J. Hyg., Camb. (1984), **92**, 263–276
Printed in Great Britain

A one-year study of trivalent influenza vaccines in primed and unprimed volunteers: immunogenicity, clinical reactions and protection

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(*Received* 18 *November* 1983; *accepted* 9 *January* 1984)

SUMMARY

Three hundred volunteers were divided into two age groups, 14–30 years and 31–60 years. Each participant was immunized intramuscularly with a subunit, whole virus or adsorbed whole virus vaccine, containing A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Singapore/222/79 influenza virus. Serum haemagglutination-inhibition (HI) antibody response, protection, and reactogenicity were studied after one and two doses of the vaccines. Primary immunization induced much higher percentages of HI antibody titres $\geqslant 100$ against all three vaccine viruses and much higher geometric mean titres (GMT) in volunteers with pre-immunization titres $\geqslant 18$ as compared to those with pre-immunization titres < 18. Secondary immunization did not result in an increase of GMTs or antibody titres $\geqslant 100$ in volunteers with pre-immunization titres < 18. On the whole, the response to the subunit vaccine was similar to that to the other two vaccines. To influenza B/Singapore/222/79 virus the response was lowest after administration of the whole virus vaccine in the age group 31–60 years. Over 50% of the HI titres $\geqslant 100$ found after immunization in the different vaccine and age groups were still present after one year. Serologically established infections during the winter months following immunization amounted to 15% in the subunit vaccine group, 6% in the whole virus vaccine group, and 10% in the adsorbed whole virus vaccine group. Local and systemic reactions to all three vaccines were mild in nature. Local reactions after primary immunization were much less frequent following administration of the subunit vaccine as compared to the other two vaccines, especially in the younger age group. In comparison to primary immunization, after booster immunization the incidence of local reactions was higher for the subunit vaccine and lower for the adsorbed whole virus vaccine. In the age group 14–30 years the incidence of local reactions after primary as well as booster immunization was much greater in females than in males, especially when the adsorbed whole virus vaccine was used.

INTRODUCTION

The phenomenon that the pattern of influenza antibody present in individuals is age-dependent has long ago been described by Francis and co-workers in their 'doctrine of original antigenic sin' (Davenport, Hennessy & Francis, 1953). Sera

collected in 1958 and 1967 from Dutch people born between 1940 and 1949 specifically showed a high frequency of anti-haemagglutinin antibody titres against the A-H1N1-1949 virus (Masurel, 1969*a*). This cohort was optimally primed for the A-H1N1 subtype of virus epidemic in the period 1947–57. In sera sampled in 1977, prior to the emergence of the A-H1N1 virus ('Russian flu') in The Netherlands, haemagglutination inhibition (HI) antibody titres against this virus were found in 74 and 5% of people born in 1940–9 and 1950–7, respectively (Masurel & Anker, 1978). After the appearance of the A-H1N1-1977 virus it was recommended by the World Health Organization to immunize people younger than 20 years twice with this virus; on the basis of the above-mentioned sero-epidemiological findings the Dutch Public Health Organization advised the same procedure, though for individuals under 30 years of age.

The use of split product and subunit vaccines has reduced systemic and local reactions upon immunization. However, it has been reported that especially in unprimed persons, immunization with these influenza vaccines induces a lower rate of anti-haemagglutinin antibody than vaccination with whole virus vaccines (Tyrrell *et al.* 1981). In contrast to whole virus vaccines, in non-primed subjects split product and subunit vaccines had to be administered in two doses four weeks apart instead of a single dose, to give satisfactory levels of HI antibody. This was established for the A/New Jersey/76 (H1N1) vaccine by (among others) the Pandemic Working Group (1977) and Parkman *et al.* (1976), and for the A/USSR/77 (H1N1) vaccine by Feery *et al.* (1979) and Potter *et al.* (1980).

In the present study an immunization trial was conducted with three different influenza vaccines in volunteers aged 14–30 years and 31–60 years to compare the serological response, clinical reactions, and efficacy in preventing serologically detectable infections. Furthermore, protective antibody was investigated immediately after and one year following immunization, and compared with that in other studies.

MATERIALS AND METHODS

Study group

Participants in the study were 301 volunteers living in the village of Dieren (population 20000) in the eastern part of The Netherlands. Of the volunteers, 108 were in the age group 14–30 years and 193 in the age group 31–60 years. To allow the allocation of the three influenza vaccines, the participants were divided into three groups with about the same pattern of distribution of HI antibody titres against the vaccine viruses in pre-immunization sera (Table 1). None of the participants had been vaccinated against influenza before.

Immunization

On 16 and 17 October, 1980 participants from both age groups were immunized with 0·5 ml influenza vaccine. Only those in the younger age group (14–30 years) received a booster dose of the same vaccine four weeks later. Vaccines were administered intramuscularly in the upper arm.

Twenty-four hours after primary and booster immunization the volunteers recorded on a questionnaire local reactions, such as redness, swelling, and pain at the injection site, and systemic reactions, such as headache, fever, and malaise. Local

or systemic reactions were considered positive if one or more local symptoms or one or more systemic symptoms were reported.

Influenza vaccines

The three vaccines used were commercially available. Alorbat (AWV) was a whole virus vaccine prepared by adsorption on to aluminium hydroxide, 0·5 ml of which contained 7 μg haemagglutinin (HA) of each of the influenza viruses A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1), and B/Singapore/222/79. Influvac (WV) was a whole virus vaccine and Sandovac (SU) a purified subunit antigen vaccine, both containing 10 μg HA of all three influenza viruses mentioned above.

Serological studies

Blood samples were collected on 6 and 7 October (pre-immunization, I), 12 and 13 November (II), 10 and 11 December, 1980 (III), in April (IV), July (V), and November 1981 (VI). The number of sera used for calculation of geometric mean titres (GMT) and of percentages of HI titres $\geqslant 100$ are shown in Table 1. The total drop-out percentage during the study period was 2·7%. All six sera from each participant were simultaneously examined in the HI test for antibody against the influenza viruses A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1), and B/Singapore/222/79. The HI tests were carried out according to the method described previously (Masurel, Ophof & De Jong, 1981). In establishing the GMT, an HI titre of < 9 was recorded as 8. A titre $\geqslant 100$ was adopted as protective in this study.

RESULTS

Fourfold HI titre increase following immunization

Table 2 shows the fourfold or greater HI titre increase against the three influenza viruses present in vaccines SU, WV and AWV.

Participants aged 14–30 years and with pre-immunization titres $\geqslant 18$ showed, after booster immunization, similar high percentages of fourfold titre increase against A-H3N2 and A-H1N1 virus, whereas against the B virus they were considerably lower. The highest percentage of fourfold increase against the B virus was seen in vaccine group SU. Booster immunization induced higher percentages of fourfold increase against A-H3N2 virus and B virus in vaccine group AWV only.

Volunteers aged 14–30 years with pre-immunization titres < 18 reached an optimum degree of fourfold increase against A-H3N2 virus after immunization. The highest frequency of fourfold increase against A-H1N1 virus was induced after booster immunization with vaccine AWV. Primary immunization with all three vaccines resulted in high percentages of fourfold increase against the B virus; booster immunization did not alter these percentages markedly.

In volunteers aged 31–60 years with pre-immunization titres $\geqslant 18$ there was a great variation between the three vaccine groups in percentage of fourfold increase against the two influenza A viruses and the influenza B virus.

Participants in the same age group with pre-immunization titres < 18 showed a high antibody response in all vaccine groups to both influenza A viruses. To the B virus the percentages of fourfold titre increase were considerably lower.

Table 1. *Number of sera used for calculation of GMTs and of percentages of HI titres* $\geqslant 100$

			No. of volunteers							
			Pre-immunization titre $\geqslant 18$				Pre-immunization titre < 18			
Age group	Virus	Vaccine	I, II & III*	IV*	V*	VI*	I, II & III	IV	V	VI
$\leqslant 30$ years	A-H3N2	SU†	13	13	13	13	25	23	21	21
		WV†	16	16	15	16	20	20	20	19
		AWV†	13	13	13	13	21	21	19	19
	A-H1N1	SU	22	22	20	20	16	12	12	12
		WV	23	23	22	22	13	13	13	13
		AWV	18	17	16	16	16	13	12	12
	B	SU	8	7	6	6	30	29	28	28
		WV	10	10	8	9	26	26	25	25
		AWV	10	10	10	10	24	23	20	20
> 30 years	A-H3N2	SU	21	21	21	21	43	42	41	40
		WV	14	14	14	13	49	48	47	47
		AWV	14	13	14	14	52	51	50	49
	A-H1N1	SU	35	35	34	34	29	29	28	27
		WV	33	32	32	31	30	29	29	29
		AWV	40	39	40	40	26	25	25	24
	B	SU	5	5	5	5	59	58	56	55
		WV	7	7	7	7	56	56	56	55
		AWV	5	5	5	4	61	60	60	60

* I, 10 days before immunization; II, 1 month after immunization (pre-booster in age group 14–30 yr); III, 2 months after immunization (post-booster in age group 14–30 yr); IV, V, and VI, 6, 9, and 13 months after immunization, respectively.

† SU, Subunit antigen vaccine; WV, whole virus vaccine; AWV, adsorbed whole virus vaccine.

Decline in number of volunteers, as seen under IV, V, and VI, was caused by drop-outs ($n = 8$) and volunteers with infections ($n = 21$).

HI titres $\geqslant 100$ *in the year following immunization*

Table 3 shows the serological response in terms of HI titres $\geqslant 100$. In volunteers aged 14–30 years with pre-immunization titres $\geqslant 18$, after primary as well as secondary vaccination, the percentages of HI titres $\geqslant 100$ against the two influenza A viruses varied between 92 and 100% and against the B virus between 70 and 90% among all three vaccine groups. Booster immunization did not result in higher percentages of titres $\geqslant 100$. One year after vaccination (VI) HI titres $\geqslant 100$ had declined only slightly for the influenza A viruses as well as the B virus.

Volunteers in the same age group with pre-immunization titres < 18 reacted, after booster immunization (III), with the highest percentage of HI titres $\geqslant 100$ against A-H3N2 virus in vaccine group WV, against A-H1N1 virus in group AWV, and against the B virus in group SU. Once again, booster immunization did not result in higher percentages of titres $\geqslant 100$. One year after immunization (VI) the percentage of titres $\geqslant 100$ against A-H3N2 virus had decreased with about 20 in

Table 2. *Fourfold HI titre increase against the influenza viruses A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Singapore/222/79 after primary and booster immunization*

			Percentage of sera with fourfold HI titre increase			
			Volunteers with pre-immunization titres ⩾ 18		Volunteers with pre-immunization titres < 18	
Age group	Virus	Vaccine	I→II*	I→III*	I→II	I→III
⩽ 30 years	A-H3N2	SU†	92	85	92	100
		WV†	81	75	100	100
		AWV†	62	77	95	95
	A-H1N1	SU	82	77	56	69
		WV	83	78	69	77
		AWV	89	83	69	94
	B	SU	50	50	90	87
		WV	40	30	77	77
		AWV	10	20	83	88
> 30 years	A-H3N2	SU	71	—	86	—
		WV	64	—	94	—
		AWV	86	—	96	—
	A-H1N1	SU	83	—	93	—
		WV	58	—	97	—
		AWV	73	—	85	—
	B	SU	40	—	68	—
		WV	29	—	57	—
		AWV	80	—	67	—

*† See Table 1.

all three vaccine groups; against A-H1N1 virus a decrease (30 %) was observed in vaccine group AWV only; against the B virus a 10 % decline was found for all three vaccine groups.

In the age group 31–60 years with pre-immunization titres ⩾ 18 the percentage of titres ⩾ 100 (II) against A-H3N2 virus after immunization with vaccine WV was 20–30 % lower than after administration of vaccines SU and AWV, while against A-H1N1 virus it was equally high in all vaccine groups, and against the B virus a difference of nearly 60 % was seen between the vaccine groups WV and AWV, the latter group showing the highest percentage. One year after immunization (VI), HI titres ⩾ 100 against the three vaccine strains had decreased at most by 14 %.

After immunization of volunteers in the same age group but with pre-immunization titres < 18, the highest percentage of HI titres ⩾ 100 against A-H3N2 virus was seen after administration of vaccines WV and AWV, against A-H1N1 virus after vaccine SU, and against the B virus after vaccines SU and AWV. One year after immunization (VI), HI titres ⩾ 100 against the influenza A viruses had decreased with 14–25 % and against the B virus with about 15 % among the three vaccine groups.

Table 3. *Frequency of HI titres* ⩾ 100 *to the influenza viruses A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Singapore/222/79 in sera sampled* 10 *days before and during one year after immunization*

			% sera with HI titres ⩾ 100											
			Volunteers with pre-immunization titres ⩾ 18						Volunteers with pre-immunization titres < 18					
Age group	Virus	Vaccine	I*	II*	III*	IV*	V*	VI*	I	II	III	IV	V	VI
⩽ 30 years	A-H3N2	SU†	23	100	92	100	100	100	0	72	76	57	57	57
		WV†	19	94	100	88	100	81	0	90	90	70	75	68
		AWV†	23	92	100	100	92	92	0	86	86	76	74	63
	A-H1N1	SU	41	100	100	100	100	100	0	56	56	50	50	50
		WV	30	96	100	96	91	91	0	54	54	54	54	54
		AWV	39	100	94	100	100	100	0	69	63	38	33	33
	B	SU	38	88	75	86	83	83	0	60	67	55	54	57
		WV	20	90	80	90	63	63	0	46	42	35	32	32
		AWV	60	80	70	70	60	60	0	54	46	43	45	40
> 30 years	A-H3N2	SU	14	86	90	81	86	86	0	53	53	33	32	33
		WV	14	64	71	64	71	54	0	65	57	52	53	47
		AWV	7	93	93	85	79	79	0	65	60	39	42	41
	A-H1N1	SU	29	94	94	91	94	94	0	93	86	76	82	78
		WV	33	88	88	84	88	84	0	73	73	59	59	59
		AWV	25	90	90	82	78	80	0	69	65	48	48	46
	B	SU	20	80	80	80	60	80	0	32	31	21	18	18
		WV	14	43	43	43	43	29	0	23	21	9	9	7
		AWV	25	100	100	100	80	100	0	30	25	18	18	17

*† See Table 1.

Table 4. *Geometric mean HI titres (GMT) to influenza A/Bangkok/1/79 (H3N2), A/Brazil/11/78 (H1N1) and B/Singapore/222/79 in sera sampled 10 days before and during one year after immunization in volunteers aged 14–30 years and 31–60 years*

Age group	Virus	Vaccine	Pre-immunization sera with HI titre ⩾ 18 GMT						Pre-immunization sera with HI titre < 18 GMT					
			I*	II*	III*	IV*	V*	VI*	I	II	III	IV	V	VI
⩽ 30 years	A-H3N2	SU†	61	870	649	594	510	463	9	175	257	132	143	112
		WV†	50	444	390	282	296	307	8	280	265	183	190	172
		AWV†	53	342	435	313	297	231	10	301	228	216	183	130
	A-H1N1	SU	98	1657	1031	863	732	697	9	166	164	260	230	166
		WV	71	884	691	540	480	469	9	122	144	98	90	93
		AWV	77	915	803	684	539	467	10	144	167	123	115	81
	B	SU	63	271	238	230	305	261	9	136	113	125	121	102
		WV	60	158	139	131	109	147	9	74	63	55	51	44
		AWV	114	226	192	151	145	137	9	107	106	81	89	69
> 30 years	A-H3N2	SU	45	637	458	277	304	257	9	130	109	62	64	57
		WV	45	417	378	209	228	161	9	243	194	144	136	113
		AWV	50	719	512	277	295	290	9	243	185	127	127	112
	A-H1N1	SU	64	848	719	502	541	539	10	497	399	220	245	164
		WV	67	374	318	244	227	256	10	197	188	121	124	129
		AWV	61	354	294	227	220	232	10	160	135	86	83	64
	B	SU	68	278	220	194	196	192	8	60	54	38	38	28
		WV	44	113	89	81	91	59	8	36	34	23	22	19
		AWV	73	584	670	412	327	395	8	52	45	31	29	26

*† See Table 1.

Geometric mean HI titres in the year following immunization

Table 4 shows the geometric means of HI titres. Primary immunization of volunteers in the age group 14–30 years with pre-immunization titres $\geqslant 18$ resulted, in vaccine group SU, in about twofold higher GMTs against A-H3N2 virus and A-H1N1 virus as compared to the vaccine groups WV and AWV. Booster immunization did not result in a rise in GMT against all three viruses, except for vaccine group AWV, in which a small increase against A-H3N2 virus was observed. One year after immunization, GMTs in vaccine group SU were much higher than those in vaccine groups WV and AWV.

After booster immunization of volunteers in the same age group but with pre-immunization titres < 18 GMTs against A-H3N2 and A-H1N1 virus were similar for the three vaccine groups. One year later (VI) the highest GMT against these two influenza A viruses was found in vaccine groups WV and SU, respectively. After both immunizations as well as after one year the lowest GMT was found in vaccine group WV for the B virus.

In the age group 14–30 years with pre-immunization titres $\geqslant 18$ GMTs were much higher than in sera from the same age group with pre-immunization titres < 18.

Volunteers aged 31–60 years with pre-immunization titres $\geqslant 18$ showed, after immunization, the highest GMT against A-H3N2 virus in vaccine group AWV, to A-H1N1 virus in vaccine group SU, to the B virus in vaccine group AWV. After one year almost the same distribution could be observed.

Participants in the age group 31–60 years with pre-immunization titres < 18 showed, after immunization, the lowest GMT against A-H3N2 virus in group SU, against A-H1N1 virus in group AWV, and against the B virus in group WV. Once again, after one year a similar distribution of GMTs was seen among the different vaccine groups.

In the same age group GMTs of volunteers with pre-immunization titres $\geqslant 18$ were also much higher than those of participants with pre-immunization titres < 18.

Infection rate in the winter months following immunization

Table 5 presents the serological response to the influenza viruses A-H3N2, A-H1N1 and B during the period December 1980–July 1981, when outbreaks of these viruses occurred in The Netherlands. In this study it was impossible to register clinical manifestations of infection in volunteers.

In the age group 14–30 years two volunteers immunized with vaccine SU showed a fourfold HI titre increase against A/Bangkok/1/79 (H3N2); four volunteers of vaccine group SU and four of vaccine group AWV had a response against A/Brazil/11/78 (H1N1), and in each of the three vaccine groups two participants showed a response against B/Singapore/79. The total percentage of infections with influenza A or B virus in this age group was 6% for vaccine group WV and 3–4 times higher for vaccine groups AWV and SU.

Volunteers aged 31–60 years showed similar infection rates for the three vaccine groups with regard to the influenza A-H3N2 and A-H1N1 viruses. In vaccine group WV no fourfold increase against influenza B occurred, while in vaccine groups SU and AWV infections could be established.

Table 5. *Infection rate of influenza A/H3N2/79, A/H1N1/78, and B/Singapore/79 virus represented by ⩾ fourfold titre increase in sera from volunteers aged* 14–30 *and* 31–60 *years sampled in the period December* 1980 *to July* 1981

Age group	Virus	Vaccine	No. of volunteers	No. (%) ⩾ fourfold increase III → IV or IV → V*	GMT Pre-infection	GMT Post-infection
⩽ 30 years	A-H3N2	SU†	36	2 (6)	49	684
		WV†	35	0	—	—
		AWV†	32	0	—	—
	A-H1N1	SU	36	4 (11)	23	362
		WV	35	0	—	—
		AWV	32	4 (13)	68	1152
	B	SU	36	2 (6)	31	170
		WV	35	2 (6)	29	216
		AWV	32	2 (6)	45	181
	All	SU	36	8 (22)	—	—
		WV	35	2 (6)	—	—
		AWV	32	6 (19)	—	—
> 30 years	A-H3N2	SU	64	2 (3)	90	362
		WV	63	2 (3)	21	385
		AWV	66	2 (3)	60	1627
	A-H1N1	SU	64	2 (3)	22	121
		WV	63	2 (3)	85	457
		AWV	66	1 (2)	30	242
	B	SU	64	3 (5)	10	63
		WV	63	0	—	—
		AWV	66	1 (2)	19	76
	All	SU	64	7 (11)	—	—
		WV	63	4 (6)	—	—
		AWV	66	4 (6)	—	—

*† See Table 1.

The 21 volunteers of both age groups with fourfold titre increases against an influenza A virus in the period December 1980–July 1981 had pre-infection titres < 100, except for one participant (HI titre: 136). The 10 volunteers with fourfold titre increases to the B virus had pre-infection titres < 70, except for one participant (HI titre: 96).

Reactogenicity

Table 6 shows the local and systemic reactions after primary immunization in males and females of the age groups 14–30 years and 31–60 years. In the former age group female volunteers showed higher percentages of side effects than male participants in all vaccine groups. Local reactions were most frequent in females of vaccine group AWV, and systemic reactions in females of vaccine group WV. Male volunteers immunized with vaccine SU showed the lowest percentage of side effects.

Absence of local or systemic reactions varied in the age group above 30 years

Table 6. *Local and systemic reactions after primary immunization in male and female volunteers in the age groups* 14–30 *and* 31–60 *years*

Age group	Vaccine	Sex	No. of volunteers	Percentage reactions: Local	Systemic	Local and systemic	No local and/or systemic
⩽ 30 years	SU†	M*	14	7	7	0	86
		F*	24	33	13	4	53
	WV†	M	19	63	5	5	37
		F	17	76	18	18	24
	AWV†	M	16	38	6	6	63
		F	18	94	11	11	6
> 30 years	SU	M	29	38	7	3	59
		F	35	43	9	6	54
	WV	M	36	42	22	11	47
		F	27	67	11	11	33
	AWV	M	25	52	24	24	48
		F	41	49	20	15	46

† See Table 1.
* M, male, F, female.

Table 7. *Percentage of local and systemic reactions after booster immunization in volunteers aged* 14–30 *years*

Vaccine	Sex	No. of volunteers	Percentage reactions: Local	Systemic	Local and systemic	No local and/or systemic
SU†	M*	14	43	0	0	57
	F*	22	77	5	5	23
WV†	M	19	58	0	0	42
	F	16	75	19	13	19
AWV†	M	16	19	0	0	81
	F	18	72	6	6	28

* See Table 6.
† See Table 1.

from 59 % in males immunized with vaccine SU to 33 % in females vaccinated with vaccine WV. The differences in percentage of reactions between males and females, and between the three vaccine groups, were much smaller than in the age group under 30 years. In volunteers older than 30 the highest frequency of local reactions was found in females of vaccine group WV, the lowest in males of vaccine group SU. The lowest percentage of systemic reactions was found for individuals immunized with vaccine SU.

Table 7 presents the percentage of side effects after booster vaccination of volunteers in the age group 14–30 years. Again, a higher percentage of local reactions was found in female participants. Male volunteers showed no systemic

reaction in any vaccine group. Males in vaccine group AWV showed the highest percentage of absence of systemic or local side effects. After primary and booster immunization in both age groups local as well as systemic reactions were mild in nature.

DISCUSSION

The results of this study in volunteers with commercially available inactivated influenza vaccines show the distribution of antibody just before and in the year following immunization. In participants younger than 30 years, prior to immunization, the number of protective HI titres against the A-H1N1 virus is more than twofold higher than against the A-H3N2 virus. For this reason the concept of twice immunizing people under 30 years of age with the A-H1N1 virus is no longer tenable. This is also illustrated by a second finding, namely that in volunteers with pre-immunization titres < 18 booster immunization did not evoke an increase in the number of participants with protective HI titres. Contrary to this are earlier findings of Feery *et al.* (1979), Potter *et al.* (1980), Kark *et al.* (1981) and Masurel, Ophof & De Jong (1981), who indicated that a second dose of vaccine virus A-H1N1 was necessary to give a more satisfactory level of serum antibody. This discrepancy could perhaps be caused by the fact that participants in the present study under 30 years of age, with pre-immunization titres < 18, for the greater part had undergone infections with viruses related to A/Brazil/11/78 (H1N1) virus since 1977, so that the vaccine virus did not encounter virgin soil.

In volunteers of the younger age group with pre-immunization titres < 18 vaccination evoked about 20% more protective HI titres $\geqslant 100$ to A/Bangkok/1/79 (H3N2) than in the older age group. This confirms the conclusion of Feery *et al.* (1979) that young adults responded better to A/Texas/1/77 (H3N2) than participants in the older age groups. In contrast, the A-H1N1 component in the three vaccines produced in the older age group an equal or higher percentage of protective HI titres as compared to the younger age group. Most likely, these findings can be explained by the fact that in volunteers with pre-immunization titres < 18 against A/Bangkok and A/Brazil, priming with the A-H3N2 subtype occurred after 1968 (Masurel, 1969*b*) and with the A-H1N1 subtype in the period 1947–56 (Masurel & André, 1978), respectively.

Our finding that one year post-immunization, in unprimed volunteers protective antibody was still present in a high percentage, is in contrast with results of Wright, Bryant & Karzon (1980) and Potter *et al.* (1980). They found that antibody induced by whole virus vaccines in the unprimed population was of short duration, even when two doses were administered. An explanation may be that in our study the younger age group had already been primed by natural infection.

A remarkable observation in the present study is that in the older age group no influenza B infections were found after immunization with vaccine WV, although this vaccine evoked by far the lowest serological response. The same phenomenon was seen by us in a trial with a whole virus vaccine carried out in 1974 (results not published). Wright, Bryant & Karzon (1980) also reported that the HI antibody response following infection or vaccination with influenza B virus did not provide a satisfactory index of immunity. In contrast, Oxford, Yetts & Schild (1982) detected in their vaccine study, using the single radial haemolysis

(SRH) technique, higher levels of antibody to influenza B viruses as compared to the HI test.

Earlier results of Wesselius-de Casparis, Masurel & Kerrebijn (1972) were corroborated by our findings that influenza A infections did not occur in connection with HI titres $\geqslant 150$ and sporadically with titres $\geqslant 100$. As regards the influenza B virus, these borderlines were at HI titres 100 and 70, respectively. Presumably an HI titre 100, as found by our technique, is comparable to an HI titre 40 and an enzyme immunoassay titre 3200 in other studies (Hobson *et al.* 1972; Potter *et al.* 1977; Goodeve *et al.* 1983; Pyrhönen, Suni & Romo, 1981).

Bernstein & Cherry (1983) reported a lower rate of local reactions after booster immunization with subunit vaccine as compared to primary immunization. However, in the present study the incidence of local reactions was higher after revaccination with vaccine SU, which is in agreement with results by Jennings *et al.* (1981) in their study involving three types of vaccine. The correlation proposed by Parkman *et al.* (1976) and the Pandemic Working Group (1977) between reactions to influenza virus vaccination and levels of circulating HI antibody prior to immunization was not found by us. The reasons for this discrepancy are not yet clearly understood.

Another interesting finding in the present study is that in the younger age group the incidence of local reactions is much greater in females than in males, especially in vaccine group AWV. This is true for primary as well as booster immunization. In recent literature we have not found data with regard to differences in reactions after influenza vaccination between males and females. However, Feery (1977) reported a marked sex difference in frequency of reactions after smallpox vaccination; the female–male ratio of adverse reactions was 1·6:1, while the sex difference in reaction rates increased with age. The latter is in contrast with our findings in the older age group, where the sex difference in reactions was less distinctly present.

The results of this study suggest that the questions of age and sex difference in reaction rates after primary and booster immunization would be worthy aspects of further study, especially in view of the recent advice of the WHO to twice immunize young children with a trivalent influenza vaccine (World Health Organization, 1983).

The authors are indebted to Drs L. W. Bunt, J. van der Hoeve, R. J. Polée, A. R. Simonis, H. W. M. van der Velden and A. J. E. de Wit, and the local department of the Red Cross at Dieren for their help and co-operation. We thank W. J. J. Anker, G. I. Arron, R. van Beek, H. van Driel, M. Horsman, W. Kamp and G. L. van der Water for technical assistance, W. E. P. Beyer and R. A. Heijtink for comments, and R. S. Engels-Bakker for the English translation and preparation of the manuscript. Last but not least thanks are due to the volunteers, whose enthusiastic and unrelenting co-operation was a prerequisite for the success of this study.

REFERENCES

BERNSTEIN, D. I. & CHERRY, J. D. (1983). Clinical reactions and antibody responses to influenza vaccines. A comparison of split and subunit vaccines in children and young adults. *American Journal of Diseases of Children* **137**, 622.

DAVENPORT, F. M., HENNESSY, A. V. & FRANCIS, T. (1953). Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus. *Journal of Experimental Medicine* **98**, 641.

FEERY, B. J. (1977). Adverse reactions after smallpox vaccination. *Medical Journal of Australia* **2**, 180.

FEERY, B. J., GALLICHIO, H. A., RODDA, S. J. & HAMPSON, A. W. (1979). Antibody responses to influenza vaccines containing A/USSR/90/77. *Australian Journal of Experimental Biology and Medical Science* **57**, 335.

GOODEVE, A., POTTER, C. W., CLARK, A., JENNINGS, R., SCHILD, G. C. & YETTS, R. (1983). A graded-dose study of inactivated, surface antigen influenza B vaccine in volunteers: reactogenicity, antibody response and protection to challenge virus infection. *Journal of Hygiene* **90**, 107.

HOBSON, D., CURRY, R. L., BEARE, A. S. & WARD-GARDNER, A. (1972). The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *Journal of Hygiene* **70**, 767.

JENNINGS, R., POTTER, C. W., MASSEY, P. M. O., DUERDEN, B. I., MARTIN, J. & BEVAN, A. M. (1981). Responses of volunteers to inactivated influenza virus vaccines. *Journal of Hygiene* **86**, 1.

KARK, J. D., LEBIUSH, M., RANNON, L., WITZTUM, E., NILI, E. & KEDEM, M. (1981). The antigenicity of whole virus versus subunit trivalent influenza vaccines – a field trial in the Israel Defence Forces, 1978. *Medical Microbiology and Immunology* **170**, 55.

MASUREL, N. (1969*a*). Serological characteristics of a 'new' serotype of influenza A virus: the Hong Kong strain. *Bulletin of the World Health Organization* **41**, 461.

MASUREL, N. (1969*b*). Relation between Hong Kong virus and former human A2 isolates and the A/Equi2 virus in human sera collected before 1957. *Lancet* i, 907.

MASUREL, N. & ANDRÉ, F. E. (1978). Antibody response against current H1N1 influenza virus after vaccination with last season's trivalent vaccine. *Lancet* i, 144.

MASUREL, N. & ANKER, W. J. J. (1978). Influenza A in het winterseizoen 1977–78, veroorzaakt door het 'oude' H3N2-virus en het 'nieuwe' H1N1 virus. *Nederlands Tijdschrift voor Geneeskunde* **122**, 383.

MASUREL, N., OPHOF, P. & DE JONG, P. (1981). Antibody response to immunization with influenza A/USSR/77 (H1N1) virus in young individuals primed or unprimed for A/New Jersey/76 (H1N1) virus. *Journal of Hygiene* **87**, 201.

OXFORD, J. S., YETTS, R., & SCHILD, G. C. (1982). Quantitation and analysis of the specificity of post-immunization antibodies to influenza B viruses using single radial haemolysis. *Journal of Hygiene* **88**, 325.

PANDEMIC WORKING GROUP OF THE MEDICAL RESEARCH COUNCIL (1977). Antibody responses and reactogenicity of graded doses of inactivated influenza A/New Jersey/76 whole-virus vaccine in humans. *Journal of Infectious Diseases* **136**, supplement, S475.

PARKMAN, P. D., GALASSO, G. J., TOP, F. H. & NOBLE, G. R. (1976). Summary of clinical trials of influenza vaccines. *Journal of Infectious Diseases* **134**, 100.

POTTER, C. W., JENNINGS, R., NICHOLSON, K., TYRRELL, D. A. J. & DICKINSON, K. G. (1977). Immunity to attenuated influenza virus WRL 105 infection induced by heterologous, inactivated influenza A virus vaccines. *Journal of Hygiene* **79**, 321.

POTTER, C. W., CLARK, A., JENNINGS, R., SCHILD, G. C., WOOD, J. M. & MCWILLIAM, P. K. A. (1980). Reactogenicity and immunogenicity of inactivated influenza A (H1N1) virus vaccine in unprimed children. Report to the Medical Research Council Committee on influenza and other respiratory virus vaccines. *Journal of Biological Standardization* **8**, 35.

PYRHÖNEN, S., SUNI, J. & ROMO, M. (1981). Clinical trial of a subunit influenza vaccine. *Scandinavian Journal of Infectious Diseases* **13**, 95.

TYRRELL, D. A. J., SCHILD, G. C., DOWDLE, W. R., CHANOCK, R. & MURPHY, B. (1981). Development and use of influenza vaccines. *Bulletin of the World Health Organization* **59**, 165.

WESSELIUS-DE CASPARIS, A., MASUREL, N. & KERREBIJN, K. F. (1972). Field trial with human and equine influenza vaccines in children: protection and antibody titres. *Bulletin of the World Health Organization* **46**, 151.

WRIGHT, P. F., BRYANT, J. D. & KARZON, D. T. (1980). Comparison of influenza B/Hong Kong virus infections among infants, children, and young adults. *Journal of Infectious Diseases* **141**, 430.

WORLD HEALTH ORGANIZATION (1983). Recommended composition of influenza vaccines for use in the 1983–1984 season. *Weekly Epidemiological Record (WHO, Geneva)* **58**, 53.