Ten-Year Follow-Up Study for Safety of Adjuvant 65 Influenza Vaccine in Man (37469)

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Previous reports (1–17) from these laboratories described the development and clinical testing of influenza virus vaccine in an immunologic adjuvant called adjuvant 65. The earlier adjuvant 65 influenza vaccine consisted of a water-in-oil emulsion of influenza virus vaccine in peanut oil employing Arlacel A (mannide monooleate) as emulsifier and aluminum monostearate as stabilizer. Present adjuvant 65 contains chemically pure aluminum monostearate and isomannide monooleate in place of the impure components used earlier. Previously published data included the findings in long-term chronic-toxicity tests in animals (1, 8, 11, 16) and on intermediate term observations of subjects who received the adjuvant type vaccine (10, 15, 16).

The present report summarizes the findings on inspection of the local injection sites and of the records of cause of death in persons during the interim period who served as unvaccinated controls or who were given aqueous or adjuvant 65 influenza virus vaccine up to 10 yr previously.

Materials and Methods. All subjects were in institutions for the mentally retarded. Details concerning each study group and the vaccines they received were presented in detail previously (4, 6, 9, 10, 12, 15). For the present study, the arms of the subjects were examined for local lesions in the general vicinity of the injection sites and the health record of each person was reviewed. The findings were recorded, summarized, and analyzed statistically, using Fisher's exact test or chi square test.

Results. Table I lists the studies in persons in institutions who had been given aqueous or adjuvant 65 type influenza vaccine or who were held as unvaccinated controls. All together, 510 subjects who had received adjuvant 65 influenza vaccine, 369 who had been given aqueous type influenza vaccine, and 190 controls who had not received the experimental vaccines were examined. The subjects had received vaccine from 2 yr 8 mo to 10 yr previously.

There were remarkably few reactions. The only discernible reactions were very small nodules approximately 3–4 mm in diameter that were difficult to detect visually or by palpation. Four persons (0.8%) who received adjuvant vaccine, 1 person (0.3%) who received aqueous type vaccine, and 1 person (0.5%) who was not vaccinated were found to have such a nodule. The nodules were not important clinically and the differences in rate for the 3 groups were not statistically significant ($p > 0.40$ by Fisher's exact test).

The health records of 937 persons who had received adjuvant type influenza vaccine, 700 who were given aqueous type vaccine, and 371 controls were reviewed. The persons were in the same groups as shown in Table I. A total of 156 persons had died during the period between vaccination and observation and the cause of death for each person is shown in Table II. The causes of death were diverse including respiratory disease, cardiovascular disease, cancer, and a miscellaneous group of entities. The cause of death of 13 persons was not recorded because of inability to obtain consent for postmortem examination or because of death while away from the institution. The percentage of persons who died was roughly the same in all groups, viz.,

TABLE I. Long-Term Clinical Observations in Persons Who Received Influenza Virus Vaccine in Aqueous or Adjuvant 65 Formulations.

Study no. (Ref.)	Virus purification method	Kind of vaccine	Lot no.	No. persons vaccinated	Influenza antigen (units/dose) (CCA)	No. doses given	Observation time period following first vaccine dose	No. persons observed	No. with local reactions (nodules)
30A (—)	Protamine eluted	Adjuvant 65	1, C-2780	8	313	1–3	10 yr	5	0
		Aqueous	1, C-2781	7	313	3		6	0
1A (4) ($30B_1$)	Protamine eluted	Adjuvant 65	1, C-2780	25	313	2–3	9 yr, 7 mo	18	0
		Aqueous	1, C-2781	26	313	3		19	0
1B (4) ($30B_2$)	Protamine eluted	Adjuvant 65	1, C-2780	22	313	3	9 yr, 7 mo	13	0
		Aqueous	1, C-2781	21	313	3		14	0
45A (—)	Sharples	Adjuvant 65	115, C-3848	60	300	1–2	7 yr, 6 mo	44	2
		Aqueous	116, C-3854	59	300	1–2		45	0
55 (5)	Chem. purified	Adjuvant 65	140, C-4108	50	250	1–2	6 yr, 10 mo	34	0
		Aqueous	156, C-4554	50	500	1–2		31	0
		Unvacc. controls		51				35	0
63 (5)	Chem. purified	Adjuvant 65	140, C-4108	24	250	2	6 yr, 6 mo	14	0
		Aqueous	156, C-4554	22	250	2		11	0
		Unvacc. controls		24				7	0
65 (—)	Chem. purified	Adjuvant 65	183, C-4917	96	300	1–2	6 yr, 2 mo	43	0
		Adjuvant 65	184, C-4918	98	600	1–2		49	0
		Aqueous	181, C-4915	96	300	1–2		37	0
		Aqueous	182, C-4916	94	600	1–2		35	0
		Unvacc. controls		56				25	1
155 (9)	Filter purified	Adjuvant 65	11–16	434	300–600	1–2	3 yr, 7–8 mo	198	2
		Aqueous	69	275	600	1–2		129	1
		Unvacc. controls		199				100	0
218 (—)	Zonal purified	Adjuvant 65	323–4	120	50–400	1	2 yr, 8 mo	92	0
		Aqueous	325	50	40–400	2		42	0
		Unvacc. controls		41				23	0
Total									
Adjuvant 65				937				510	4 (0.8%)
Aqueous				700				369	1 (0.3%)
Controls				371				190	1 (0.5%)

TABLE II. Cause of Death in Persons Who Received Influenza Virus Vaccine in Aqueous or Adjuvant 65 Formulations and in Persons Who Served as Uninoculated Controls.[a]

Cause of death	Group [no. died (%)]			
	Adjuvant 65	Aqueous	Uninoculated controls	Total
Respiratory				
Pneumonia	22 (28%)	22 (44%)	12 (44%)	56
Respiratory arrest	8 (10%)	3 (6%)	3 (11%)	14
Pulmonary infarction	2 (3%)			2
Cardiovascular				
Congestive heart failure with pulmonary edema	11 (14%)	7 (14%)	2 (7%)	20
Myocardial infarction	8 (10%)	4 (8%)	2 (7%)	14
Generalized arteriosclerosis	9 (11%)	2 (4%)	1 (4%)	12
Cancer				
Seminoma of rt. testicle with metastases to chest		1 (2%)		1
Vesicular carcinoma with metastases to peritoneum			1 (4%)	1
Metastatic carcinoma—primary site unknown		1 (2%)	1 (4%)	2
Epidermoid carcinoma of vagina	1 (1%)			1
Gastric carcinoma	1 (1%)			1
Other				
Drowning			1 (4%)	1
Peritonitis—secondary to ruptured viscus	1 (1%)	3 (6%)		4
Poison ingestion	1 (1%)			1
Generalized convulsion with respiratory arrest			2 (7%)	2
Fulminating hepatitis	2 (3%)			2
Chronic glomerulonephritis		1 (2%)		1
Hydrocephalus	1 (1%)			1
Cerebral edema—etiology unknown		1 (2%)		1
Large subdural hematoma with bronchopneumonia			1 (4%)	1
Malnutrition	2 (3%)	1 (2%)		3
Skull fracture (parietal bone) with brain injury	1 (1%)			1
Intestinal obstruction	1 (1%)			1
Unknown	8 (10%)	4 (8%)	1 (4%)	13
Total persons who died	79	50	27	156
Total persons in the group	937	700	371	
Percent who died	8.4%	7.1%	7.3%	

[a] No statistically significant differences were found among the overall rates for the three groups ($p > 0.30$ by chi square test).

8.4% in the adjuvant 65 type vaccine, 7.1% in the aqueous type vaccine, and 7.3% in the unvaccinated control groups. The differences in rates between the 3 groups were not significantly different ($p > 0.30$ by chi square test). Importantly, there were no cancers in the arms of any of the subjects. Two persons in each category had died of cancer.

Discussion. A most important consideration for use of any vaccine is its safety. The

extensive studies to date have failed to reveal any clinically important local or systemic reactions following use of influenza vaccine in adjuvant 65 (1–17). This finding was further substantiated in the present study in human subjects who were followed for as long as 10 yr. Clearly, no clinically important untoward effect was noted and these findings are wholly consistent with those of the short-term toxicity (1, 3, 15, 16) and chronic toxicity (1, 8, 15, 16) tests carried out in various species of animals over a long period of time. The safety of the vaccine is further assured by the ease with which the components are metabolized (7) and by the fact that all but the last trace of adjuvant material is removed from the injection site and from the body within 60 days after vaccination, as evidenced by radioactive tracer studies.

Summary. Clinical follow-up was made of more than 2000 persons who had received adjuvant 65 type influenza or aqueous type influenza vaccine or who had served as unvaccinated controls in studies in which vaccines were given up to 10 yr previously. No long-term effect was noted other than the occurrence in no more than 0.8% of persons observed of a persistent nodule that was difficult to palpate and that was no larger than a small pea. The rates for occurrence of nodules in vaccinated persons were not significantly greater than in the unvaccinated controls. There was no significant difference in the percentage of persons who died between the groups for respiratory disease, cardiovascular disease, cancer, or for miscellaneous cause ($p > 0.15$ for each category by Fisher's exact test). All evidence points to safety of the adjuvant type vaccine both on short- and long-term observation.

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1. Hilleman, M. R., Progr. Med. Virol. **8**, 131 (1966).

2. Woodhour, A. F., Metzgar, D. P., Stim, T. B., Tytell, A. A., and Hilleman, M. R., Proc. Soc. Exp. Biol. Med. **116**, 516 (1964).

3. Peck, H. M., Woodhour, A. F., Metzgar, D. P., McKinney, S. E., and Hilleman, M. R., Proc. Soc. Exp. Biol. Med. **116**, 523 (1964).

4. Stokes, J., Jr., Weibel, R. E., Drake, M. E., Woodhour, A. F., and Hilleman, M. R., N. Engl. J. Med. **271**, 479 (1964).

5. Woodhour, A. F., Metzgar, D. P., Lampson, G. P., Machlowitz, R. A., Tytell, A. A., and Hilleman, M. R., Proc. Soc. Exp. Biol. Med. **123**, 778 (1966).

6. Weibel, R. E., Woodhour, A. F., Stokes, J., Jr., Metzgar, D. P., and Hilleman, M. R., N. Engl. J. Med. **276**, 78 (1967).

7. Porter, C. C., and Titus, D. C., Proc. Soc. Exp. Biol. Med. **124**, 500 (1967).

8. Peck, H. M., Woodhour, A. F., and Hilleman, M. R., Proc. Soc. Exp. Biol. Med. **128**, 699 (1968).

9. Stokes, J., Jr., Weibel, R. E., Woodhour, A. F., McAleer, W. J., Potkonski, L. A., and Hilleman, M. R., J. Amer. Med. Ass. **207**, 2067 (1969).

10. Weibel, R. E., Woodhour, A. F., Stokes, J., Jr., Friedman, A., McAleer, W. J., and Hilleman, M. R., Amer. J. Med. **48**, 464 (1970).

11. Hilleman, M. R., "International Symposium on Adjuvants of Immunity," Utrecht, 1966, p. 13. (Symp. Series Immunobiol. Standard.) Karger, Basel (1967).

12. Woodhour, A. F., McAleer, W. J., Friedman, A., Weibel, R. E., Stokes, J., Jr., and Hilleman, M. R., Proc. Soc. Exp. Biol. Med. **131**, 501 (1969).

13. Hilleman, M. R., Bull. W. H. O. **41**, 623 (1969).

14. Woodhour, A. F., Friedman, A., Tytell, A. A., and Hilleman, M. R., Proc. Soc. Exp. Biol. Med. **131**, 809 (1969).

15. Hilleman, M. R., Woodhour, A., Friedman, A., Weibel, R. E., and Stokes, J., Jr., Ann. Allergy **30**, 152 (1972).

16. Hilleman, M. R., Woodhour, A. F., Friedman, A., and Phelps, A. H., Ann. Allergy **30**, 477 (1972).

17. Woodhour, A. F., Friedman, A., Weibel, R. E., and Hilleman, M. R., presented: Symp. Int. Ass. Biological Standardization 39th "Influenza Vaccines for Men and Horses," Nov. 20–23, 1972, London, England, in press.

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