PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 200–204 (1974)

Studies on Vaccination of Infants Against Influenza with Influenza Hemagglutinin[1] (38069)

A. V. HENNESSY AND F. M. DAVENPORT

Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan 48104

There is little information available on the optimum dose and schedule for the vaccination of infants against influenza, since administration of inactivated influenza virus vaccines to infants occasions a high rate of severe febrile reactions (1). Therefore, when it was found that large doses of ether extracted polyvalent influenza hemagglutinin vaccine could be given to very young infants without inducing unacceptable reactions, a systematic study was initiated on antibody response of infants to administration of aqueous or aluminum phosphate adsorbed polyvalent influenza hemagglutinin vaccines employing various vaccination schedules (2). The results constitute the body of this report.

Materials and Methods. Viruses. Strains of influenza viruses used for measuring antibody levels were selected from the collection of the Strain Study Center, Commission on Influenza, School of Public Health, Ann Arbor, Michigan. When testing for antibody against A_2/Japan/305/57, the antigenically identical strain A_2/AA/23/57 was substituted since it is more avid and is less sensitive to inhibitor. Vaccines were prepared commercially using lines of virus obtained from the Division of Biological Standards, National Institutes of Health, Bethesda, Maryland.

Vaccines. Purified hemagglutinin concentrates were prepared as previously described (3). The vaccine used in the first study contained 250 chick cell agglutinating (CCA) units per 0.25 ml divided as follows: A_2/Japan/305/57 and A_0/PR/8/34, 50 units each: Swine/1976/31, A_2/Japan/170/62, A_1/AA/1/57, B/Lee/40, B/GL/54 and B/Md/59, 25 units each.

The second study employed two vaccines —one aqueous and the other aluminum phosphate adsorbed, each containing 300 CCA units per 0.5 ml divided as follows: A_0/PR/8/34 and A_1/AA/1/57, 50 CCA units each; A_2/Taiwan/1/64 and B/Md/1/59 100 CCA units each.

Subjects, measurements, bleeding schedule. Infants attending the Well Baby Clinic of the University of Michigan Hospital were subjects of the studies. Consent for participation was obtained in writing from parents prior to giving vaccine. Parents were instructed to take rectal temperature at 6 hr intervals during the first 24 hr after vaccination. Body temperatures of 100°F or greater were classified as fever. Reports of vaccine reaction were obtained from parents by telephone 24 hr after the Clinic visit and again at the next Clinic visit. In the first study, blood specimens were obtained before each vaccination and 1 mo after the last. In the second study, blood specimens were obtained before the first inoculation, 1 mo after the second, and before and 1 mo after the "booster."

Hemagglutination-inhibition. Antibody was measured by means of the standard hemagglutination-inhibition (HAI) technique (4). Sera from the first study were treated with trypsin and periodate (5) be-

[1] This investigation was conducted under the sponsorship of the Commission on Influenza, Armed Forces Epidemiological Board, and was supported by the U. S. Army Medical Research and Development Command, Department of the Army, under Research Contract DADA 17-70-C-0050.

TABLE I. Geometric Mean HAI Antibody Response of 24 Infants Vaccinated With Hemagglutinin Vaccine (Schedule I*).

Bleeding	Test Strains							
	A/Swine/1976/31	A/PR/8/34	A_1AA/1/57	A_2/AA/23/57	A_2/Jap/170/62	B/Lee/40	B/GL/54	B/Md/59
Pre vacc.	8	8	8	8	8	8	8	8
sera	(0) **	(3)	(2)	(13)	(5)	(2)	(14)	(3)
1 mo post 1st vacc.	8 (0/24) ***	8 (0/24)	8 (0/24)	8 (1/24)	8 (0/24)	8 (2/24)	8 (0/24)	8 (0/24)
1 mo post 2nd vacc.	8 (4/24)	8 (5/24)	8 (1/24)	8 (1/24)	8 (7/24)	21 (15/24)	10 (5/24)	9 (6/24)
1 mo post 3rd vacc.	11 (3/24)	8 (0/24)	8 (0/24)	8 (2/24)	15 (7/24)	29 (5/24)	16 (4/24)	15 (5/24)

* Months 0, 1, 2, 3.
** Distribution of prevaccination antibody positives.
*** Proportion showing 4-fold or greater titer rise.

TABLE II. Geometric Mean HAI Antibody of 19 Infants Vaccinated With Polyvalent Hemagglutinin Vaccine (Schedule II*).

Bleeding	Test Strains							
	A/Swine/1976/31	A/PR/8/34	A_1/AA/1/57	A_1/AA/23/57	A_2/Jap/170/62	B/Lee/40	B/GL/54	B/Md/59
Pre. vacc.	8	8	8	9	8	8	9	8
sera	(1) **	(4)	(1)	(11)	(8)	(5)	(8)	(5)
2 mo post 1st vacc.	8 (0/19) ***	8 (0/19)	8 (0/19)	8 (1/19)	8 (1/19)	9 (7/19)	9 (2/19)	8 (1/19)
1 mo post 2nd vacc.	9 (5/19)	9 (4/19)	8 (1/19)	8 (2/19)	9 (5/19)	47 (13/19)	19 (8/19)	22 (10/19)
1 mo post 3rd vacc.	10 (0/19)	9 (0/19)	8 (0/19)	11 (1/19)	10 (2/19)	56 (0/19)	21 (0/19)	26 (2/19)

* Months 0, 2, 3, 4.
** Distribution of prevaccination antibody positives.
*** Proportion showing 4-fold or greater titer rise.

fore testing with type A strains and by heating at 56°C for 30 min when type B strains were employed. In the second study, sera were treated with trypsin and periodate before testing with A_1/AA/1/57 and A_2/TW/1/64 and with RDE when PR8 and B/Md/1/59 were used (6). The sera were stored at 4°C.

Results. In a previous publication on vaccination of infants with polyvalent influenza hemagglutinin, it was reported that two inoculations, several months apart, of either 500, 1000 or 2000 CCA units of antigen, induced low levels of antibody in most infants and that fever, readily controlled by aspirin, was encountered in a few (2). Since 3 injections are commonly recommended for initial immunization of infants with DPT (Diphtheria, Pertussis, Tetanus), it was of interest to extend the studies using 2 different schedules of 3 injections in order to determine whether antibody response to an initial series of injections could be improved thereby. In order to ascertain whether incidence of fever could be further reduced, a dose of 250 CCA units was given at each interval.

The first schedule was three doses at monthly intervals. The subjects were 24 infants whose ages ranged from 8 wks–24 wks (median age 14 wks, average age 14 wks, s.d. 5 wks) on admission to the study. Table I shows the geometric HAI pre- and postvaccination titers, the distribution of prevaccination antibody positives and the proportion of infants demonstrating a four-fold or greater rise in titer at each vaccine interval.

In prevaccination serum samples antibody was not detectable or was present at very low levels. Only against A_2/AA/23/57 and B/GL/54 was antibody found in more than half the sera. Antibody found in prevaccination sera most probably represents persistence of maternally transferred antibody. The first inoculation did not increase mean titer values. However a few infants experienced an increase in antibody levels. After the second dose mean titers increased only to influenza B viruses while the proportion of infants showing a four-fold or greater rise in serum titer was increased to all strains. The third inoculation had only a slight effect on levels of antibody and frequency of further antibody rise.

In order to increase the opportunity for antibody conditioning and thus higher postvaccination antibody levels, a different schedule was adopted. The period between the first and second dose was increased to 2 mo while the interval between the second and third was maintained at 1 mo. Nineteen infants whose range was 8 wks–26 wks (median age 17 wks, average age 16 wks, s.d. 6 wks) on admission to the study were vaccinated employing this schedule. The results are shown in Table II. The findings are remarkably similar to those found with the previous vaccination schedule except that postvaccination levels at the end of the series tended to be slightly higher. Again, the third inoculation had only a slight effect. However, it is clear that delaying the second inoculation by 1 mo did not provide a distinct immunologic advantage. Both schedules gave a better response to an initial series than was previously reported for a single dose of vaccine (2). No suppressive influence of maternal antibody was discernible since neither the frequency nor levels of antibody measured in the prevaccination sera with each test strain correlated with the antibody response of the infants. No correlation was apparent between antibody response and age of infant. The response to B strains was better than to A strains and this was also seen in a former study and in results presented in the following section (2). The explanation for this phenomenon is not known since that difference was not apparent when the same vaccine was given to adults (7).

Vaccine associated fever in young infants. Thirty-five percent of infants had a febrile response of 100° or more following the first inoculation as judged by that exacting criterion of identifying febrile responses. After the second or third dose the percentages were 32 and 32, respectively. If only temperatures of 101° or greater are considered, the overall febrile rate was reduced to 5%. In previous studies with higher doses, the percentage of febrile reactions of 101° or greater was 10%. The lower dose used in the present study appeared to be effective in minimizing responses.

Effect of a mineral carrier and delay of vaccination to an older age on the antibody response of infants to polyvalent influenza hemagglutinin. Brown and Kendrick have reported that there was a markedly higher antibody response in infants who received their last dose of an initial series of DPT at or after the seventh month of life than in infants who completed the initial series earlier (8). It was of interest, therefore, to ascertain whether higher titers of postvaccination influenza antibodies could be achieved by initiating the initial series at a later age than that used in our earlier studies. At the same time, it was decided to determine whether aluminum phosphate functions as an adjuvant for hemagglutinin vaccine in infants as it does in mice (9). The dose of absorbed and unabsorbed vaccine was 300 CCA units. The initial series was 2 inoculations given a month apart, since the earlier investigation had shown that a third dose contributed only slightly to antibody response. A third inoculation of the same vaccine was administered as a "booster" 9–12 mo after the initial series.

Table III shows pre- and postgeometric mean antibody titers and the proportion of infants demonstrating a four-fold or greater increase in titer for both the initial series and the "booster" inoculation. Eleven infants received the adsorbed vaccine and 12, the aqueous preparation. The infants ranged in age from 6 to 12 mo (median age 9 mo). None of the 23 infants had measurable antibody in their preprimary serums which is consistent with the loss of maternal antibody with age. When given aluminum phosphate adsorbed vaccine, a high proportion of infants developed antibody to all test strains. The response following aqueous vaccine was similar although the mean titers to A_1/AA/1/57 and A_2/TW/1/64 were lower than those observed after aluminum phosphate adsorbed antigen, while the mean titer to B/Md/1/59 was higher. Hence, for vaccination with an initial series, no distinct immunologic advantage is apparent after use of aluminum phosphate adsorbed vaccine. The titers achieved are higher than those found for younger infants after the initial series and indicate that for hemagglutinin vaccine as well as for DPT the immunologic response improved with age.

In the interval between the initial series and the "booster," titers in both vaccine groups had fallen to low or immeasurable levels. Following the "booster" titer values against all strains in both vaccine groups were higher than after the initial series, suggesting a secondary response. The levels of A_2/TW/1/64 antibody found after the "booster" dose are the result not only of vaccination but in part also reflect the effect of natural infection with Hong Kong virus during the period of the study. Five of the children in the aluminum phosphate group and 5 in the aqueous group showed serologic evidence of infection with Hong Kong virus during this interval. Therefore, results obtained with the Taiwan strain were disregarded. Infants who received aluminum phosphate adsorbed vaccine responded with

TABLE III. Geometric Mean HAI Antibody Responses of Infants Following a Primary Series and After a "Booster" Dose.

Vaccine	Bleedings	A_0/PR/8/34	A_1/AA/1/57	A_2/TW/1/64	B/Md/1/59
	0	8	8	8	8
Aluminum phosphate adsorbed	Post primary	18 (7/11) *	18 (7/11)	29 (10/11)	42 (11/11)
	Pre booster	8	8	8	9
	Post booster	64 (9/11) *	46 (9/11)	147 (11/11)	111 (11/11)
	0	8	8	8	8
Aqueous	Post primary	18 (8/12) *	11 (7/12)	18 (9/12)	75 (11/12)
	Pre booster	8	8	10	8
	Post booster	45 (10/12) *	18 (7/12)	64 (10/12)	85 (11/12)

* Proportion showing 4-fold or greater rise in titer.

higher titers and thus demonstrated a better secondary response than did infants who were given aqueous vaccine.

Discussion. In infants, influenza hemagglutinin vaccine behaves in many ways as do other inactivated vaccines such as DPT. Two or more inoculations of vaccine gave a better initial antibody response than did a single inoculation of larger amounts of antigen. Increasing the time period between the first and second doses in the initial series resulted in a slightly better antibody response. Beginning the initial series at or after the sixth month of life resulted in higher antibody levels and a greater proportion of sero converters than when the initial series was begun earlier in infancy.

The improved secondary response following adsorbed vaccine indicates that in infants as well as in mice, aluminum phosphate adsorbed antigen is a better conditioner for the secondary response during the initial series than is aqueous vaccine (10). In adults, whose response is essentially a secondary one, no difference is observed in the antibody levels following either aqueous or mineral carrier adsorbed influenza hemagglutinin or virus vaccine (11).

Antibody levels achieved in young infants after an initial series are very low and possibly would contribute little to protection against influenza. However, the higher antibody levels observed in older infants after an initial series and the still higher ones induced by "booster" inoculation might afford a significant degree of protection in this age group since, after the initial series, about half of the infants had titers of 32 or greater and after the "booster" with aluminum phosphate adsorbed vaccine, three-fourths of the infants produced antibody at a level of 32 or greater, a titer value considered protective for young adults (12). It should be appreciated that though protective levels of antibody have been established for older age groups by controlled vaccine field trials, protective levels for infants have not been determined. Thus, judgment as to the value of antibody levels induced by a vaccine in this age group cannot be made with certainty.

Summary. Infants 2–6 mo of age were given 3 doses of influenza hemagglutinin vaccine. One group received 250 CCA units of vaccine at intervals of 1 mo. A second group was given 250 CCA units with an interval of 2 mo between the first and second dose and an interval of 1 mo between the second and third dose. A slightly better antibody response was obtained with this schedule.

In a second experiment, infants 6–9 mo of age were given an initial series of either 300 CCA units of aqueous or aluminum phosphate adsorbed hemagglutinin vaccine. These infants exhibited a better antibody response than younger ones. When after 9–12 mo a third dose of the same vaccine was given, a secondary response was achieved. The response of infants who received adsorbed vaccine was slightly higher. The response of infants to polyvalent influenza hemagglutinin is similar to their response to other inactivated vaccines.

1. Davenport, F. M., and Hennessy, A. V., *in* "Viral Infections of Infancy and Childhood" (H. Rose, ed.) Hoeber-Harper, New York (1960).

2. Hennessy, A. V., and Davenport, F. M., J. Amer. Med. Ass. **200**, 896 (June 5, 1967).

3. Davenport, F. M., Hennessy, A. V., Brandon, F. M., Webster, R. G., Barrtt, C. D., Jr., and Lease, G. O., J. Lab. Clin. Med. **63**, 5 (January, 1964).

4. Committee on Standard Serological Procedures in Influenza Studies, J. Immunol. **65**, 347 (1950).

5. Communicable Disease Center, CDC Influenza Surveillance, Bulletin 38, U. S. Department of Health, Education and Welfare (1958).

6. Hilleman, M. R., and Werner, J. H., J. Immunol. **71**, 110 (1953).

7. Hennessy, A. V., and Davenport, F. M., J. Immunol. **97**, 235 (1966).

8. Brown, G. C., and Kendrick, P. L., Amer. J. Pub. Health **50**, 1529 (October, 1960).

9. Davenport, F. M., Proc. Soc. Exp. Biol. Med. **127**, 340 (1968).

10. Hennessy, A. V., and Davenport, F. M., Proc. Soc. Exp. Biol. Med. **138**, 396 (November, 1971).

11. Davenport, F. M., Hennessy, A. V., and Askin, F. B., J. Immunol. **100**, 1139 (1968).

12. Meiklejohn, G., Kempe, C. H., Thalman, W. G., Lennette, E. H., Amer. J. Hyg. **55**, 12 (1952).

Received Oct. 12, 1973. P.S.E.B.M., 1974, Vol. 146.