Epidemic Influenza Vaccine and Antiserum

H. M. Powell, Lilly Research Laboratories

Preparation of influenza vaccine

Influenza PR8 type A virus was grown 48 hours in the allantoic fluid of eleven-day incubated eggs in the usual way. After the eggs had been chilled in the ice box overnight, the virus-containing allantoic fluid was drawn off with practically no admixture with red blood cells. The virulence of this virus in successive lots averaged 10^{-5} for Swiss mice inoculated intranasally in the usual way.

Each lot of allantoic fluid virus was divided into two portions and treated as follows in the preparation of virus vaccine:

(1) Addition of 'Merthiolate' (Sodium Ethyl Mercuri Thiosalicylate, Lilly) 1:20,000 and incubation one week at 37° C. Three lots of vaccine were prepared in this way.

(2) By a common current method comprising addition of formalin 0.1 per cent plus phenol 0.3 per cent. Three lots of vaccine were prepared in this way.

The vaccine preserved with 'Merthiolate' became nonvirulent for Swiss mice when incubated at 37° C. for one week, and the vaccine preserved with formalin and phenol became non-virulent very quickly without incubation. Continued incubation at 37° C. for four weeks was used as a stability test of the immunizing qualities of these vaccines. During this period of four weeks of incubation of the various lots of vaccine, two active immunization tests were done in Swiss mice. The first test was done after incubation for one week, and the second test was done at the completion of incubation for four weeks. In each immunization test, a group of ten Swiss mice each received 0.1 cc of the vaccine intraperitoneally. This dose was repeated one week later.

One week after injecting the last immunizing doses, decimal dilutions of active virus from 10^{-1} to 10^{-6} were administered intranasally to subdivided groups of both immunized and control mice. The results of these tests are expressed in Table 1 in two ways; first by a fraction, the numerator being the number of mice which died and the denominator being the number of mice used; and second by a total score, dead mice being given a score of 5 while survivors examined at autopsy were given scores of 4, 3, 2, 1, and 0, respectively, corresponding to readings of decreasing lung consolidation. Low scoring immunized mice and high scoring control mice obviously are desirable in immunity tests.

Potency and stability of influenza vaccine

It is observed in Table 1 that influenza virus vaccine treated with 'Merthiolate' shows strong immunizing action, and this is impaired very little by incubation at 37° C. for four weeks. Vaccine treated with formalin and phenol, on the other hand, is impaired greatly in immunizing action by incubation at 37° C. for four weeks.

Influenza type B virus vaccine has been prepared in the same manner as type A with essentially the same laboratory results. A constant difference, however, has been a lower degree of mouse virulence of the B virus.

Pooled types A and B virus vaccines have been tested in mice with results essentially similar to those obtained above. Human tests have not yet been done.

Most of the immunity tests with influenza virus vaccine heretofore reported have given information on activity of the agent at the time of preparation but not necessarily at the time of actual use. Four weeks of heating at 37° C. would not usually occur prior to use; however, variable storage temperatures in transportation and handling prior to use are frequently encountered.

It appears from our stability tests that non-virulent influenza vaccine treated with 'Merthiolate' has a better margin of immunizing effectiveness than non-virulent influenza vaccine treated with formalin and phenol. This conclusion, based on comparative degrees of active immunity obtained against infection with active virus in Swiss mice, obviously means somewhat more than comparative titers of antibodies as measured *in vitro*.

Preparation of influenza antiserum

Rabbit bivalent antiserum against influenza virus types A and B has been prepared for experimental use by methods already described. (1) This serum is of high titer and is intended for prophylactic use by repeated inhalation by intranasal spray whenever epidemic influenza begins to appear in a community. Production of such antiserum is as practicable as production of other antisera, and prophylactic use at the last moment ahead of an epidemic would be both possible and preferable. Reports already published (1) indicate this antiserum when used as described is effective in preventing epidemic influenza types A and B. These types comprise the great majority of all cases of epidemic influenza. There is no evidence thus far that this antiserum is effective in cases which have already developed influenza. In other words, the antiserum, as well as the vaccine, is intended for prophylactic use, but the optimum time of use of each agent would be entirely different. Further details regarding this influenza antiserum are found in the report (1) already referred to.

Summary

1. A stable epidemic influenza vaccine in fluid form has been described. Effectiveness in human subjects has not yet been assayed.

2. Rabbit antiserum against both influenza types A and B viruses has been prepared for prospective inhalation prophylaxis. Effectiveness in human subjects has been indicated by recent publications.

Reference

1. Powell, H. M. Rabbit antiserum against influenza A and B viruses for inhalation prophylaxis against epidemic influenza. J. Ind. State Med. Assoc., **37**:18-20 (1944).

Table 1. Tests for immunity incited by type A influenza virus vaccines in Swiss mice.

virus vaccine preparation	dilution of test virus	vaccine incubated 1 week at 37° C.				vaccine incubated 4 weeks at 37° C.			
		vaccinated mice		controls		vaccinated mice		controls	
		dead/used	score	dead/used	score	dead/used	score	dead/used	score
'Merthiolate' treated vaccine	10^{-1}	1/5	8	5/6	28	1/6	10	4/6	27
	10^{-2}	0/6	2	6/6	30	0/6	7	5/6	28
	10^{-3}	0/6	2	6/6	30	0/6	6	5/6	28
	10^{-4}	0/6	2	5/6	28	0/6	2	4/6	23
	10^{-5}	0/6	2	5/6	26	0/5	0	4/6	24
formalin and phenol treated vaccine	10^{-1}	4/6	22	5/6	29	3/3	15(30)*	2/2	10(30)
	10^{-2}	3/6	17	6/6	30	3/4	16(24)	2/2	10(30)
	10^{-3}	1/6	8	6/6	30	1/4	9(13)	2/2	10(30)
	10^{-4}	0/6	4	6/6	30	0/4	1 (1)	2/2	10(30)
	10^{-5}	1/6	6	6/6	28	0/4	0 (0)	1/2	9(27)

* Insufficient mice were available for the full quota of test animals on the second vaccine heated for 4 weeks. Actual scores are indicated, and computed scores, on basis of 6 mice per group, are shown in parentheses.