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Comparative trials of live attenuated and detergent split influenza virus vaccines

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SUMMARY

Comparative clinical trials of live attenuated and detergent-split subunit influenza virus vaccines were undertaken with 1048 volunteers in Western Australia. Volunteers were divided into three main groups, each of which received either live virus vaccine or a saline control administered intranasally, or subunit vaccine injected subcutaneously. No differences were recorded between the three groups in their post-vaccination symptoms. Serum samples were collected at various times up to 50 weeks after vaccination, and antibody titres were measured by haemagglutination-inhibition (HI) tests and, for 231 volunteers, by virus neutralization tests. The two vaccines were almost equivalent in inducing seroconversion in vaccinees with pre-trial HI titres of 96 or less, but the subunit vaccine stimulated a higher geometric mean HI antibody titre. The longevity of the HI antibody response was greater for the live virus vaccine. The height of the response and the longevity of neutralizing antibody were the same for both vaccines. Both vaccines provided a high degree of protection against epidemic A/England/42/72 influenza, and some protection against A/Port Chalmers/1/73 influenza.

INTRODUCTION

It is widely believed that the most effective procedure for immunization against influenza may be by means of live attenuated virus vaccines. Killed subunit vaccines currently in use have only provided 60–75% protection against epidemic influenza in open populations, although higher protection rates have been achieved by mass vaccination, particularly in closed communities. In comparative trials in man and in laboratory animals, live virus vaccines administered intranasally have induced better levels of protection than killed vaccines given subcutaneously (Beare, Hobson, Reed & Tyrrell, 1968; Freestone *et al.* 1972; Potter *et al.* 1972), and despite significantly lower titres of humoral antibody against the viral haemagglutinin, have induced equivalent protection compared with an adjuvant vaccine (Freestone *et al.* 1972). It is known that intranasal inoculation of vaccine can elicit the production of local IgA antibody in the upper respiratory tract (Alford, Rossen, Butler & Kasel, 1967; Slepushkin *et al.* 1971) and the production of IgA and IgG antibodies in the lower respiratory tract (Waldman *et al.* 1973*a*), both of which may be of considerable importance in stimulating satisfactory degrees of protection.

Suitably attenuated live virus vaccines, however, present two potential dangers: reversion to full virulence, and an increase in virulence after person-to-person transmission. Any live influenza vaccine therefore must be shown to be stable and non-reactogenic, and must give rise to little or no transmission. Evidence of transmission to close contacts from virus shedding has been observed previously with live influenza vaccines (McDonald, Zuckerman, Beare & Tyrrell, 1962), but has not been found within a closed population (Davenport *et al.* 1971; Beare, Habershon, Tyrrell & Hall, 1973; Lamy *et al.* 1973).

This report describes the results of clinical trials of a live attenuated influenza virus vaccine in three communities in Western Australia. The primary objective of these trials was to ascertain the immunological response, and where possible, the degree of protection, elicited by intranasal inoculation of the live virus vaccine compared with a subcutaneous killed subunit vaccine. A number of other characteristics were also investigated, including the period after vaccination during which virus was shed; transmission of vaccine virus from vaccinees to unvaccinated individuals in the same household; the sero-conversion frequencies in subjects with residual immunity gained from previous exposure to influenza; and the relative lengths of immunity in response to live and subunit vaccines.

MATERIALS AND METHODS

Volunteers

A total of 1048 volunteers were drawn from three localities in Western Australia: Busselton, a coastal resort town 160 miles south of Perth, with a high population of retired people and with a large transient summer tourist population (246 volunteers); Collie, a coal-mining town 130 miles south-east of Perth, with a stable population (553 volunteers); and staff and students of the University of Western Australia in Perth (249 volunteers). There was little difference in mean ages of volunteers from Busselton (44·4 years) and from Collie (43·8 years), but the volunteers from Perth were considerably younger (23·3 years).

Male volunteers between the ages of 18 and 55, who were in good health and were not in the influenza high risk categories, were permitted to receive the live virus vaccine. The vaccine was not administered to women because of reports suggesting a link between influenza infections during the first trimester of pregnancy with congenital malformations and with childhood leukaemia although fetal risk must be considered very slight (reviewed by MacKenzie & Houghton, 1974). However, women were included in the trials as subunit vaccinees, or as placebo controls to examine the possibility of post-vaccinal transmission. Volunteers aged over 55 were included as subunit vaccinees.

The volunteers were assigned to three groups, shown in Table 1, and vaccinated with live virus vaccine, subunit vaccine or a saline control. A further three small groups were vaccinated with various dilutions of the live virus vaccine.

Table 1. *Number of volunteers and their vaccine groups*

	Live virus vaccine	Placebo controls	Subunit vaccine	Live virus vaccine 1/5	Live virus vaccine 1/10	Live virus vaccine 1/50
Group ...	(A)	(B)	(C)	(D)	(E)	(F)
Total no. of subjects	415	262	311	21	21	18
Number of males	415	122	153	21	21	18
Number of females	0	140	158	0	0	0
No. of subjects aged between 18 and 55	415	262	180	21	21	18
No. of subjects with pre-trial HI titre of $\leqslant 96$	392	233	294	20	20	15

Vaccines

Live virus vaccine

The live virus vaccine, 'Alice' strain, was developed by Recherche et Industrie Thérapeutique, Belgium as a stable inhibitor-resistant variant of MRC-2, a recombinant isolated by Dr G. C. Schild, WHO Influenza Centre, Mill Hill, London, from a cross between A/Eng/42/72 (H3N2) and A/PR8/34 (H0N1). This recombinant retained the neuraminidase and haemagglutinin antigens of the A/Eng/42/72 parent.

The vaccine was obtained as a freeze-dried preparation, and reconstituted before use with physiological saline. It was administered intranasally as drops to the subject in a supine position, 0·3 ml. in each nostril, with a total dose of $10^{7\cdot3}$ egg infectious units. A second dose was given 14 days later.

A few volunteers were given 1/5, 1/10 and 1/50 dilutions of the vaccine in physiological saline.

Subunit vaccine

The killed subunit vaccine was a commercially available preparation manufactured by the Commonwealth Serum Laboratories, Melbourne, and was administered by deep subcutaneous injection.

Two doses of 1·0 ml. containing 16,000 haemagglutinating units of A/England/42/72 and 8,000 haemagglutinating units of B/Roma/1/67, were given 4 weeks apart.

Placebo controls

Placebo inoculations of physiological saline were administered intranasally as two doses, 14 days apart, in the same quantities as for the live virus vaccine.

Clinical schedules

Blood samples were collected from volunteers immediately before vaccination to determine pre-trial humoral antibody titres, and subsequently before administration of the second dose of vaccine (13 days for live virus vaccinees and placebo controls; 27 days for subunit vaccinees), and at 7, 30 and 50 weeks after vaccination. All serum samples were stored at $-20°$ C.

Volunteers from Collie and Perth were asked to complete a daily symptoms chart to record any 'influenza-like' symptoms for 7 days after each dose of vaccine. The possible symptoms included running nose, cough, sore throat, headache, malaise, joint pains, muscular pain, local pain at the site of injection, and a raised temperature. Volunteers who did not receive symptoms charts were closely questioned at their next visit to the clinic.

A few volunteers in Perth and Busselton were also asked to take nasal and/or throat swabs at various intervals from 6–12 hr. to 4 days after vaccination for virus isolation studies. The swabs were returned to the laboratory in chilled medium containing 0·5 % gelatin.

Epidemic survey

An influenza epidemic occurred in Western Australia between October and mid-December 1973 (approximately 12–24 weeks after vaccination). The nature of these trials made it impossible to keep volunteers under medical supervision, and therefore no direct assessment of the severity of infections could be made among the volunteers. In an attempt to determine whether clinical symptoms did occur, a questionnaire was sent to each volunteer in Collie and Busselton requesting information of 'influenza-like' symptoms suffered by volunteers or members of their families. The symptoms requested were similar to those included in the post-vaccinal symptoms charts.

Virus isolation

The medium surrounding nasal and throat swabs was inoculated into the allantoic cavity of 10-day-old embryonated eggs, four eggs for each swab. The allantoic fluids were harvested after 48 hr. incubation at 37° C., and examined for the presence of virus by haemagglutination (HA). Negative samples were passaged a second time. Viruses isolated were tested for inhibitor-resistance in the presence of equine serum.

Haemagglutination-inhibition assays

Serum samples were titrated in parallel for haemagglutination-inhibition (HI) antibody after treatment with cholera filtrate to destroy non-specific virus inhibitors. Four HA units of influenza strain MRC-7, which contained the antigenic determinants of A/England/42/72, were incubated with serial twofold serum dilutions overnight at 4° C., before the addition of a 0·5 % suspension of fowl erythrocytes. Post-epidemic sera were also tested against 4 HA units of A/Perth/2/73 which was antigentically similar to A/Port Chalmers/1/73. All titres were expressed as the reciprocals of the dilution at which haemagglutination was completely inhibited.

Neutralization assays

Neutralization assays were performed in WHO-type plastic trays by the membrane-on-shell technique of Fazekas de St Groth & White (1958). Twofold serum dilutions were mixed with 30–100 egg infectious units of MRC-7, and incubated for 4 hr. at 37° C. A 0·025 ml. amount of each of the virus–serum mixtures was then transferred into a well of a WHO-type perspex tray containing a piece of shell 1 cm. square with the chorioallantoic membrane attached to it. After 48 hr.

Table 2. *Summary of symptoms recorded after first dose of vaccine*

	Live virus vaccine	Placebo controls	Subunit vaccine	Live virus vaccine		
				1/5	1/10	1/50
Group ...	(A)	(B)	(C)	(D)	(E)	(F)
No. of symptoms charts returned	108	153	151	12	16	16
No. with no symptoms recorded	64	84	79	10	12	8
No. recording symptoms of:						
Rhinorrhoea	24*	50	49	1	3	4
Cough	6	13	14	1	0	0
Sore throat	23	33	21	0	2	1
Headache	21	34	26	1	1	3
Malaise	12	23	15	2	1	0
Joint pains	10	11	10	1	1	2
Muscular pains	4	11	6	1	0	0
Pain at site of injection	0	0	42	0	0	0
Fever	4	3	6	0	0	0

* No. of subjects recording specific symptoms on days 1, 2 or 3 after vaccination.

incubation with continuous shaking, the shell fragments were removed and the multiplication of the virus ascertained by haemagglutination. All titres were expressed as the reciprocal of the dilution at which complete neutralization was observed.

RESULTS

Post-vaccinal symptoms

Symptom charts were returned by 456 volunteers after their first dose of vaccine and 212 volunteers after their second dose. An analysis of the symptoms recorded during the 3 days after their first dose is shown in Table 2. Less than half the volunteers in each group recorded one or more symptoms, and no significant differences were observed in the pattern of reactions either between vaccinees and placebo controls, or between the recipients of the two vaccines. Fewer symptoms were recorded after the second dose of vaccine, but once again a similar pattern of reaction was found between the different vaccine groups.

It would appear, therefore, that the live virus vaccine was well tolerated and was non-reactogenic.

Post-vaccination virus isolations

The results of nasal and throat swabs taken *by vaccinees* after receiving the first dose of vaccine were uncertain because a labelling error made it impossible to distinguish between live virus vaccinees and placebo controls. The virus isolation frequencies after the second dose of vaccine are shown in Table 3. A small amount of residual virus was observed in nasal swabs taken 6–12 hr. after vaccination from five live virus vaccinees, but the quantity of infectious virus was very low and

Table 3. *Virus isolations from nasal or throat swabs after the administration of live virus vaccine or placebo*

Day post-vaccination	Vaccine group	Type of swab	No. of swabs	No. of isolations
0*	Live virus vaccine	Nasal	76	5
		Throat	24	0
	Placebo	Nasal	30	1
		Throat	17	0
1	Live virus vaccine	Nasal	60	0
		Throat	24	0
	Placebo	Nasal	27	0
		Throat	17	0
4	Live virus vaccine	Nasal	24	0
		Throat	18	0
	Placebo	Nasal	17	0
		Throat	13	0

* Day 0 = 6–12 hr. after vaccination.

Table 4. *HI titres of volunteers receiving live virus vaccine*

		Pre-vaccination titres							
		<6	6	12	24	48	96	192	384
Post-vaccination titres*	<6	8	—	—	—	—	—	—	—
	6	9†	2	—	—	—	—	—	—
	12	12	4	9	—	—	—	—	—
	24	32	11	8†	4	—	—	—	—
	48	21	20	16	4	9†	—	—	—
	96	21	13	22	20	4	5	—	—
	192	13	6	13	18	7	2	2	—
	384	6	4	8	12	4	8	2	4
	768	3	7	7	5	3	3	2	2
	1536	0	0	1	1	1	3	0	1
	3072	1	0	0	0	0	1	0	0
	6144	0	1	0	0	0	0	0	0
Sero-conversion (%)		76	91	80	87·5	54	68	33	15

* The highest titres at 2 or 7 weeks after vaccination have been used in the construction of this table.

† Two volunteers in each of these groups had been exposed to subunit vaccine within 2 months of the start of the trials.

required two egg passages to be detectable by haemagglutination. Virus was also isolated from a nasal swab taken by a placebo control. All virus strains were horse-serum-inhibitor resistant.

Serological response to vaccination

A fourfold or greater rise in the serum HI antibody titre was assumed to be indicative of sero-conversion. The HI antibody responses after vaccination as a function of the pre-vaccination titres are shown in Table 4 (live virus vaccine),

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Table 5. *HI titres of volunteers receiving subunit vaccine*

		Pre-vaccination titres							
		<6	6	12	24	48	96	192	384
Post-vaccination titres*	<6	6	—	—	—	—	—	—	—
	6	1	1	—	—	—	—	—	—
	12	3	5	1	—	—	—	—	—
	24	9	2	5	7	—	—	—	—
	48	11	13	2	2	3	—	—	—
	96	12	7	12	5	5	2	—	—
	192	5	5	11	4	2	2	2	—
	384	7	10	15	8	3	0	1	2
	768	7	5	10	8	6	2	3	0
	1536	3	1	7	3	1	0	0	0
	3072	4	2	6	5	2	3	2	1
	6144	2	5	6	5	0	2	1	0
	12288	2	1	3	2	4	1	0	0
Sero-conversion (%)		86	89·5	92	82	69	67	67	33

* The highest titres at 4 or 7 weeks after vaccination have been used in the construction of this table.

Table 6. *HI titres of volunteers receiving placebo inocula*

		Pre-vaccination titres						
		<6	6	12	24	48	96	192
Post-vaccination titres*	<6	45	—	—	—	—	—	—
	6	19	20	—	—	—	—	—
	12	3	8	54	—	—	—	—
	24	4	0	10	24	—	—	—
	48	3	1	1	4	18	—	—
	96	1	0	1	0	2	8	—
	192	2	0	0	0	0	3	10
	384	0	0	2	0	0	0	2
	768	0	0	0	0	0	0	0
	1536	0	0	0	0	1	0	0
Sero-conversion (%)		13	3·5	6	0	5	0	0

* The post-vaccination titres are the highest titres 2 or 7 weeks after vaccination.

Table 5 (subunit vaccine) and Table 6 (placebo controls). It was not possible to preselect volunteers on the basis of their immune status, and indeed it was preferable to ascertain the efficacy of the vaccines to elicit sero-conversion in both the presence and absence of residual levels of HI antibody. However, approximately 44 % of volunteers had no pre-existing immunity (HI titre of $\leqslant 6$).

The ability of the vaccines to invoke sero-conversion in volunteers with pre-vaccination HI antibody titres of 96 or less, and the vaccine dose after which sero-conversion occurred, are shown in Table 7. The geometric mean HI titres and the

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Table 7. *Sero-conversion after administration of live virus and subunit vaccines in volunteers with an initial HI titre of* $\leqslant 96$

Vaccine	No. of volunteers	Sero-conversion: After first dose	Sero-conversion: After second dose	Sero-conversion: Total	Sero-conversion (%)
Live virus	386	185	127	312*	81
Subunit	294	211	40	251	85
Placebo	233	4	12	16	7
1/5 normal dose of live virus	20	1	10	11	55
1/10 normal dose of live virus	20	0	11	11	55
1/50 normal dose of live virus	15	0	8	8	53

* A further six volunteers did not sero-convert, but they had been exposed to subunit vaccine two months before the start of these trials.

longevity of the responses are shown in Fig. 1. There was little difference in the ability of the two vaccines to elicit sero-conversion (81 % of live virus vaccinees to 85 % of subunit vaccinees), but whereas 84 % of the subunit vaccinees, who sero-converted, did so after the first dose of vaccine, only 59 % of live virus vaccinees sero-converted at the same stage. Moreover, the geometric mean HI titres induced by the subunit vaccine were considerably higher than those induced by the live virus vaccine. These results are not unexpected and reflect the route of inoculation and nature of the two vaccines; the killed subunit vaccine being subcutaneous and the live attenuated vaccine being intranasal. To elicit sero-conversion, therefore, the live virus vaccine must initiate a mild upper respiratory tract infection, and the two dose protocol is employed to ensure that the majority of vaccinees are successfully infected. Conversely, the second dose of subunit vaccine is used essentially as a booster dose.

The results shown in Fig. 1 also suggest that the longevity of the HI response may be greater after infection with the live virus vaccine. Although the subunit vaccine induced higher geometric mean titres, the titres fell more rapidly over the 50-week study period. If the curves are extrapolated, the subunit vaccinees should regain their pre-trial geometric mean titre about 120 weeks post-vaccination and the live virus vaccinees at approximately 180 weeks post-vaccination.

Three diluted doses of live vaccine were given to a few volunteers (1/5, 1/10 and 1/50 of the normal dose), but they were not as successful in eliciting sero-conversion. It would appear, therefore, that the amount of live virus present in the normal vaccine dose was the minimal effective quantity.

The height of the immune response of volunteers who had sero-converted after exposure to the two vaccines was analysed in terms of their pre-trial immune status; volunteers with no HI antibody (pre-trial titres of $\leqslant 6$), and volunteers with residual HI antibody from a previous exposure to influenza (pre-trial HI titres of 24 or greater). Those volunteers with a pre-trial HI titre of 12 were excluded.

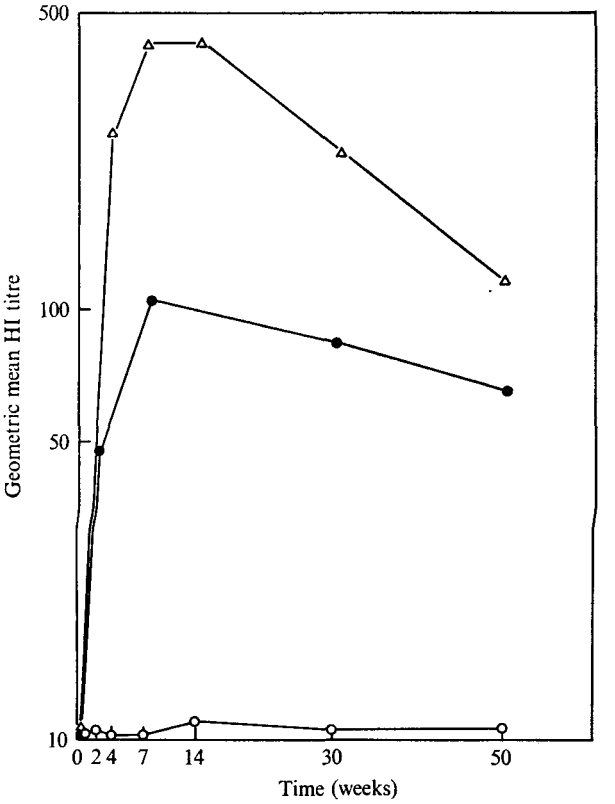

Fig. 1. Geometric mean serum HI titres. ●–●, Live virus vaccinees; △–△, subunit vaccinees; ○–○, placebo volunteers.

The results are shown in Fig. 2. Both vaccines induced a greater response in vaccinees with no prior immunity (a 50-fold and a 16-fold increase in the geometric mean titres for the subunit and live virus vaccines respectively), than in those vaccinees with pre-existing HI antibody (20-fold and 7·5-fold respectively), although the mean titres were considerably higher in the latter because of the anamnestic response. There was no apparent longevity of the HI response (Fig. 2) for either of the two live virus vaccine groups, or for the subunit vaccinees with pre-existing HI antibody, but a significant decrease in longevity was observed in the geometric mean titres of subunit vaccinees with pre-trial titres of $\leqslant 6$.

The effect of age on the immune response induced by the vaccines was examined with the subjects grouped as younger than 21, 21–40, 41–55, and older than 55 (Table 8). With the live virus vaccine, the 41–55 age group responded with higher titres and with a slower rate of antibody decline than the other two age groups, but a higher percentage of volunteers did not show sero-conversion. The ability of the subunit vaccine to elicit sero-conversion increased with age, and only 8% of the vaccinees in the older than 55 group failed to sero-convert. The youngest age group, however, responded poorly to the subunit vaccine and the geometric mean titres fell rapidly during the 50-week study period, but it should be noted that the number of volunteers in this group was small.

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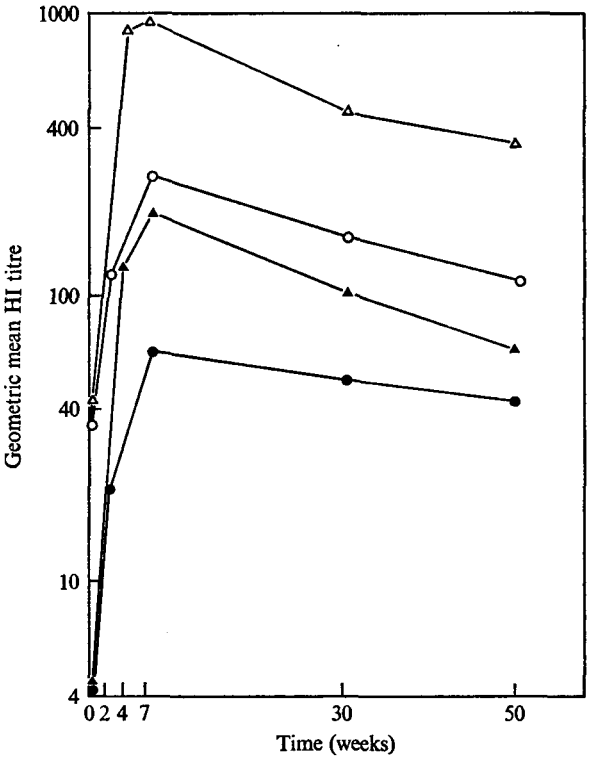

Fig. 2. The effect of pre-vaccination immune status on serum HI antibody responses to live virus and subunit vaccines. ●–●, Geometric mean titres of live virus vaccinees with pre-vaccination titres of ⩽6; ○–○, geometric mean titres of live virus vaccinees with pre-vaccination titres of ⩾24; ▲–▲, geometric mean titres of subunit vaccinees with pre-vaccination titres of ⩽6; △–△, geometric mean titres of subunit vaccinees with pre-vaccination titres of ⩾24.

Live virus vaccine was administered to nine subjects 4 weeks after they had received an initial dose of subunit vaccine. All of the vaccinees sero-converted after receiving the subunit vaccine, and three showed further sero-conversion after exposure to the live virus vaccine despite high titres of HI antibody (Table 9).

Neutralizing antibody titres were measured in parallel in serum samples drawn from 231 volunteers (124 live virus vaccinees, 86 subunit vaccinees, and 21 placebo controls). A number of the volunteers who had sero-converted to HI antibody failed to show a fourfold rise in neutralizing antibody; 27·5 % of live virus vaccinees and 23 % of subunit vaccinees. The geometric mean neutralizing titres throughout the 50-week study period are depicted in Fig. 3. Little difference was observed between the two vaccines in the longevity of their respective geometric mean neutralizing titres, or, in contrast to the geometric mean HI titres, in their magnitude.

Transmission studies

An investigation into the possibility of virus transmission was undertaken between live virus vaccinees and placebo controls in the same household, who were defined as being 'at risk'. Placebo volunteers 'at risk' were compared to placebo

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Table 8. *The effect of age on response to live virus and subunit vaccines*

	Live virus vaccine			Subunit vaccine			
Age	⩽20	21–40	41–55	⩽20	21–40	41–55	>55
Pre-vaccine	12·5 (51)*	9 (142)	10 (100)	12 (18)	10 (54)	10 (68)	12 (118)
2 or 4 weeks	76 (49)	41 (142)	41 (98)	184 (17)	218 (49)	337 (64)	322 (110)
7 weeks	116 (48)	101 (128)	140 (85)	296 (16)	390 (47)	371 (61)	443 (104)
30 weeks	69 (34)	83 (94)	100 (66)	136 (10)	203 (36)	206 (61)	216 (105)
50 weeks	57 (29)	54 (67)	94 (55)	44 (8)	157 (24)	142 (46)	140 (77)
Volunteers not sero-converting (%)	20	21	38	28	22	21	8
Volunteers with pre-existing HI antibody (%)	37	23	34	22	26	19	35

* Figures in parentheses are the number of serum samples tested for each point.

Table 9. *HI titres of volunteers receiving subunit vaccine initially followed by live virus vaccine four weeks later*

Volunteer no.	Pre-trial titre	Titre 4 weeks after receiving subunit vaccine	Titre 3 weeks after receiving live virus vaccine
1	12	192	192
2	12	384	768
3	48	384	1536*
4	24	768	3072*
5	6	48	48
6	6	768	3072*
7	6	6144	6144
8	12	1536	1536
9	<6	48	96

* Sero-conversion after administration of live virus vaccine.

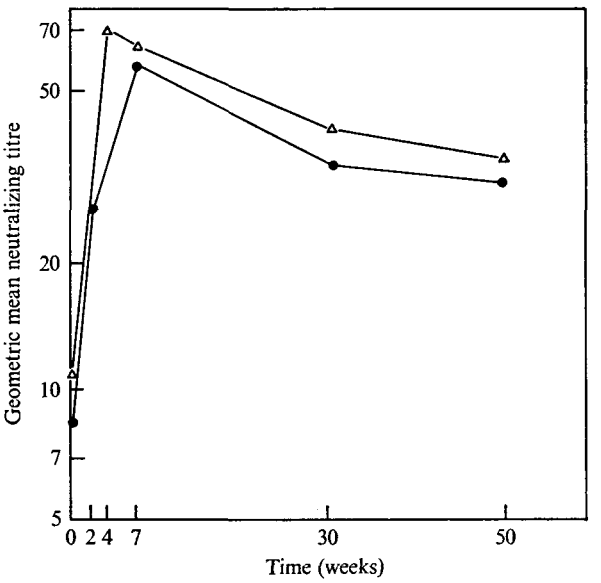

Fig. 3. Geometric mean neutralizing antibody titres of live virus and subunit vaccinees. ●–●, Live virus vaccinees; △–△, subunit vaccinees.

volunteers who had no known familial relationship with live virus vaccinees, and the numbers of each group showing a fourfold serum HI antibody rise 2 or 7 weeks after vaccination are shown in Table 10. Of the 9 'at risk' placebo volunteers who were found to have sero-converted, 7 were wives of live virus vaccinees (there were 57 wives in the 'at risk' group), 1 was the fiancée of a live virus vaccinee, and 1 was a brother of a live virus vaccinee. One of the above wives was actively involved in the clinical trials as a member of the laboratory staff. Three of the placebo volunteers in the 'no known risk' group who sero-converted were closely associated with many live virus vaccinees during working hours in a confined space. These results suggested that a small amount of transmission may occur between close contacts, such as husband and wife.

Table 10. *Risk of virus transmission to placebo controls after administration of live virus vaccine*

Placebo volunteers (pre-trial titre ⩽96)	Total no.	No. sero-converting 2 or 7 weeks post-vaccination	Sero-converting (%)
'At risk'	69	9*	13
No known risk	166	6†	3·6

* 'At risk' placebo volunteers sero-converting included seven wives of live virus vaccinees. One wife was a member of the Laboratory staff in the vaccine trials.

† Three placebo volunteers sero-converting in this group were closely associated with many live virus vaccinees in an office or at the coal-mine pit-face.

Epidemic influenza – an attempt to estimate the protective effect of live virus and subunit vaccines

An influenza epidemic occurred in Western Australia with the majority of cases falling between mid-October and mid-December 1973. The epidemic was unusual for two reasons: it was much later in the year than normal; and two antigenically distinct strains of influenza were isolated. The two strains were antigenically related to A/England/42/72 and A/Port Chalmers/1/73. It was not possible to monitor the volunteers during this period, and in order to assess the protective effect of the two vaccines, evidence of infection was sought from retrospective questionnaires for vaccinees to record 'influenza-like' symptoms, and from sero-conversion (HI antibody) during the epidemic period.

Serological evidence

A fourfold or greater rise in serum HI antibody titres between 7 and 50 weeks after vaccination was construed as evidence of infection with epidemic influenza. All sera were titrated against both strains of virus using MRC-7 (antigenically similar to A/England/42/72) and A/Perth/2/73 (antigenically similar to A/Port Chalmers/1/73). The number of volunteers who showed sero-conversion, their pre-epidemic HI titres, and the strain of epidemic influenza involved, are shown in Table 11. Approximately 28% of the volunteers with no pre-epidemic HI antibody (placebo controls and vaccinees who did not show sero-conversion) sero-converted to one or other influenza strain, and both strains occurred with equal frequency. The results in Table 11 tended to suggest that the live virus vaccine was marginally more effective than the subunit vaccine in providing protection to A/England/42/72, but the numbers were too small to be significant.

Evidence from questionnaires

Volunteers in Busselton and Collie were asked by retrospective questionnaire whether they had experienced any 'influenza-like' symptoms during the epidemic period, including rhinorrhoea, headache, cough, sore throat, muscle and joint pains, or fever. The answers were graded by Dr V. Balmer, Medical Director (Australia), Smith, Kline and French Laboratories, as follows:

(1) Fever *or* joint and muscle pains plus one other symptom – influenza.
(2) Any two or more other symptoms – possibly influenza.
(3) One or no symptoms – no influenza.

Table 11. *Serological evidence of epidemic influenza*

Pre-epidemic titre	Vaccinees who had sero-converted after: Live virus vaccine — No. of vaccinees	Live virus vaccine — Epidemic sero-conversion A/Eng/42/72	Live virus vaccine — Epidemic sero-conversion A/Perth/2/73	Subunit vaccine — No. of vaccinees	Subunit vaccine — Epidemic sero-conversion A/Eng/42/72	Subunit vaccine — Epidemic sero-conversion A/Perth/2/73	Placebo volunteers and vaccinees who had failed to sero-convert — No. of vaccinees	Epidemic sero-conversion A/Eng/42/72	Epidemic sero-conversion A/Perth/2/73
< 6	0	0	0	0	0	0	75	12	12
6	0	0	0	0	0	0	48	5	5
12	1	0	0	0	0	0	92	7	8
24	37	0	6	13	0	3	45	2	2
48	55	0	1	39	1	3	48	1	2
96	73	1	0	51	1	0	23	1	1
> 96	104	0	0	134	0	1	46	0	0
Totals	270	1	7	237	2	7	377	28	30

Table 12. *Epidemic influenza – summary of questionnaires returned, and severity of cases showing sero-conversion during the epidemic period*

	No influenza* — No. of questionnaires returned	Serological evidence A/England	Serological evidence A/Perth	Possible epidemic influenza* — No. of questionnaires returned	Serological evidence A/England	Serological evidence A/Perth	Epidemic influenza* — No. of questionnaires returned	Serological evidence A/England	Serological evidence A/Perth
Live virus vaccinees who sero-converted after vaccination	86	0	2	30	0	2	34	1	1
Subunit vaccinees who sero-converted after vaccination	113	0	1	38	0	0	40	2	2
Placebo volunteers and volunteers who failed to sero-convert	115	14	9	24	3	3	46	7	11

* Epidemic influenza – questionnaire replies recording fever *or* joint and muscle pains plus one other symptom. Possible epidemic influenza – questionnaire replies recording any two other symptoms.

The questionnaires of those volunteers who showed serological evidence of epidemic influenza were examined to determine the severity of the infections, and the results are shown in Table 12 with the total number of questionnaires returned, their grading, and their vaccine grouping. Four of six live virus vaccinees who had serological evidence of epidemic influenza (5 cases of A/Perth/2/73 and 1 case of A/England/42/72) had mild or subclinical infections, whereas four out of five subunit vaccinees with serological evidence of epidemic influenza (3 cases of A/Perth/2/73 and 2 cases of A/England/42/72) recalled 'influenza-like' symptoms. However, the returned questionnaires reinforced the contention that little useful information on influenza attack rates can be obtained from retrospective self-diagnosis.

DISCUSSION

The clinical trials described above were designed to assess the efficacy of a live influenza virus vaccine, the 'Alice' strain, in terms of its reactogenicity, antigenicity, transmissibility, and protective capacity, and to compare it with a commercially available killed subunit vaccine.

Three major immune defence mechanisms are involved in the acquisition of protection; humoral antibodies, local secretory antibodies, and cell-mediated immunity (CMI). The humoral HI antibody response has been the most commonly employed criterion for estimating the potential effectiveness of influenza vaccines, and has been shown to correlate with protection (Hobson, Beare & Gardner, 1971). Humoral neuraminidase-inhibiting (NI) antibody has also been implicated in protection (Murphy, Kasel & Chanock, 1972). However, local antibody (HI, NI and neutralizing antibodies) in the upper and lower respiratory tracts may be more important still by preventing viral infection at the site of entry (Alford *et al.* 1967; Waldman *et al.* 1973*a*). It is believed that CMI plays a part in immunity to influenza because it has not been possible to account completely for resistance to infection with live influenza vaccines by the presence of humoral or local antibody (Habershon *et al.* 1973). Waldman and his colleagues (Waldman & Henney, 1971; Waldman, Spencer & Johnson, 1972) have shown in guinea-pigs that local CMI in the respiratory tract can be stimulated independently of systemic CMI, and is dependent on route of immunization. Thus bronchial lymphocytes were stimulated much more after intranasal inoculation than after subcutaneous inoculation of killed vaccine. Similar results were observed in man (Waldman, Gadol, Olsen & Johnson, 1973*b*; Jurgensen *et al.* 1973).

Significant differences exist, therefore, in the type of immune response induced by intranasal inoculation of live virus vaccine and deep subcutaneous administration of the killed subunit vaccine. In the former, humoral and local antibody and local CMI are stimulated, whereas in the latter, the response is almost totally restricted to humoral antibody and CMI. These differences may explain the better protection rates observed for intranasal live virus vaccines in comparative trials with subcutaneous killed vaccines in man and animals (Beare *et al.* 1968; Potter *et al.* 1972; Freestone *et al.* 1972).

In this study the ability of the two vaccines to elicit a fourfold increase in

humoral HI antibody in vaccinees with a pre-vaccination titre of 96 or less was similar, with 81 % of live virus vaccinees and 85 % of subunit vaccinees showing sero-conversion. If the response < 6 to 12 is also included as positive sero-conversion, the percentage of live virus vaccinees is increased to 84 % and the subunit vaccinees to 86 %. However, as might be expected, parenteral immunization induced considerably higher geometric mean HI titres. In a trial in which live virus vaccine was compared with killed oil adjuvant vaccine, a similar difference in HI titres was found, but the two vaccines were equally effective in providing protection (Freestone *et al.* 1972). The difference in titres, therefore, should not be considered as a measure of protective capacity.

A number of authors have suggested that natural infection with influenza might confer longer-lasting immunity than parenteral immunization, and this concept has been proposed as a potential advantage of live virus vaccines. The longevity of the immune response in these trials indicated that the humoral HI antibody induced by the subunit vaccine fell more rapidly than that induced by the live virus vaccine, and that if the curves of the geometric mean HI titres were extrapolated, subunit vaccinees would regain pre-vaccination titres 120 weeks after vaccination and live virus vaccinees 180 weeks after vaccination. The results, therefore, indicated that the live virus vaccine should confer longer lasting immunity than the subunit vaccine.

The effect of age on response to the two vaccines was difficult to assess. That a higher proportion of vaccinees over the age of 55 were found to show sero-conversion after administration of subunit vaccine even in the absence of pre-vaccination HI antibody, was probably caused by an anamnestic response to prior exposure to other influenza strains. The reasons for the high number of live virus vaccinees in the 41–55 age group not responding are not fully understood, but a large proportion of these vaccinees were coal-miners who were vaccinated at the pit-head before starting work and to whom the vaccine was administered in a sitting position rather than a supine position.

Immunization with live virus vaccines has been found previously to induce sero-conversion in vaccinees with little or no HI antibody, but has been relatively ineffective in boosting the immunity in vaccinees with prior experience (Hobson, Beare & Gardner, 1971; Hobson *et al.* 1973). In these trials, however, the live virus and subunit vaccines were equally effective in inducing sero-conversion in vaccinees with pre-vaccination HI titres of 24–96, but for volunteers with titres above 96 the effectiveness of the live virus vaccine decreased. Nevertheless, of seven vaccinees who responded to an initial vaccination of subunit vaccine with HI titres of 192 or greater, three showed sero-conversion after receiving the live virus vaccine as their second dose. This latter result suggested that subjects in the influenza high risk categories, for whom an initial dose of live virus vaccine might be considered unwise, could receive the live virus vaccine as a second dose.

No significant differences were observed in the geometric mean neutralizing antibody titres between the live virus and subunit vaccinees. The relative importance of humoral neutralizing and HI antibody titres in assessing vaccine effectiveness has not been elucidated, but Tyrrell and his colleagues (McDonald *et al.* 1962)

have suggested that neutralizing antibodies might provide a better measurement of antibody response and protective capacity. If this is so, these results indicate that both vaccines should be equally effective in protecting against epidemic influenza.

Suggestive evidence of a small amount of post-vaccinal transmission was obtained between live virus vaccinees and placebo volunteers living in the same household. In previous clinical trials with other live influenza virus vaccines, low level transmission was reported between close contacts (McDonald *et al.* 1962), but could not be demonstrated between volunteers in closed communities (Davenport *et al.* 1971; Habershon *et al.* 1973; Lamy *et al.* 1973). In a trial of 'Alice' live influenza vaccine carried out at The Centre for Disease Control, Atlanta, Georgia, no evidence of transmission was observed in wives of 31 live virus vaccinees who had received doses of vaccine similar to those employed in this study (R. J. Rubin, G. R. Noble, L. Corey, D. Brandling-Bennett, H. Kaye, M. T. Coleman, W. J. Brown, W. R. Dowdle and M. B. Gregg (1974), personally communicated by A. Prinzie). Shedding of virus by live virus vaccinees was infrequent, and the virus titres were considerably lower than the amount believed to be necessary to elicit sero-conversion. Thus, although serological evidence for transmission was obtained in this study, virological evidence of infection by an 'inhibitor-resistant' strain is needed to support such a conclusion.

The influenza epidemic that occurred during the trial period was caused by two serologically distinct influenza variants, closely related to A/England/42/72 and A/Port Chalmers/1/73. Serological evidence of infection during the epidemic in the placebo group and in vaccinees who had not shown post-vaccinal sero-conversion gave an attack rate of 7·4% (28 cases in 377 subjects) for the A/England variant, and 7·96% (30 cases in 377 subjects) for the A/Port Chalmers variant. On a random basis, the expected number of live virus vaccinees with serological evidence of infection, therefore, would have been 20 and 21·5 for A/England and for A/Port Chalmers respectively, and for the subunit vaccinees, 17·5 and 19. However, the actual number of live virus vaccinees with serological evidence of infection during the epidemic period, and who had previously shown post-vaccinal sero-conversion, were 1 and 7 for the two epidemic strains respectively, representing a reduction in the attack rate of 95% and 67%, and for the subunit vaccinees, 2 and 7, representing a reduction in the attack rate of 88·5% and 63%. The validity of these attack rates should be considered with caution in the absence of any clinical correlation.

The problems inherent in assessing influenza infections from self-diagnosed retrospective questionnaires has been discussed elsewhere (MacKenzie & Houghton, 1974). The criteria employed in grading replies must also be considered of doubtful value, particularly in distinguishing mild and severe infections. Nevertheless, it was interesting to note that 4 out of 6 live virus vaccinees who had serological evidence of influenza suffered mild or subclinical infections whereas 4 out of 5 subunit vaccinees claimed to have suffered severe infections.

Thus little apparent difference was observed in the efficacy of the two vaccines. Waldman *et al.* (1973*a*) have suggested, however, that more effective immunization can be attained by the nasal route if vaccine is permitted to penetrate to the

lower respiratory tract. The frequency of sero-conversion after inoculation of the live virus vaccine, therefore, could possibly be improved if the vaccine was administered as a nasal spray with small aerosol particles rather than by nasal drops.

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REFERENCES

Alford, R. H., Rossen, R. D., Butler, W. T. & Kasel, J. A. (1967). Neutralizing and haemagglutination-inhibiting activity of nasal secretions following experimental human infection with A2 influenza virus. *Journal of Immunology* **98**, 724–31.

Beare, A. S., Habershon, R. B., Tyrrell, D. A. J. & Hall T. S. (1973). Recombinant live influenza vaccine virus – tests of transmissibility in Man. *Journal of Biological Standardization* **1**, 233–6.

Beare, A. S., Hobson, D., Reed, S. E. & Tyrrell, D. A. J. (1968). A comparison of live and killed influenza virus vaccines. *Lancet* ii, 418.

Davenport, F. M., Hennessy, A. V., Minuse, E., Maassab, H. F., Anderson, G. R., Mitchell, J. R., Heffelfinger, J. C. & Barrett, C. D. (1971). Pilot studies on mono and bivalent live attenuated influenza virus vaccines. *Proceedings of the Symposium on Live Influenza Vaccines, Zagreb*, pp. 105–13.

Fazekas de St Groth, S. & White, D. O. (1958). An improved assay for the infectivity of influenza viruses. *Journal of Hygiene* **56**, 151–62.

Freestone, D. S., Hamilton-Smith, S., Schild, G. C., Buckland, R., Chinn, S. & Tyrrell, D. A. J. (1972). Antibody responses and resistance to challenge in volunteers vaccinated with live attenuated, detergent split and oil adjuvant A2/Hong Kong/68 (H_3N_2) influenza vaccines. *Journal of Hygiene* **70**, 531–43.

Habershon, R. B., Molyneux, M. E., Slavin, G., Loewi, G. & Tyrrell, D. A. J. (1973). Skin test with influenza virus. *Journal of Hygiene* **71**, 755–60.

Hobson, D., Beare, A. S. & Gardner, A. W. (1971). Haemagglutination-inhibition serum antibody titres as an index of the response of volunteers to intra-nasal infection with live attenuated strains of influenza virus. *Proceedings of the Symposium on Live Influenza Vaccine, Zagreb*, pp. 73–85.

Hobson, D., Baker, F. A., Curry, R. L., Beare, A. S. & Massey, P. M. O. (1973). The efficacy of live and inactivated vaccines of Hong Kong influenza virus in an industrial community. *Journal of Hygiene* **71**, 641–7.

Jurgensen, P. F., Olsen, G. N., Johnson, J. E., Swenson, E. W., Ayoub, E. M., Henney, C. S. & Waldman, R. H. (1973). Immune response of the human respiratory tract. II. Cell-mediated immunity in the lower respiratory tract to tuberculin and mumps and influenza virus. *Journal of Infectious Diseases* **128**, 730–5.

Lamy, F., Prinzie, A., Peetermans, J., Vascoboinic, E. & Huygelen, C. (1973). Clinical evaluation of a live attenuated influenza virus vaccine ('Ann' strain). *International Symposium on Influenza Vaccines for Men and Horses, London, 1972. Symposium Series on Immunobiological Standard* **20**, 282–8. Basle: Karger.

McDonald, J. C., Zuckerman, A. J., Beare, A. S. & Tyrrell, D. A. J. (1962). Trials of live influenza vaccine in the Royal Air Force. *British Medical Journal* i, 1036–42.

MacKenzie, J. S. & Houghton, M. (1974). Influenza infections during pregnancy: association with congenital malformations and with subsequent neoplasms in children, and potential hazards of live virus vaccines. *Bacteriological Reviews* **38**, 356–70.

Murphy, B. R., Kasel, J. A. & Chanock, R. M. (1972). Association of serum antineuraminidase antibody with resistance to influenza in man. *New England Journal of Medicine* **286**, 1329–32.

Potter, C. W., Oxford, J. S., Shore, S. L., McLaren, C. & Stuart Harris, C. H. (1972). Immunity to influenza in ferrets. I. Response to live and killed virus. *British Journal of Experimental Pathology* **53**, 153–67.

Slepushkin, A. N., Schild, G. C., Beare, A. S. Chinn, S., & Tyrrell, D. A. J. (1971). Neuraminidase and resistance to vaccination with live influenza A2 Hong Kong vaccines. *Journal of Hygiene* **69**, 571–8.

Waldman, R. H. & Henney, C. S. (1971). Cell-mediated immunity and antibody responses in the respiratory tract after local and systemic immunization. *Journal of Experimental Medicine* **134**, 482–94.

Waldman, R. H., Jurgensen, P. F., Olsen, G. N., Ganguly, R. & Johnson, J. E. (1973*a*). Immune response of the human respiratory tract. I. Immunoglobulin levels and influenza virus vaccine antibody response. *Journal of Immunology* **111**, 38–41.

Waldman, R. H., Gadol, N., Olsen, G. N. & Johnson, J. E. (1973*b*). Respiratory tract cell-mediated immunity to influenza. *International Symposium on Influenza Vaccines for Men and Horses, London, 1972. Symposium Series on Immunobiological Standard* **20**. Basle: Karger.

Waldman, R. H., Spencer, C. S. & Johnson, J. E. (1972). Respiratory and systemic cellular and humoral immune responses to influenza virus vaccine administered parenterally or by nose drops. *Cellular Immunology* **3**, 294–300.