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Studies with Inactivated Influenza Vaccines Purified by Zonal Centrifugation*

1. Adverse Reactions and Serological Responses

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High (3000 CCA units) and standard low (300 CCA units) doses of formalin-inactivated influenza vaccines purified by zonal ultracentrifugation were evaluated in a double-blind manner for adverse reactions and antibody responses in a high school population and in 2 adult populations.

The fewest local and systemic reactions were produced by subcutaneous administration of the low doses of purified monovalent A2/Aichi/2/68, A2/Japan/170/62, and B/Massachusetts/3/66 vaccines. The high doses of A2/Japan and A2/Aichi vaccines produced 2–3-fold greater incidence of adverse reactions but did not exceed that observed with the commercial vaccines. The 3000 CCA units of B/Mass. vaccine produced more frequent and more severe systemic reactions than the polyvalent vaccine.

Neither the A2/Japan vaccine nor the commercial vaccines stimulated a significant serum antibody response to A2/Aichi.

The homologous serum antibody response to an Aichi vaccine was excellent in all 3 populations. A2/Aichi vaccines produced higher heterologous A2/Japan titres than did the A2/Japan vaccines. It was demonstrated that high doses of purified influenza vaccine can be given safely, and that 10-fold increases in vaccine concentration produce 2–3-fold increases in antibody titre.

The application of zonal ultracentrifugation to the production of inactivated influenza virus vaccines resulted in a product that is free of major impurities (Reimer et al., 1967) and that causes fewer reactions in humans than the commercially available Sharples vaccine (Peck, 1968). The availability of these highly purified influenza virus antigens thus made it possible, after a 20-year lapse (Salk, 1948), to reinvestigate the effect of large doses of aqueous vaccine in man. Initial studies in prison volunteers demonstrated that up to 4800 CCA units of vaccine prepared by zonal ultracentrifugation could be administered subcutaneously with a minimum of adverse effects; higher doses of vaccine were shown to produce higher and more broadly reacting serum antibody titres (Mostow et al., 1969). This report and the one that follows [3] describe a series of 3 double-blind field trials designed to evaluate the protective efficacy of subcutaneous administration of high doses of inactivated influenza vaccines. The first report describes the effect of purification and increased vaccine dosage on the occurrence of adverse reactions and the stimulation of serum antibody. The second describes the efficacy of these vaccines during naturally occurring outbreaks of A2/Hong Kong/68/influenza.

MATERIALS AND METHODS

All vaccines were prepared from formalin-inactivated egg-grown influenza viruses. The vaccines, methods of production, and potency in CCA units as determined separately by the manufacturer and by

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TABLE 1

SUMMARY OF STUDY POPULATIONS AND DISTRIBUTION OF SUBCUTANEOUS DOSES OF INACTIVATED INFLUENZA VACCINES, 1968–69

Vaccine	CCA units		Vaccine recipients		
	Manufacturer	DBS [a]	Georgia State Prison	Retirement community, Calif.	Orange County, Calif., schools
Highly purified [b]					
A2/Aichi/2/68 (low dose)	300	232	48	576	417
A2/Aichi/2/68 (high dose)	3 000	2 225	46	596	417
A2/Japan/170/62 (low dose)	300	244	49	577	462
A2/Japan/170/62 (high dose)	3 000	2 421	49	611	432
B/Massachusetts/3/66 (low dose)	300	234	44	—	440
B/Massachusetts/3/66 (high dose)	3 000	2 548	45	—	123
Commercial [c]					
Divalent [d]	600	370	—	600	—
Polyvalent [e]	600	365	45	566	—
Total	—	—	326	3 526	2 291

[a] Division of Biologics Standards, National Institutes of Health, Bethesda, Md.
[b] Prepared by zonal centrifugation.
[c] Prepared by Sharples centrifugation.
[d] Containing A2/Japan/170/62, A2/Taiwan/1/64, and B/Mass./3/66.
[e] Containing A2/Japan/170/62, A2/Taiwan/1/64, A/PR/8/34, A1/Ann Arbor/1/57, and B/Mass./3/66.

the Division of Biologics Standards, National Institutes of Health, are listed in Table 1. Vaccines purified by zonal ultracentrifugation were especially prepared for this project by Eli Lilly and Company. The commercial vaccines were obtained from retail stock prepared by another manufacturer.

Volunteers without a history of sensitivity to eggs or egg products were recruited from 3 populations, their informed consent having previously been obtained. The study groups consisted of (1) approximately 350 young and middle-aged males from among 2500 inmates at the Georgia State Prison; (2) approximately 3500 elderly men and women from among 11 000 residents of a California retirement community located near Long Beach; and (3) approximately 2300 boys and girls from among 4500 students attending 2 high schools and 2 junior high schools in southern Orange Country, Calif.

Immunization procedure

Volunteers at the Georgia State Prison were distributed into 7 groups that were equivalent on the basis of age and prevaccination titre to A2/Japan/170/62. Volunteers from the other 2 populations were randomly divided without regard to prevaccination titre into 6 vaccine groups of equal size. All vaccines were administered subcutaneously in 1-ml amounts by jet-injector gun. Members of each group received a single dose of one of the vaccines. All vaccines were supplied to the field investigators in coded and shielded vials, and the codes (different for each population) were not broken until all results were tabulated and calculations completed.

Specimens

In the Georgia State Prison study, serum specimens were collected 1 week prior to the administration of vaccine and 21 days after vaccination. In the retirement community and the schools, serum specimens were obtained from random samples of vaccinated persons (25% and 50%, respectively) at the time of vaccination and again 21 days after vaccination.

Serological tests

Haemagglutination-inhibition (HI) tests were performed at the National Communicable Disease Center by standard microtitre techniques (Davenport & Minuse, 1964). Sera were treated overnight

TABLE 2
ADVERSE REACTIONS TO INFLUENZA VACCINES, GEORGIA STATE PRISON

Reaction	Occurrence of reactions (%) in recipients of the following vaccines						
	B/Mass./3/66		A2/Japan/170/62		A2/Aichi/2/68		Polyvalent
	Low dose	High dose	Low dose	High dose	Low dose	High dose	
Local							
Erythema >50 mm	4	20	2	16	0	9	33
Induration >20 mm	14	36	10	22	19	30	56
Tenderness	9	29	12	35	4	28	44
Sting at time of injection	14	49	12	31	8	22	24
Systemic							
Fever ≥99.6°F (37.6°C)	2	22	0	6	4	4	7
Fever ≥100.0°F (37.8°C)	0	9	0	2	2	2	4
Four or more systemic reactions	9	36	10	14	13	21	20

with receptor-destroying enzyme (*Vibrio cholerae*) to remove non-specific inhibitors.

Evaluation of reactions to vaccine

In the Georgia State Prison study each volunteer was questioned immediately before injection and at 8, 24, and 48 hours after vaccination about the occurrence of headache, eye pain, malaise, generalized muscle pain, loss of appetite, nausea, and chills. Oral temperatures were also recorded. Measurements of erythema and induration at the injection site and complaints of local tenderness were recorded at 8, 24, and 48 hours. Each subject was also asked at the 8-hour post-vaccination interview whether the injection had produced an immediate " sting ".

At the retirement community each vaccinated person was given a questionnaire concerning the occurrence of the same systemic symptoms and local reactions as listed above for the Georgia State Prison study and requested to complete and return it 4 days after vaccination. All participants in the school study were requested to complete the same questionnaire under the supervision of their teachers on the 4th day after vaccination. In addition, school absenteeism was tabulated for vaccinated and unvaccinated students for the 3 days immediately following injection.

RESULTS

Adverse reactions to vaccine

Georgia State Prison. Local reactions to low doses of all highly purified vaccines were minimal (Table 2). Local reactions to high doses of the highly purified vaccines were greater than those to the low doses, but were generally less severe than those associated with the commercially available polyvalent Sharples vaccine. The high dose of the B/Mass./3/66 vaccine was an exception in that it caused a markedly greater sting at the time of injection. Systemic reactions to all vaccines were relatively infrequent, again with the exception of the high dose of B/Mass./3/66 vaccine, which was associated with a significant number of febrile reactions.

Retirement community. The questionnaire was returned by 90% of those vaccinated. Two samples were analysed. One included questionnaires from all persons who had been bled (25% of the total number of volunteers). The second consisted of questionnaires from a 33% random sample of all non-bled volunteers. The results were comparable and the data were pooled for presentation.

Of the recipients of low doses (300 CCA units) of the monovalent A2/Japan and A2/Aichi vaccines, 66% and 69%, respectively, reported no reaction (Table 3). Similarly, the responses to high doses (3000 CCA units) of the A2/Japan and A2/Aichi vaccines were almost identical; 46% and 49%, respectively, of the recipients reported no reaction. In contrast, only 32% of the recipients of the commercially available divalent and polyvalent vaccines (600 CCA units) had no reactions.

Schools. Completed questionnaires were returned by 88% of the vaccinated students. Over-all, adverse reactions to the A2/Japan and A2/Aichi vaccines

TABLE 3
ADVERSE REACTIONS TO INFLUENZA VACCINES, CALIFORNIA RETIREMENT COMMUNITY

Reaction	Occurrence of reactions (%) in recipients of the following vaccines					
	A2/Aichi/2/68		A2/Japan/170/62		Commercial	
	Low dose	High dose	Low dose	High dose	Divalent	Polyvalent
None	69	49	66	46	32	32
One or more local reactions	24	44	22	44	63	62
One or more systemic reactions	17	28	17	28	33	39
Local and systemic reactions	9	21	5	18	28	33

TABLE 4
ADVERSE REACTIONS TO INFLUENZA VACCINES, ORANGE COUNTY, CALIF., SCHOOLS

Reaction	Occurrence of reactions (%) in recipients of the following vaccines					
	A2/Aichi/2/68		A2/Japan/170/62		B/Mass./3/66	
	Low dose	High dose	Low dose	High dose	Low dose	High dose
None	34	23	35	23	36	17
One or more local reactions	49	61	51	70	50	72
One or more systemic reactions	37	56	41	47	44	75
Local and systemic reactions	23	39	30	40	30	64

were reported almost twice as frequently (Table 4) as by the members of the retirement community (Table 3).

Of the recipients of 300 CCA units of highly purified A2/Japan and A2/Aichi vaccines, 35% and 34%, respectively, reported no reaction; with both vaccines, 23% of the recipients of 3000 CCA units reported no reaction. Systemic reactions were associated much more frequently with the high dose of B/Mass. vaccine (75% of the recipients had some reaction) than with the other vaccines (to which 37%–56% of the recipients reported reactions), thus confirming the observations at the Georgia State Prison. Reactions to vaccination in the school-age group were reflected in school absenteeism. In the first 2 schools a 4-fold increase in absenteeism occurred on the day following vaccination among the recipients of one of the vaccines. On the basis of this observation the administration of that vaccine was discontinued in the remaining 2 schools. Absenteeism returned to normal on the second day following vaccination. Absenteeism among the remaining vaccine groups was no higher than among nonvaccinated students. The vaccine causing high absenteeism was later identified, upon decoding, as the high dose of B/Mass./3/66.

Summary of the 3 groups. The data from all 3 populations showed that (1) the low doses of highly purified vaccine were less reactogenic than the high doses; (2) the high doses of the A2/Aichi and A2/Japan vaccines were less reactogenic than the Sharples vaccines (divalent and polyvalent); and (3) the high dose of the B/Mass. vaccine produced much more frequent systemic reactions than did the other vaccines.

Serological responses to vaccine

All sera were examined for HI antibody to A2/Aichi/2/68, A2/Japan/170/62, and B/Mass./3/66 antigens. The results are summarized in Table 5. A significant increase in the number of persons with antibody to the Aichi antigen was produced only by the Aichi vaccine. The number of recipients responding with 4-fold or greater rises in homologous anti-

TABLE 5

HAEMAGGLUTINATION-INHIBITION ANTIBODY RESPONSES IN STUDY POPULATIONS RECEIVING SUBCUTANEOUS DOSES OF COMMERCIAL INFLUENZA VACCINES AND HIGH AND LOW DOSES OF HIGHLY PURIFIED VACCINES

Vaccine	Test antigens								
	A2/Aichi/2/68			A2/Japan/170/62			B/Mass./3/66		
	GSP [a]	CRC [b]	OCS [c]	GSP [a]	CRC [b]	OCS [c]	GSP [a]	CRC [b]	OCS [c]
A. Proportion (%) of persons showing antibody rises ≥4-fold [d]									
Highly purified									
A2/Aichi/2/68									
300 CCA	78	76	88	62	20	71	7	0	1
3000 CCA	93	88	96	67	45	92	5	3	4
A2/Japan/170/62									
300 CCA	15	0	6	40	43	67	0	5	11
3000 CCA	16	3	23	57	53	80	5	3	12
B/Mass./3/66									
300 CCA	0		3	3		2	23		66
3000 CCA	0		3	5		8	60		71
Commercial									
Divalent		3			37			48	
Polyvalent	11	12		22	33		50	39	
B. Pre- and post-immunization geometric mean titres [e]									
Highly purified									
A2/Aichi/2/68									
300 CCA	<10/69	<10/60	<10/64	81/456	29/54	37/282	12/15	15/15	<10/<10
3000 CCA	<10/126	<10/229	<10/153	99/728	32/103	37/640	16/19	21/21	<10/<10
A2/Japan/170/62									
300 CCA	<10/<10	<10/<10	<10/<10	80/202	26/71	36/234	10/10	<10/10	<10/12
3000 CCA	<10/<10	<10/<10	<10/<10	101/490	34/108	41/432	12/14	16/18	<10/15
B/Mass./3/66									
300 CCA	<10/<10		<10/<10	92/86		37/45	15/29		<10/61
3000 CCA	<10/<10		<10/<10	90/111		38/53	12/53		<10/82
Commercial									
Divalent		<10/<10			22/58			17/61	
Polyvalent	<10/<10	<10/<10		86/202	32/75		10/34	19/74	

[a] Georgia State Prison.
[b] California retirement community.
[c] Orange County, Calif., schools.
[d] Between pre- and post-immunization bleedings.
[e] Of pre- and post-immunization blood samples.

body was dose-related. Of adults receiving 300 CCA units of Aichi vaccine, 76%–78% had 4-fold or greater rises in titre, compared with 88%–93% of adults receiving 3000 CCA units. The Aichi vaccines were also as effective as the homologous A2/Japan vaccines in stimulating A2/Japan antibody.

Vaccinees receiving high doses of A2/Japan and B/Mass. vaccines had fewer 4-fold or greater rises (40%–60%) than those receiving the high dose of Aichi vaccine. These results probably reflect previous natural infection or vaccination with viruses antigenically closely related to the former 2 vaccine strains. The prevaccination titres to the A2/Japan antigen (Table 5B) were high—the range of geometric mean titres (GMT) was 22–34 in the retirement community, 37–41 in the schools, and 80–101 at the Georgia State Prison. In contrast, prevaccination titres to the Aichi antigen were low, the GMT being <10 for all populations.

The effect of prevaccination antibody is also evident in the GMT increases resulting from immunization. The high dose of Aichi vaccine induced a 21–45-fold increase in the GMT of A2/Aichi antibody, whereas the high dose of A2/Japan vaccine induced only a 3.2–4.9-fold increase in the GMT of homologous antibody. Nevertheless, with or without the presence of pre-existing antibody, the high doses of all 3 vaccines produced essentially twice the homologous GMT recorded for the low dose of each vaccine (Table 5B).

The geometric mean titres produced by the commercial vaccine and by the low doses of purified A2/Japan and B/Mass. vaccines did not appreciably differ in those populations where comparison could be made (Table 3).

DISCUSSION

The above data reveal that large doses of highly purified influenza vaccine can be given safely. The data also support the thesis that most reactions to influenza vaccine are due to nonviral impurities. There was no evidence that purification of influenza vaccine either enhanced or reduced antigenicity. The observation of minimal adverse reactions with high doses of vaccines contrasts sharply with earlier observations using large doses of Sharples centrifuged vaccine (Salk, 1948). The vaccines used in our study also seemed to be safe for older children. Although the number of adverse reactions reported by students was nearly twice that reported by adults, class attendance was not affected by any of the vaccines other than the high dose of B/Mass./3/66 vaccine.

The frequent systemic reactions associated with the dose of 3000 CCA units of/B/Mass. vaccine may have been related in some way to the manufacture of this single vaccine lot. However, the possibility that B/Mass. vaccine might inherently cause more reactions than the A2 vaccines cannot be excluded. In either event, new criteria for safety and purity should be developed now that vaccines of greater potency are possible.

Since both the high and low doses of A2/Japan vaccine failed to stimulate appreciable serum antibody against the Aichi strain, it is unlikely that these vaccines could provide significant protection against Hong Kong influenza. However, the Aichi vaccine appeared to be a good antigen. The low dose produced an excellent serum antibody response and a 10-fold increase in vaccine dosage produced substantially (2-fold) higher antibody titres. However, serum antibody titres do not necessarily indicate protection against disease. An evaluation of the protective efficacy of the vaccines during a natural epidemic of Hong Kong influenza is presented in the following paper.[1]

[1] See the paper by Schoenbaum et al. on page 531 of this issue.

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