Effect of $AlPO_4$ on Antibody Response[1] (35905)

A. V. HENNESSY, M. E. PATNO, AND F. M. DAVENPORT

Departments of Epidemiology and Statistics, School of Public Health, University of Michigan, Ann Arbor, Michigan 48104

It has been reported previously that in mice, the primary antibody response to influenza hemagglutinin vaccine is enhanced when aluminum phosphate adsorbed antigen is used (1). However, the effect of adsorbtion to a mineral carrier on the secondary response of mice to influenza hemagglutinin is not known. Accordingly experiments were initiated to investigate this question systematically. The results constitute the body of the report.

Materials and Methods. Vaccines. The hemagglutinin (HA) concentrate used to prepare the aqueous and aluminum phosphate adsorbed vaccines was made by a commercial firm according to the method previously described (2). Two vaccine formulations were employed. The set of vaccines given in the first experiment and as a conditioning dose in the second contained 200 chick cell agglutinating (CCA) units/ml of A2/Taiwan/1/64 hemagglutinin while the set given as the "booster" dose in the second and for both doses in Expt. 3 contained 150 CCA units/ml of the same hemagglutinin. The aqueous vaccines were suspensions of hemagglutinin in phosphate buffered saline containing 1:4000 formalin and 1:100,000 merthiolate as a preservative. The mineral carrier adsorbed vaccines were identical to their respective aqueous mates except that the hemagglutinin was completely adsorbed on 3.8 mg/ml of aluminum phosphate.

Mice. Albino swiss mice, weighing 12–20 g, were obtained from a commercial source and used within 3 weeks of receipt.

Vaccination and bleeding schedule. One milliliter of 10^0, 10^{-1}, or 10^{-2} dilutions of either an aqueous or an aluminum phosphate adsorbed hemagglutinin vaccine was given intraperitoneally to groups of 14 mice. The mice were rested for 60 days and a second injection of the same strength was given. However, at the time of the booster dose 7 mice in each group received aqueous vaccine and 7 received mineral carrier adsorbed vaccine. Fourteen days later, all mice were bled for determination of hemagglutination-inhibiting antibodies. The experiment was repeated on two separate occasions. Vaccines with mineral carrier were diluted in buffered saline containing 3.8 mg/ml of aluminum phosphate. Aqueous vaccines were diluted in buffered saline.

Hemagglutination-inhibition titrations (HI). A standard pattern method was employed (3). The concentration of chicken erythrocytes was 0.5%. Mouse sera were treated with trypsin and potassium periodate before testing (4).

Results. The results of the three experiments are shown in Fig. 1. On the left side of the chart are plotted, as logs to the base 2, the geometric mean A2/Taiwan/1/64 antibody titers found in 14 day "postbooster" sera of mice who had received a primary inoculation of aqueous hemagglutinin vaccine. The geometric mean titers for each dilution found in the three experiments are grouped together. The crosses represent the serum titers of mice given aqueous vaccine as a booster dose and the open circles are the serum titers of mice given aluminum phosphate adsorbed hemagglutinin as a booster. The postbooster geometric mean antibody titers found in the sera of mice who received aluminum phosphate adsorbed vaccine as a primary inoculation are plotted in an identi-

[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.

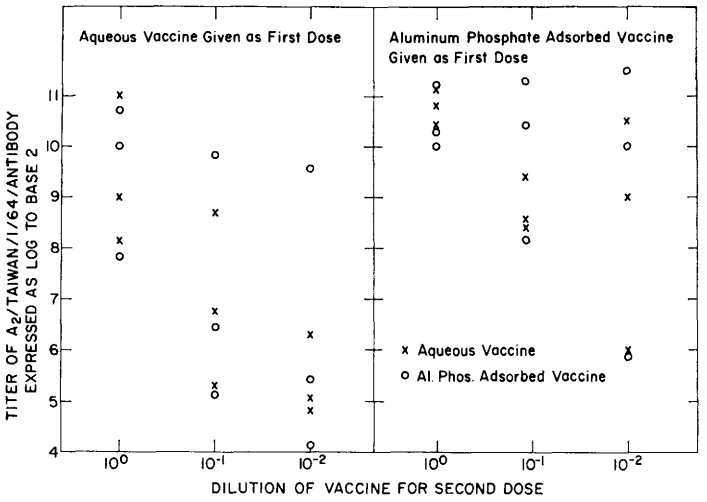

FIG. 1. Geometric mean HI antibody response of previously vaccinated mice given a second dose of HA vaccine (derived from A_2/Taiwan/1/64).

cal manner on the right side of the chart.

The postbooster mean serum antibody titer of mice who received aluminum phosphate adsorbed vaccine as a primary inoculation are higher than those of the corresponding mice who received aqueous vaccine as a primary stimulus. Also, the postbooster serum titers of mice immunized first with mineral carrier vaccine tend to be more uniform and decline less as the vaccines are diluted than do the serum titers of mice given aqueous vaccine as a first dose. However, within each dilution group, no difference in antibody response is discernable between booster doses of either aluminum phosphate adsorbed or aqueous vaccine. Statistical treatment of the data by means of analysis of variance is given in Table I.

The data presented indicate that aluminum phosphate adsorbed influenza hemagglutinin vaccine is a better conditioner for the secondary response in mice than is aqueous vaccine and that it does not act as an adjuvant when given as a booster inoculation. Similar findings have been reported by Henneberg *et al.* (5) for aluminum oxide adsorbed and aqueous poliomyelitis vaccines given to monkeys.

Discussion. Findings in mice closely parallel those found in man with mineral carrier adsorbed and aqueous influenza hemagglutinin vaccines. No difference could be ascer-

TABLE I. Statistical Treatment of the Antibody Responses by Analysis of Variance.

Source	*df*	S. Sq.	M. Sq.	*F*
Experiments	2	28.2483	14.1241	4.38[a]
Treatments	11	96.4584	8.7689	3.92[b]
Adsorbed vs aqeous				
Initial dose	1	41.9689	41.9689	18.74[b]
Booster dose	1	1.9090	1.9090	.85 NS[c]
Comparisons among dilutions	9	52.5806	5.8412	2.61[a]
Error	22	49.1474	2.239	
Total	35	173.8543		

[a] Significant at the 5% level.
[b] Significant at the 1% level.
[c] NS = not significant.

tained between antibody response of young adults, which is essentially a secondary one, to either type of vaccine (6). In infants, as well as in mice, aluminum phosphate adsorbed hemagglutinin was observed to be a better conditioner for a secondary response than aqueous hemagglutinin suspensions (7). However, in infants, unlike the findings in mice, aluminum phosphate adsorbed hemagglutinin did not give a better primary antibody response than did aqueous vaccine (1, 7).

The mouse and human data indicate that where primary vaccination is to be reinforced by a booster dose, aluminum phosphate adsorbed influenza hemagglutinin is the vaccine of choice for the primary immunization. Either aqueous or mineral carrier vaccine could be given as a booster.

Summary. Groups of mice were inoculated once with graded dilutions of either aluminum phosphate adsorbed or aqueous influenza hemagglutinin vaccine containing A2/Taiwan/1/64 as a primary immunization. Sixty days later each group was divided: one half was given aqueous vaccine and the other, aluminum phosphate adsorbed hemagglutinin. The dose was the same strength as the first. The postbooster antibody response was higher in those groups who had received mineral carrier adsorbed vaccine as the first inoculation ($p = .01$) indicating that aluminum phosphate adsorbed hemagglutinin is a better conditioner for the secondary response than is aqueous vaccine.

1. Davenport, F. M., Proc. Soc. Exp. Biol. Med. **127**, 587 (1968).

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

3. Comm. on Stand. Serol. Procedures in Influenza Studies, J. Immunol. **65**, 347 (1950).

4. Jensen, K. E., Influenza Surveillance Communicable Dis. Center Bull. **38**, (1958).

5. Henneberg, G., Dresher, J., Godgluck, G., and Grutzner, L., Amer. J. Hyg. **75**, 44 (1962).

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

7. Hennessy, A. V., and Davenport, F. M., Submitted for publication.

Received May 26, 1971. P.S.E.B.M., 1971, Vol. 138.