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Response to influenza vaccine in adjuvant 65-4

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SUMMARY

A comparison was made of the antibody response and subjective reactions to zonally-purified influenza vaccine in aqueous suspension and in peanut oil adjuvant 65-4. Both preparations contained 700 CCA units of A/Aichi/2/68, and 300 CCA units of B/Mass/1/71.

Subjective reactions were recorded by asking the volunteers to complete a record daily for 5 days. Pain at the injection site was recorded by 64% of the recipients of the oil adjuvant vaccine compared with 35% of the aqueous recipients, but local redness was more frequent after aqueous vaccine. Systemic symptoms were recorded a little more frequently after aqueous than oil adjuvant vaccine.

When measured 7½ weeks after a single dose of vaccine, the HAI geometric mean antibody titre (G.M.T.) to the A/Hong Kong/1/68 antigen (antigenically similar to the A/Aichi/2/68 antigen in the vaccine) increased 2·7-fold after aqueous and 16·4-fold after adjuvant vaccine. Sixty-two weeks after vaccination the antibody titres remained higher in those given adjuvant vaccine. The G.M.T. to B/Mass/1/71 increased 1·9-fold 7½ weeks after aqueous vaccine and 3·7-fold after adjuvant vaccine.

The antibody response to both influenza A and B antigens was broader in the recipients of adjuvant vaccine. The G.M.T. to A/England/42/72 increased 2·8-fold after aqueous and 13-fold after adjuvant vaccine; and to B/England/847/73 it increased 1·3-fold after aqueous and 1·9-fold after adjuvant vaccine.

INTRODUCTION

The protection given by influenza vaccine is incomplete and is not believed to be long-lasting (Eickhoff, 1971). The potent oil-emulsion adjuvants have not found wide favour for use with influenza antigens (Stuart-Harris, 1969; Murray, 1970) owing to the occasional development of persistent local reactions at the site of

injection (Medical Research Council, 1964) and also because they may sometimes give rise in animals to tumours and, possibly, autoimmune reactions (Stokes *et al.* 1964). To overcome these drawbacks Merck, Sharp and Dohme workers have developed a peanut oil preparation, Adjuvant 65, in which all the components are metabolizable (Hilleman, 1966; Hilleman, Woodhour, Friedman & Weibel, 1973).

Adjuvant 65 has been progressively purified and in the latest preparation, Adjuvant 65-4, both the aluminium monostearate stabilizer and the mannide mono-oleate emulsifier have been synthesized and are more than 99% pure (Hilleman *et al.* 1973). Laboratory studies have failed to reveal teratogenicity of components of Adjuvant 65, and tumours which may be produced in certain strains of mice are believed to be due to a sensitivity in these strains to the oncogenic effect of physico-chemical irritation – an effect equally produced by diphtheria/tetanus/pertussis vaccine (Hilleman, 1970; 1972, personal communication). In addition, follow-up for nearly 20 years of U.S. armed forces personnel given mineral oil influenza vaccines has failed to reveal evidence of any harmful effects (Beebe, Simon & Vivona, 1972). The opportunity was therefore taken to examine the response to adjuvant 65-4 influenza vaccine in comparison with aqueous vaccine.

MATERIALS AND METHODS

Vaccines. These were prepared and supplied by Merck, Sharp and Dohme Research Laboratories.

(1) Zonally-purified influenza vaccine in aqueous suspension. Each 0·5 ml. dose contained:

A2/Aichi/2/68 (H3, N2), equivalent to 700 CCA units.
B/Mass/1/71, equivalent to 300 CCA units.

(2) Oil adjuvant vaccine. Each 0·5 ml. dose contained the same antigens in the same final concentrations as the aqueous vaccine, suspended as a water-in-oil emulsion in Adjuvant 65-4, the composition being:

Influenza vaccine	50%
Peanut oil (United States Pharmacopeia)	45%
Isomannide mono-oleate emulsifier, synthetic	3%
Aluminium monostearate stabilizer, synthetic	2%

The adjuvant vaccine consisted of a creamy white emulsion dispensed in a dose of 0·5 ml. loaded in a 2 ml. syringe fitted with a needle of 1·0 mm. external diameter; the syringes were kept before use at 4° C., and stood at room temperature during the vaccination session.

Volunteers. These were employees of a heavy industrial factory (Cryoplants), 18 years of age or over. They were randomly allocated to receive either aqueous or oil adjuvant vaccine according to odd or even birth dates. Vaccines were given intramuscularly into the deltoid muscle. Pregnancy or hypersensitivity to eggs were regarded as contraindications to vaccination. A blood sample was collected before vaccination from each volunteer who agreed; a second blood sample was taken

Table 1. *Participants in adjuvant* 65-4 *study*

Vaccine	No. vaccinated	Returned inquiry form		1st blood		2nd blood		3rd blood	
		No.	%	No.	%	No.	%	No.	%
Aqueous	187	133	71	128	68	84	45	43	23
Adjuvant 65-4	182	151	83	145	80	104	57	52	29

$7\frac{1}{2}$ weeks later, and a third sample 62 weeks later. Volunteers were told which vaccine they had received only when they attended for the $7\frac{1}{2}$ week blood sample and their reaction record had been collected.

Reactions to vaccination. These were recorded by providing each volunteer with a card on which to record daily for 5 days, including the day of vaccination, the presence of the following symptoms which were pre-printed on the card:

> No symptoms; pain at site of injection; redness at site of injection; headache; fever (i.e. hot or shivery); pains in back, arms or legs; symptoms of a 'cold'; other.

Reactions recorded in this way are referred to as 'subjective reactions' (Smith, Fletcher & Wherry, 1974). In addition, the subjects were asked to report to the health centre if they developed an adverse reaction that caused them any concern.

Antibody titrations. Sera were stored at $-20°$ C. The antibody content of the paired samples was tested by haemagglutination-inhibition (W.H.O., 1953). The antigens studied are given in the Results. The third samples were titrated in parallel with the corresponding previous samples for which sufficient serum remained.

In calculating geometric mean titres (G.M.T.) it was assumed that individual titres lay at the next mid-point of the logarithmic intervals, and titres of less than 1/10, or greater than 1/5120 at the corresponding mid-points below and above.

RESULTS

Participants

The proportion of volunteers who returned the reaction inquiry form was 12 % greater among the oil-adjuvant recipients than aqueous vaccine recipients, and a similar excess was evident in the proportion who provided a 1st and also a 2nd blood sample (Table 1). The differences are presumably a chance factor, no other explanation being apparent. The greater number returning the reaction inquiry form might in part be due to the greater amount of local pain caused by the oil-adjuvant vaccine (see below), but the excess is the same as that of the proportion providing 1st blood samples – collected before the vaccines were given.

The age and sex composition of the two groups was similar; there were few female employees in the factory, and only 43 were included in the study.

Table 2. *Number and percentage of vaccinees recording freedom from subjective symptoms after vaccination*

		Days after vaccination					
		0*	1	2	3	5	Total
Aqueous	No.	70	65	88	99	115	133
	%	53	49	66	74	86	100
Oil adjuvant	No.	42	71	95	106	122	151
	%	28	47	63	70	81	100

* Day 0 = day of vaccination.

Table 3. *Number of vaccinees recording symptoms at 0 to 4 days after receiving each vaccine*

		Day					
		0	1	2	3	4	Total
Local pain	Oil	92	61	35	18	10	216
	Aq.	38	30	16	7	4	95
Local redness	Oil	3	6	3	2	0	14
	Aq.	16	30	20	13	5	84
Headache	Oil	10	10	11	11	4	46
	Aq.	9	17	8	5	1	40
Fever	Oil	9	3	6	7	2	27
	Aq.	17	10	3	1	1	32
Muscle pains	Oil	16	16	9	9	3	53
	Aq.	13	14	8	4	2	41
'Cold'	Oil	13	18	13	13	12	69
	Aq.	7	16	12	12	10	57

Reactions

The adjuvant vaccine was given without difficulty and no difference in acceptability of the two vaccines was apparent at the vaccination session. None of the subjects reported to the health centre with an adverse reaction, but many recorded the presence of subjective reactions, although neither the local nor systemic symptoms were severe and in no instance did they interfere with work or cause a volunteer to be absent.

The incidence of reactions was greater in those given adjuvant vaccine – 44 (33 %) of the recipients of aqueous vaccine recorded freedom from all symptoms compared with only 31 (21 %) of adjuvant vaccine recipients. On the day of vaccination (day 0) only 28 % of adjuvant vaccine recipients were free from symptoms, compared with 53 % of aqueous vaccine recipients (Table 2). The differences were mainly attributable to local symptoms (Table 3). On the day of vaccination, for example, 92 recipients of adjuvant vaccine (61 %) complained of local pain at the injection site compared with only 38 of those given the aqueous vaccine (29 %), and the number complaining of local pain remained higher for the whole 5-day period in which symptoms were recorded. Ninety-six of the adjuvant vaccinees

Table 4. *Antibody to A/Hong Kong/1/68 before and 7½ weeks after vaccination with the two vaccines*

	Aqueous		Adjuvant 65-4	
	Before vaccine	7½ weeks after vaccine	Before vaccine	7½ weeks after vaccine
Total sera	82	82	104	104
No. of sera with titre of				
< 10	9	0	9	0
10	0	0	4	0
20	6	5	11	0
40	13	3	15	0
80	17	8	20	3
160	14	18	16	6
320	14	23	17	16
640	3	11	8	12
1280	3	6	4	30
2560	2	7	0	24
5120	1	1	0	13
Geometric mean titre	132	399	117	1543

Table 5. *Reciprocal geometric mean antibody titres in the blood samples collected 62 weeks after vaccination, titrated in parallel with the corresponding first and second samples*

	Aqueous vaccine (43 subjects)			Adjuvant vaccine (52 subjects)		
Antigen	Before vacc.	7½ weeks after vacc.	62 weeks after vacc.	Before vacc.	7½ weeks after vacc.	62 weeks after vacc.
A/HK/1/68	199	494	373 (1:1·3)*	158	1935	1076 (1:1·8)
A/Eng/42/72	81	222	137 (1:1·6)	52	893	262 (1:3·4)
B/Mass/1/71	34	67	55 (1:1·2)	32	126	89 (1:1·4)

* Figures in parentheses show the ratio between the G.M.T. at 62 weeks and that at 7½ weeks.

(64 %) complained of local pain at some time in the 5 days compared with 47 of the aqueous vaccinees (35 %). Local redness, however, was recorded about 5 times more commonly by recipients of aqueous vaccine than by those receiving adjuvant vaccine.

Antibody response

Response to A/Hong Kong/1/68. Before vaccination only 18 (10 %) of the 186 subjects had no detectable antibodies (< 1/10) to an antigen closely related to that in the vaccine (Table 4). The G.M.T. rose from 1/132 to 1/399 (3-fold) 7½ weeks

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Table 6. *Antibody to A/England/42/72 before and 7½ weeks after vaccination with the two vaccines*

	Aqueous		Adjuvant 65-4	
	Before vaccine	7½ weeks after vaccine	Before vaccine	7½ weeks after vaccine
Total sera	82	82	104	104
No. of sera with titre of				
< 10	16	6	19	2
10	7	0	11	0
20	11	3	18	2
40	21	10	23	1
80	9	23	18	11
160	12	21	8	14
320	4	13	7	23
640	2	3	0	22
1280	0	3	0	17
2560	0	0	0	10
5120	0	0	0	2
Geometric mean titre	48	143	43	572

later in those given aqueous vaccine, and from 1/117 to 1/1543 (13-fold) in the recipients of adjuvant vaccine. Among those given adjuvant 65-4, 90 (87%) had a 4-fold or greater antibody rise compared with 37 (45%) of those given aqueous vaccine.

Ninety-five subjects provided a blood sample 62 weeks after vaccination. At that time the mean antibody titres were 2·9-fold higher in those who had adjuvant vaccine than in those given aqueous vaccine (Table 5).

Response to A/England/42/72. The A/Eng/42/72 (H3, N2) variant of the influenza virus first caused epidemic influenza in Britain in the 1972–3 winter, i.e. after the first sera were collected in the present study on 27 October 1972. The strain showed only partial serological cross-reaction with A/Hong Kong strains (Pereira *et al.* 1972). Thirty-five subjects (19%) had no detectable pre-vaccination antibody at a serum dilution of 1/10 and in 82 (44%) the titre was less than 1/40 (Table 6).

In response to vaccination the G.M.T. of those given aqueous vaccine increased only 3-fold, from 1/48 to 1/143, whereas in those given adjuvant 65-4 vaccine the increase was 13-fold, from 1/43 to 1/572. Among those given adjuvant vaccine 89 (86%) had a 4-fold or greater response compared with 34 (41%) of the recipients of aqueous vaccine.

Sixty-two weeks after vaccination, the mean antibody titres were twice as high in the adjuvant 65-4 vaccine group as in the aqueous vaccine group (Table 5).

Response to influenza B/Mass/1/71. The antibody titres to influenza B before vaccination were low, the G.M.T. being 1/29. A 1·9-fold increase in G.M.T. was shown by the recipients of the aqueous vaccine when tested 7½ weeks after vaccination, compared with a 3·7-fold increase after adjuvant 65-4 vaccine (Table 7).

Table 7. *Antibody to B/Mass/1/71 before and* $7\frac{1}{2}$ *weeks after vaccination with the two vaccines*

	Aqueous		Adjuvant 65-4	
	Before vaccine	$7\frac{1}{2}$ weeks after vaccine	Before vaccine	$7\frac{1}{2}$ weeks after vaccine
Total sera	83	83	104	104
No. of sera with titre of				
< 10	20	2	25	0
10	18	11	23	5
20	12	18	15	17
40	11	20	25	20
80	14	22	11	32
160	7	6	2	17
320	1	3	3	7
640	0	1	0	4
1280	0	0	0	1
2560	0	0	0	1
Geometric mean titre	30	57	27	101

Table 8. *Antibody to B/England/847/73 before,* $7\frac{1}{2}$ *and* 62 *weeks after vaccination with the two vaccines*

	Aqueous			Adjuvant 65-4		
	Before vaccine	$7\frac{1}{2}$ weeks after vaccine	62 weeks after vaccine	Before vaccine	$7\frac{1}{2}$ weeks after vaccine	62 weeks after vaccine
Total sera	41	41	43	51	51	51
No. of sera with titre of						
< 20	27	24	27	36	26	26
20	1	2	3	6	1	7
40	6	4	6	2	12	8
80	3	5	3	5	5	5
160	3	3	3	1	2	2
320	1	1	0	1	2	2
640	0	1	1	0	1	1
1280	0	0	0	0	2	0
⩾ 2560	0	1	0	0	0	0
Geometric mean titre	27	36	28	22	41	33

Of those given the aqueous vaccine 20 (24%) had a 4-fold or greater response, compared with 51 (49%) of the recipients of adjuvant vaccine.

Sixty-two weeks after vaccination the G.M.T. remained slightly higher in the adjuvant vaccine recipients (Table 5). At that time 15/40 (38%) of the aqueous recipients had a titre of less than 1/40 compared with 11/51 (22%) of the adjuvant recipients.

Response to influenza B/England/847/73. The B/Eng/847/73 virus is antigenically

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similar to the B/Hong Kong strains which appeared in the United Kingdom in 1973 (P.H.L.S., 1973; W.H.O., 1973). The antibody response to the heterologous antigen was small with little increase in G.M.T. after aqueous or adjuvant vaccine (Table 8). However, a 4-fold or greater response was found in 14/51 subjects given the adjuvant vaccine compared with only 1/41 of the aqueous recipients.

DISCUSSION

The serological response to influenza vaccine in adjuvant 65-4 was better than to aqueous vaccine without adjuvant. If it is assumed that those with a titre of HAI antibodies of 1 in 40 or more are immune (Hobson, Curry, Beare & Ward-Gardner, 1972) then none of those given oil adjuvant vaccine was susceptible to the A/Hong Kong/1/68 strain of influenza when tested $7\frac{1}{2}$ weeks after vaccination. In the year after vaccination the mean antibody titres of the adjuvant vaccine recipients fell to a greater extent than in the aqueous recipients (Table 5) but, nevertheless, their mean titres were still greater than those of the subjects given aqueous vaccine.

The potency of the adjuvant 65-4 vaccine was also evident in the responses to the A/Eng/42/72 strain, which had undergone a considerable antigenic shift from the Hong Kong strain. This was important in the present study because at the time when influenza vaccine was being used in the autumn of 1972, the available vaccines contained antigens similar to the A/HK/1/68 strain, despite the very high probability that most influenza in the coming winter would be caused by a variant similar to A/Eng/42/72. After the adjuvant vaccine only 4 of 48 (8%) persons who had pre-vaccination titres of less than 1/40 to the A/Eng/42/72 variant remained at such low titres $7\frac{1}{2}$ weeks after vaccination, compared with 9 of 34 (26%) similar persons given aqueous vaccine (Table 6). Pereira *et al.* (1972) found that of 88 persons with antibody to the A/England strain at a titre of less than 1/40, 45 responded to aqueous A/Hong Kong vaccine, leaving 43 (49%) still at such low titres 2 weeks after vaccination.

The response to B/Mass/1/71 antigen, of which 300 CCA units were present in the vaccine, was lower than the response to the influenza A antigen. Nevertheless, the adjuvant vaccine stimulated higher antibody titres against influenza B than the non-adjuvant vaccine, and broadened the response to induce a 4-fold rise in G.M.T. to the distantly related B/Eng/847/73 strain in about a quarter of the recipients.

Complaints of local pain after vaccination were reported by 64% of those given adjuvant vaccine compared with 35% of those given aqueous vaccine. The pain, although mild and insufficient to cause loss of work, is a possible drawback, since minor reactions are probably an important factor affecting acceptance of vaccine by healthy adults in subsequent years (Smith *et al.* 1974). On the other hand, visible erythema over the injection site was more common after aqueous vaccine, and the number of subjective general reactions was also slightly greater.

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