus displayed severe illness and 6/12 (50%) of controls had severe illness. Though the vaccine was probably somewhat protective, the performance was below that which is expected (6) for vaccine evaluated in the course of natural challenge with an antigenically similar epidemic strain of virus.

*Treatment of sera to remove nonspecific inhibitors.* Human and animal sera contain mucoprotein inhibitors which are unassociated

TABLE III. Influence of Serum Treatment on Specificity of the Hemagglutination-Inhibition Test for Antiviral Antibody.

| Serum tested | Serum sample | Type B influenza test virus | Reciprocal of hemagglutination-inhibition titer of serum treated with: Untreated | Heat | Trypsin-heat | Trypsin-periodate-heat | Periodate |
|---|---|---|---|---|---|---|---|
| Rooster #76359, immunized *vs* B/Phil/295/65 | Pre-vacc. | Phil/295/65 | 0* | 0 | 0 | 80 | 80 |
| | " | 593 | 0 | 0 | 0 | 80 | 80 |
| | " | Md/1/59 | 0 | 0 | 0 | 0 | 0 |
| | Post-vacc. | Phil/295/65 | 5,120 | 10,240 | 5,120 | 2,560 | 5,120 |
| | " | 593 | 5,120 | 10,240 | 10,240 | 5,120 | 5,120 |
| | " | Md/1/59 | 5,120 | 10,240 | 10,240 | 5,120 | 5,120 |
| Child 205, age 4 yr, immunized *vs* B/Md/1/59 in heptavalent vaccine | Pre-vacc. | Phil/295/65 | 0 | 0 | 0 | 320 | — |
| | " | 593 | 0 | 0 | 0 | 320 | — |
| | " | Md/1/59 | 0 | 0 | 0 | 0 | — |
| | Post-vacc. | Phil/295/65 | 40 | 20 | 10 | 1,280 | — |
| | " | 593 | 40 | 20 | 10 | 640 | — |
| | " | Md/1/59 | 160 | 80 | 80 | 80 | — |
| Child 137, age 5 yr, not vaccinated | — | Phil/295/65 | 20 | 40 | 10 | 1,280 | 320 |
| | — | 593 | 20 | 40 | 20 | 2,560 | 640 |
| | — | Md/1/59 | 10 | 20 | 10 | 0 | 10 |
| Child 19, age 11 mo, not vaccinated | — | Phil/295/65 | 10 | 10 | 0 | 640 | — |
| | — | 593 | 10 | 20 | 0 | 640 | — |
| | — | Md/1/59 | 10 | 10 | 0 | 0 | — |
| Child 113, age 4 yr, not vaccinated | — | Lee/40 | 0 | 0 | — | 0 | — |
| | — | IBl/50 | 0 | 0 | — | 0 | — |
| | — | Va/301/55 | 0 | 0 | — | 0 | — |
| | — | Md/1/59 | 0 | 0 | — | 0 | — |
| | — | Can/380/61 | 0 | 0 | — | 0 | — |
| | — | Miami/2/61 | 0 | 0 | — | 0 | — |
| | — | Taiwan/2/62 | 0 | 0 | — | 0 | — |
| | — | Phil/295/65 | 0 | 0 | — | 80 | — |
| | — | 593 | 0 | 10 | — | 320 | — |
| | — | 537 | 0 | 0 | — | 20 | — |
| | — | 607 | 0 | 0 | — | 10 | — |
| | — | 301 | 0 | 0 | — | 40 | — |

* 0 titer = <1:10.

with specific antibody and which give false positive tests in the hemagglutination-inhibition test(7). The various strains of influenza virus differ markedly with respect to sensitivity to inhibitor and it is always essential that they be removed lest spurious results be obtained.

The sera from normal chickens do not contain influenza B antibody and the sera from unvaccinated children in the first few years of life are often devoid of influenza B antibody or have it present in small amount. Such sera are the best vehicle for testing for nonspecific inhibitor and for its removal. The results of studies to find the optimal procedure for serum treatment in tests using the 1965 influenza B and other strains of virus are summarized in Table III. The untreated, heat-treated and trypsin-heated prevaccination rooster sera were free of inhibitor against the 1965 influenza B strains and against the B/Md/1/59 virus. These sera, which were initially devoid of inhibitor, acquired inhibitor for the 1965 B viruses but not for B/Md/1/59 following treatment with trypsin-periodate-heat or with periodate alone. It was apparent from this that an inhibitor for the 1965 viruses was generated upon exposure of the serum to periodate. The same phenomenon of conversion from little or no inhibitor to high titer inhibitor against 1965 B viruses by treatment with periodate, either with or without exposure to trypsin, was shown in numerous examples in the Table with the human sera. The phenomenon was limited to the 1965 influenza B viruses and was not demonstrated for any of the viruses of earlier vintage. Though serum treatment appeared unneces-

TABLE IV. Cross-Hemagglutination-Inhibition Tests with Chicken Antisera Employing 1965 Influenza B Isolates and Prototype Strains from Previous Years.

| Chicken antiserum | | Reciprocal of adjusted* hemagglutination-inhibiting antibody titer against: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Prototype B strains | | | | | | | 1965 B strains | | | | |
| Strain | Reciprocal of homologous HI titer (actual) | Lee/40 | IBl/50 | Va/301/55 | Md/1/59 | Can/380/61 | Miami/2/61 | Taiwan/2/62 | Phil/295/65 | Phil/301/65 | Phil/537/65 | Phil/593/65 | Phil/607/65 |
| Prototype strains | | | | | | | | | | | | | |
| B/Lee/40 | 1,280 | *640* | 40 | <10 | 40 | 40 | 40 | 10 | 10 | 20 | 20 | 20 | 20 |
| B/IBl/50 | 320 | 160 | *640* | 160 | 160 | 40 | 20 | 80 | 40 | 80 | 80 | 80 | 40 |
| B/Va/301/55 | 2,560 | 160 | 320 | *640* | 640 | 640 | 640 | 160 | 320 | 640 | 640 | 640 | 320 |
| B/Md/1/59 | 2,560 | 20 | 10 | 40 | *640* | 80 | 80 | 20 | 40 | 80 | 80 | 80 | 40 |
| B/Can/380/61 | 640 | 160 | 160 | 640 | 2560 | *640* | 640 | 160 | 640 | 2560 | 2560 | 1280 | 320 |
| B/Miami/2/61 | 1,280 | 320 | 160 | 640 | 2560 | 640 | *640* | 1280 | 320 | 1280 | 1280 | 1280 | 640 |
| B/Taiwan/2/62 | 2,560 | <10 | 10 | 160 | 160 | 80 | 40 | *640* | 80 | 320 | 320 | 320 | 80 |
| 1965 strains | | | | | | | | | | | | | |
| B/Phil/295/65 | 10,240 | 10 | <10 | 320 | 160 | 320 | 320 | 160 | *640* | 320 | 160 | 320 | 320 |
| B/Phil/301/65 | 2,560 | 80 | 20 | 320 | 640 | 320 | 320 | 640 | 160 | *640* | 640 | 640 | 320 |
| B/Phil/537/65 | 2,560 | 20 | 10 | 160 | 640 | 160 | 160 | 640 | 320 | 640 | *640* | 640 | 320 |
| B/Phil/593/65 | 2,560 | 10 | 10 | 80 | 640 | 80 | 160 | 160 | 160 | 640 | 640 | *640* | 160 |
| B/Phil/607/65 | 320 | 20 | 10 | 80 | 320 | 160 | 160 | 80 | 640 | 640 | 320 | 640 | *640* |

* Adjusted to a titer of 1:640 in tests with homologous virus.

sary, the trypsin-heat method was routinely employed since it did not destroy specific antibody and since nonspecific inhibitors for other than 1965 strains might have been encountered.

*Antigenic comparison of 1965 influenza B viruses with prototype strains. Chicken antiserum.* The findings in cross-HI tests to compare the antigenic composition of the various influenza B viruses are summarized in Table IV. The titers of the antisera tested with homologous virus ranged from 1:320 to 1:10,240. To facilitate comparisons between strains, each serum was assigned a homologous titer value of 1:640 and the values obtained in tests with heterologous strains were reduced or elevated by the appropriate correction factor. The most significant fact was that the antiserum against the B/Md/1/59 virus used in the heptavalent vaccine showed only 1/8 to 1/16 as high antibody titer when tested with the 1965 B viruses as with the homologous virus. Antisera against the 1965 strains, by contrast, showed the same or no less than 1/4 the titer against B/Md/1/59 as against the homologous viruses. The 1965 influenza B viruses appeared to be relatively homogenous antigenically.

*Human sera.* Table V presents a summary of the HI findings in tests with prototype and 1965 B strains of sera from children who were given heptavalent respiratory vaccine containing the B/Md/1/59 viral antigen. All the children showed excellent antibody responses against the homologous B/Md/1/59 strain used in the vaccine; post vaccination titer against homologous virus showed 4- to 32-fold increases in antibody titer compared with the prevaccination specimen. The prevaccination serum titers against the B/Md/1/59 and the 1965 viruses were roughly comparable, with 4-fold difference in only 4 instances. By contrast, the post vaccination antibody titer was 8-fold lower against the 1965 virus than against homologous B/Md/1/59 in 6 instances, 4-fold lower in 15 instances, 2-fold lower in 12 instances, and the same in 7 instances. While these results reflected the same differences as shown in tests with chicken antisera, the magnitude was not as great. The serologic responses to all the influenza B viruses recovered since 1959 were generally less than against the homologous 1959 strain in the vaccine.

The antigenic difference between the B/Md/1/59 virus and the 1965 strain was emphasized further in tests which were made of the pre and post vaccination sera from the

TABLE V. Hemagglutination-Inhibiting Antibody Responses Against 1965 and Prototype Influenza B Viruses in Children Who Received Heptavalent Respiratory Virus Vaccine Containing B/Md/1/59 Influenza Virus Antigen.

| Vaccine No. | Vaccine Age (yr) | Serum specimen | Reciprocal of hemagglutination-inhibiting antibody titer against: Prototype B strains Lee/40 | IBl/50 | Va/301/55 | Md/1/59 | Can/380/61 | Miami/2/61 | Taiwan/2/62 | 1965 B strains Phil/295/65 | Phil/301/65 | Phil/537/65 | Phil/593/65 | Phil/607/65 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 5 | Pre-vacc. | 0* | 0 | 10 | 40 | 10 | 10 | 0 | 10 | 20 | 10 | 10 | 10 |
| | | Post-vacc. | 10 | 20 | 80 | *160* | 80 | 40 | 20 | 80 | 80 | 40 | 80 | 80 |
| 38 | 4 | Pre-vacc. | 10 | 0 | 0 | 20 | 10 | 10 | 0 | 20 | 20 | 20 | 20 | 20 |
| | | Post-vacc. | 10 | 0 | 20 | *80* | 40 | 40 | 20 | 20 | 40 | 40 | 40 | 40 |
| 70 | 5 | Pre-vacc. | 20 | 10 | 20 | 40 | 20 | 20 | 10 | 20 | 20 | 20 | 20 | 20 |
| | | Post-vacc. | 20 | 20 | 80 | *320* | 80 | 40 | 20 | 80 | 80 | 40 | 80 | 80 |
| 138 | 5 | Pre-vacc. | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 10 | 0 | 10 | 10 |
| | | Post-vacc. | 20 | 10 | 160 | *320* | 160 | 80 | 20 | 40 | 40 | 40 | 40 | 80 |
| 385 | 4 | Pre-vacc. | 10 | 0 | 0 | 10 | 0 | 0 | 0 | 10 | 10 | 10 | 10 | 20 |
| | | Post-vacc. | 20 | 10 | 80 | *160* | 80 | 40 | 10 | 40 | 40 | 40 | 40 | 80 |
| 113 | 4 | Pre-vacc. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 10 |
| | | Post-vacc. | 0 | 0 | 160 | *80* | 80 | 80 | 40 | 80 | 80 | 40 | 80 | 80 |
| 205 | 4 | Pre-vacc. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | Post-vacc. | 0 | 0 | 20 | *40* | 40 | 40 | 20 | 40 | 20 | 20 | 40 | 40 |
| 219 | 5 | Pre-vacc. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 10 | 10 | 10 |
| | | Post-vacc. | 0 | 0 | 20 | *80* | 20 | 20 | 80 | 20 | 20 | 10 | 20 | 20 |
| 100 | 11 mo | Unvacc. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 10 | 10 | 10 | 10 |
| 107 | 9 " | " | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 10 | 10 | 10 | 10 | 10 |

* 0 titer = <1:10.

particular vaccinated children in the vaccination study from whom the influenza B viruses were isolated. The findings given in Table VI showed that the post vaccination titers against homologous B/Md/1/59 virus ranged from 1:80 to 1:320 while those against the 1965 strains were only 1:20 or 1:40. The final antibody titer against homologous virus was 4-fold higher 10 times, 8-fold higher four times, and 16-fold higher twice than against the 1965 viruses.

*HI antibody against influenza B viruses in normal adults.* The antigenic deviation of influenza B viruses of recent vintage from the 1959 B/Md/1 strain raised the question of the general level of immunity against contemporary influenza B in the adult human population. Accordingly, 19 sera taken from employees at the Merck & Co., Rahway, N. J., plant during June, 1965 were tested for the HI antibody against the 1965 and prototype strains. The findings presented in Table VII showed that there was appreciable antibody against nearly all the influenza viruses except the old 1940 Lee virus. The levels of antibody were somewhat higher in persons recently vaccinated than among those who were not. The levels of antibody were roughly of the same order of magnitude as those of the children who became ill and excreted influenza B virus. Judged by antibody titer alone, it would appear that some of the adults were susceptible to influenza B infection though other factors such as composite immunity, which increases with age, and level of antibody in the respiratory secretion may also be important in determining susceptibility.

*Discussion.* Immunity to influenza virus generally depends upon a sufficiently high level of specific antibody in the respiratory secretions to be protective against infection with viruses which are circulating currently in the population. The level of antibody in the respiratory secretions is roughly proportional, in most instances, to the amount of antibody in circulating blood of the same individual. Because of this, vaccination against

influenza may be accomplished by elevating the level of circulating antibody by means of parenteral administration of killed virus vaccine. Protection against influenza, therefore, might be accomplished with regularity by periodic administration of a vaccine of constant strain composition were it not for the oustanding capacity for antigenic change in the viruses in nature, thereby rendering prior immunity noneffective. This is especially true of the influenza A and to a lesser extent of the influenza B viruses(8). Shift in antigenic constitution of strains is often reflected in the capacity of antisera prepared against the new viruses to cross-react well with strains of perhaps the previous 5 to 10 years but for antisera prepared against the older strains to cross-react poorly, if at all, with new strains. Because of such antigenic shifts in the influenza strains in nature, it is necessary to update the vaccine in order to keep it consistent with the antigenic composition of strains currently in circulation in the population.

There are no precise criteria at this time for appraising the significance of antigenic

TABLE VI. Hemagglutination-Inhibiting Antibody Response to Vaccination in Children from Whom Influenza B Viruses Were Subsequently Isolated.

| Case No. | Age (yr) | Serum specimen | Reciprocal of hemagglutination-inhibiting antibody titer against: Vaccine strain B/Md/1/59 | 1965 B strains B/Phil/295/65 | 1965 B strains B/Phil/537/65 |
|---|---|---|---|---|---|
| 151 | 5 | Pre-vacc. | 40 | 40 | 20 |
| | | Post-vacc. | 80 | 20 | 20 |
| 248 | 5 | Pre-vacc. | 20 | 10 | 10 |
| | | Post-vacc. | 320 | 40 | 40 |
| 254 | 4 | Pre-vacc. | 20 | 20 | 20 |
| | | Post-vacc. | 320 | 40 | 40 |
| 379 | 5 | Pre-vacc. | 20 | 20 | 10 |
| | | Post-vacc. | 160 | 40 | 40 |
| 453 | 4 | Pre-vacc. | 40 | 20 | 20 |
| | | Post-vacc. | 640 | 40 | 40 |
| 495 | 5 | Pre-vacc. | 20 | 20 | 20 |
| | | Post-vacc. | 160 | 40 | 40 |
| 503 | 5 | Pre-vacc. | 40 | 20 | 20 |
| | | Post-vacc. | 80 | 20 | 20 |
| 537 | 5 | Pre-vacc. | 20 | 20 | 10 |
| | | Post-vacc. | 80 | 20 | 20 |

TABLE VII. Hemagglutination-Inhibiting Antibody Titers Among Normal Human Adults Against 1965 and Prototype Strains of Influenza B Viruses of Previous Eras.

| Volunteer | Age | Last influenza vaccination | Reciprocal of hemagglutination-inhibiting antibody titer against: Prototype B strains Lee/40 | IBl/50 | Va/301/55 | Md/1/59 | Can/380/61 | Miami/2/61 | Taiwan/2/62 | 1965 B strains Phil/295/65 | Phil/301/65 | Phil/537/65 | Phil/593/65 | Phil/607/65 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 17 | None | 0* | 20 | 40 | 40 | 20 | 40 | 40 | 20 | 20 | 20 | 40 | 20 |
| 13 | 18 | " | 0 | 10 | 20 | 40 | 20 | 20 | 20 | 20 | 20 | 20 | 40 | 20 |
| 22 | 19 | " | 10 | 40 | 160 | 40 | 160 | 80 | 10 | 20 | 40 | 40 | 40 | 40 |
| 18 | 20 | " | 0 | 20 | 20 | 40 | 40 | 40 | 0 | 20 | 20 | 20 | 40 | 40 |
| 11 | 21 | " | 0 | 20 | 40 | 20 | 20 | 20 | 10 | 20 | 20 | 20 | 40 | 20 |
| 6 | 22 | " | 0 | 40 | 20 | 20 | 10 | 20 | 10 | 20 | 20 | 20 | 40 | 20 |
| 14 | 24 | " | 0 | 40 | 20 | 20 | 10 | 10 | 10 | 20 | 20 | 20 | 40 | 20 |
| 8 | 44 | " | 0 | 10 | 20 | 20 | 10 | 20 | 20 | 20 | 20 | 20 | 40 | 20 |
| 19 | 49 | " | 20 | 10 | 20 | 40 | 40 | 40 | 10 | 20 | 40 | 40 | 40 | 40 |
| 21 | 53 | " | 20 | 20 | 80 | 80 | 80 | 80 | 40 | 40 | 40 | 80 | 80 | 40 |
| 12 | 29 | 1957 | 80 | 80 | 80 | 40 | 40 | 40 | 80 | 40 | 40 | 40 | 80 | 80 |
| 24 | 40 | 1963 | 20 | 20 | 40 | 40 | 40 | 40 | 10 | 40 | 40 | 40 | 40 | 40 |
| 27 | 42 | " | 20 | 40 | 40 | 80 | 40 | 80 | 10 | 40 | 40 | 40 | 40 | 40 |
| 20 | 44 | 1962 | 10 | 10 | 20 | 40 | 40 | 40 | 10 | 20 | 40 | 40 | 40 | 40 |
| 5 | 48 | 1964 | 80 | 40 | 160 | 160 | 80 | 80 | 160 | 160 | 160 | 160 | 160 | 160 |
| 9 | 52 | " | 40 | 40 | 80 | 40 | 40 | 40 | 160 | 80 | 80 | 80 | 80 | 80 |
| 23 | 54 | 1963 | 0 | 10 | 20 | 20 | 20 | 20 | 0 | 20 | 20 | 20 | 20 | 20 |
| 25 | 57 | 1964 | 40 | 10 | 40 | 160 | 80 | 80 | 20 | 40 | 80 | 80 | 80 | 80 |
| 17 | 61 | " | 10 | 20 | 20 | 40 | 40 | 40 | 10 | 20 | 20 | 40 | 40 | 40 |

* 0 titer = <1:10.

deviation of influenza B virus, as measured in the laboratory, in relation to protective efficacy of the vaccine. The data in this report are of value in that they shed light on the degree of antigenic deviation of influenza B virus which is associated with reduction of vaccine efficacy to apparent borderline protective level. Based on the present findings, it appears that the 1965 influenza B viruses deviated significantly from the prototype B/Md/1/59 vaccine strain. This was most evident in cross-HI tests with chicken antiserum and, as expected, was less apparent in tests with paired sera from children who were given a vaccine containing the B/Md/1/59 strain. Antigenic difference of a 1965 influenza B strain (B/Georgia/1/65) from B/Md/1/59 has also been shown by others (9). Though there are no rigid standards which can be applied, it has been noted by one of us (M.R.H.) that generally when chicken antiserum against a type A vaccine strain shows 1/8 or less HI antibody titer when tested with a new virus compared with homologous virus, then the vaccine may show only borderline protective efficacy in man. This was the case in the failure of the A/PR8/34 and A/Weiss/43 strains to provide the expected level of protection against A1 virus in 1947(10,11). Difference of this magnitude was also shown in the present tests. Additionally, it generally appears that the titer of circulating HI antibody against the infecting strain of virus must be around 1:32 or greater in order to be minimally protective against the disease in nature. The present studies showed that the post vaccination sera taken from the children who subsequently became infected with influenza B virus all titered 1:20 or 1:40 against the 1965 B viruses. The rates for occurrence of influenza B viruses in the throat secretions of the vaccinated and control children in the study were so nearly alike as to indicate only weak protective efficacy of the vaccine(6). Some protection was suggested in that the virus recovery rate was 31% lower from vaccinees than from controls (though not significant statistically) and since the illness was most often milder in vaccinees than in controls. The level of antibody against 1965 influenza B strains in most of the sera from normal adults which were tested was in the borderline 1:20 to 1:40 titer range and this is consistent with the sporadic appearance of influenza B during the respiratory season of 1964-65. Apparent failure of protection against influenza B virus by ordinary polyvalent influenza vaccine was recorded in an epidemic which occurred in Jefferson County, Colorado, during January through March of 1965(12).

Influenza A and B viruses are commonly susceptible to nonspecific inhibition of hemagglutination by mucoprotein substances which are present in sera and which give rise to false positive results in the tests(7). For antibody assay by the HI procedure it is necessary, therefore, to remove these inhibitors which confuse or obscure the true levels of antibody. To remove inhibitor, the sera may be treated(13) with cholera filtrate containing receptor destroying enzymes (RDE), or with trypsin or periodate(5). These methods of treatment are usually quite satisfactory. The findings in the present study with 1965 influenza B viruses present a unique situation in which exposure to periodate "created" inhibitor rather than destroyed it. Only one previous example(13) of this effect known to the authors was the apparent development of inhibitor to influenza C virus in a human serum specimen treated with cholera filtrate. This finding emphasizes the need to ascertain the effect of serum-treatment for each virus prior to routine use in diagnostic tests or in strain analyses.

*Summary.* Strains of influenza B virus recovered from children who had acute respiratory illnesses during the spring of 1965 showed antigenic deviation from the B/Md/1/59 strain of influenza virus. This finding was most evident in comparative hemagglutination-inhibition tests with chicken antisera but was also shown in the relatively poor antibody response against 1965 B strains compared with homologous virus in children who were given 3 doses of a heptavalent respiratory virus vaccine containing B/Md/1/59 antigen. Further evidence was given in the demonstration of less than expected efficacy of the vaccine in protecting children against

influenza B in 1965 in a controlled field study. Sera from normal adults collected in June of 1965 often showed low antibody titers and this was consistent with the sporadic occurrence of influenza B which was recorded in the U.S.A. during the 1964-65 respiratory disease season. The findings in the study may be of value as a guideline for judging the significance of future antigenic deviation of influenza B viruses. The 1965 influenza B viruses were unique in being sensitive to a nonspecific inhibitor in chicken and human sera which was "created" by treatment with periodate. This finding emphasized the importance of establishing the utility, with each virus, of the methods used to remove nonspecific inhibitors prior to their routine application.

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## Persistent Polioviral Infection of the Intact Amniotic Membrane. II. Existence of a Mechanical Barrier to Viral Infection. (31648)

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In the animal host dissemination of virus within a given tissue is often limited, and diffuse cellular destruction is seldom observed. The liberation of interferon(1) and other antiviral substances by infected cells may account in part for some instances of viral inhibition, although it seems unlikely that such mechanisms are solely responsible for the localization of viral lesions. To assess the susceptibility of animal cells to a given virus *in vivo* one must consider the possibility that viral particles may not always have free access to these cells. Thus the arrangement of the stroma or a layer of mucus covering the cells may prevent viral adsorption to potentially susceptible cells.

In contrast to this *in vivo* cellular resistance, cells cultivated under artificial conditions *in vitro* are usually susceptible to infection by numerous viruses. Poliovirus type I for example multiplies readily in human amnion monolayer cell cultures inducing complete cellular destruction(2). If, however, the natural arrangement of cells of the membrane is left undisturbed and the membrane maintained in a nutritive medium, the behaviour of poliovirus in this system is entirely different from that observed in monolayer cell