J. Hyg., Camb. (1972), 70, 531
Printed in Great Britain

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*

A report to the Medical Research Council Committee on Influenza and other Respiratory Virus Vaccines

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(*Received* 17 *February* 1972)

SUMMARY

Forty-nine subjects were vaccinated with either live attenuated, detergent split, or oil adjuvant A2/Hong Kong influenza vaccines, or a saline influenza B vaccine as control. Respiratory symptoms occurred more frequently in subjects who received the live vaccine but in total there was little difference between the symptoms in the four groups. Antibody titres in nasal washings and serum were measured by haemagglutination inhibition, neuraminidase inhibition and virus neutralization tests. The oil adjuvant vaccine stimulated larger antibody responses than the other procedures. Six weeks after vaccination the volunteers were challenged with partially attenuated live A2/Hong Kong influenza virus administered intranasally. The live attenuated and oil adjuvant vaccines provided the best protection against challenge.

INTRODUCTION

Many studies have been made of various influenza vaccines including unsplit inactivated virus in saline, detergent-split material, oil-adjuvant preparations of unsplit inactivated virus and live attenuated virus. Not one is agreed to provide a consistent high level of immunity against infection and there has been no study in which the efficacy of all four types have been compared. This is partly because of the difficulty of employing some of the methods used to determine the efficacy of vaccination and of interpreting the results of most of them. Most studies have measured circulating haemagglutination inhibiting (HI) antibody. However, it is known that vaccination produces also antineuraminidase (AN) antibody, and that

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both types of antibody appear in nasal secretion and serum and may be important in mediating resistance to infection (Slepushkin *et al.* 1971). Some workers emphasize the importance of neutralizing antibody. The relative importance of the site and type of antibody in protection against infection has not yet been established, although there is consistent evidence that the presence of circulating HI antibody is correlated with resistance to infection (Hobson, Beare & Gardner, 1972). Immunity induced by vaccination has also been examined by studies of the incidence of influenza in vaccinated subjects subsequently exposed to natural infection. Such studies have often been unsuccessful because the expected outbreak of influenza failed to occur. However, an artificial as opposed to a natural challenge procedure overcomes this difficulty and limits the number of subjects needed (e.g. Beare, Hobson, Reed & Tyrrell, 1968; 1969).

In order to acquire a better understanding of the immunogenicity of the various types of influenza vaccine in a partly immune population – typical of an interpandemic period – an intensive study was made in volunteers vaccinated with live attenuated, detergent split and oil-adjuvant influenza vaccines of the A2/Hong Kong serotype, who were subsequently challenged with live virus of the same serotype.

MATERIALS AND METHODS

A total of 49 healthy employees aged 21 to 65 years at the Beckenham Laboratories of the Wellcome Foundation volunteered for this study. Informed consent was obtained from all subjects. Subjects were allocated to one of four groups and the groups were matched as far as possible according to the history of influenza, vaccination against influenza in earlier years, age and sex (Table 1). No volunteer had been recently immunized against influenza, but five subjects suffered an influenza-like illness at or about the time that the first study specimens were collected. It was subsequently shown that four of these subjects were infected with influenza A, which was prevalent at the time the study began in December 1969. These subjects were accepted into the rest of the trial, but the results obtained were not included in the final analysis.

Subjects were vaccinated with one of three A2/Hong Kong/68 (or equivalent) influenza vaccines or a control influenza virus B vaccine. Six weeks after the vaccination all subjects were challenged with a partially attenuated A2/Hong Kong/1/68 influenza strain. Reactions to vaccination and challenge procedures were monitored for 10 days after administration.

Vaccines

Group A. Monovalent live attenuated influenza. A dose of $10^{5\cdot5}$ EID 50 of A2/Hong Kong/1/68 (H_3N_2) was given intranasally as nose drops in a total volume of 1 ml. (0·5 ml. to each nostril). This strain was attenuated by 11 passages in leucosis-free eggs in the presence of equine serum (γ-inhibitor), at 33° C. The development and characteristics of this strain have been described in detail elsewhere (Beare & Bynoe, 1969).

Group B. Standard bivalent inactivated saline deoxycholate-split influenza

Table 1. *Composition of experimental groups*

	Live A	Split A+B	Oil adjuvant A	Unsplit B
No. of subjects	11	13 (9)	12	13
No. of females	3	3 (1)	4	4
No. of males	8	10 (8)	8	9
Average age females (years)	29	30	41	27
Average age males (years)	32	44	35	30
No. given influenza vaccine last year	7	6 (5)	7	7
No. who had recent influenza-like illness	2	2 (0)	3	4

() = number of subjects challenged.

vaccine was given subcutaneously in a 1 ml. volume. This vaccine contained, before detergent treatment, A2/Northern Territories/60/68 (H_3N_2), 8000 HA units, and B/Victoria/2/65, 3000 HA units and was provided by Wellcome Research Laboratories.

Group C. Monovalent oil-adjuvant double-emulsion vaccine was given intramuscularly in an 0·375 ml. volume. This vaccine contained A2/England/344/68 (H_3N_2) 3500 Ha units in a double emulsion of Drakeol and Arlacel. A2/Northern Territories/60/68 and A2/England/344/68 are both A2/Hong Kong/68 serotypes as indicated by the addition of (H_3N_2) as recommended recently by the World Health Organisation (1971).

Group D. Control group. A monovalent inactivated saline vaccine was given subcutaneously in a 1 ml. volume. This vaccine contained B/England/5/66, 7000 HA units, and together with vaccine for Group C was kindly provided by Evans Medical Limited.

Challenge virus

A dose of 10^5 EID 50 of monovalent live influenza A2/Hong Kong/1/68 was given intranasally as nose drops as for Group A. This strain was partly attenuated by six passages at temperatures down to 25° C. This and the vaccine for Group A were kindly provided by Dr A. S. Beare.

Specimens collected

Blood was collected at or shortly before the beginning of the experiment, 3 and 6 weeks after vaccination, and again 2 weeks after challenge – 8 weeks after vaccination.

Sets of nasal washings were collected on each of three successive days, one set before, one at 3 and one at 6 weeks after vaccination. A total of 20 ml. of phosphate-buffered saline was applied in small volumes, successively to each nasal cavity, with the subject in a sitting position. Five to 10 ml. of nasal effluent were usually

collected although recovery rates were somewhat variable. Specimens were initially stored at −20° C. then tested for the presence of blood (with Hemostix, Ames Limited). Negative specimens were dialysed against distilled water, pooled and freeze-dried, and then reconstituted in saline to one-tenth the volume of the original washing. Nasal washings for virus isolations were collected from the subjects who were vaccinated with live virus and from all subjects on the 1st, 2nd and 3rd days of challenge. These were mixed with an equal volume of nutrient broth and stored at −70° C. They were subsequently tested by inoculation of 0·2 ml. volumes into the allantoic cavity of 10-day-old chick embryos.

Antibody assays

Haemagglutination inhibition (HI) test. The virus used was an inhibitor-resistant strain of A2/Hong Kong/1/68. The sera were inactivated for 30 min at 56° C. but the nasal washings were not heated. Twofold dilutions were made in 0·2 ml. volumes in WHO plastic plates, using phosphate-buffered saline as diluent (World Health Organization, 1953). Four HA units of virus and 1% human group O red cells were used. The serum-virus mixtures were held at room temperature for 30–60 min. before the addition of red cells. This method was compared with the use of cholera-filtrate treatment and inhibitor-sensitive virus and was simpler and gave more satisfactory results when antibody titres were low.

Neuraminidase-inhibition (AN) tests. For neuraminidase-inhibition tests the method used was essentially that described by Schild & Newman (1969) with the following modifications to increase the sensitivity of the test. (*a*) The concentration of neuraminidase (purified virus) was adjusted so that on incubation with excess substrate for 16 hr. at 37° C. the amount of *N*-acetyl/neuraminic acid released per 0·08 ml. of virus gave an OD_{549} reading of 0·4–0·5 OD units. (*b*) For the enzyme neutralization test virus and serum dilutions were incubated at room temperature for 3 hr. The virus used was a recombinant A/FPV/Dutch/27 (Havl)–A/Hong Kong/68 (N_2). The use of this recombinant eliminated the possibility of non-specific inhibition of neuraminidase activity by antibody to Hong Kong haemagglutinin (H_3).

Neutralization (N) tests. The virus was a calf-kidney-adapted strain of A2/HK/68. The sera were used after inactivation at 56° C. for 30 min; washings were not heated. Fourfold serial dilutions were mixed with an equal volume of a dilution of allantoic fluid containing an estimated 10 TCD 50 of virus. The mixtures were held at room temperature for 20 min and 0·2 ml. was inoculated into each of two tubes of secondary calf kidney cells. Many tests were repeated because the dose of virus was too high or too low, but in general the results were reproducible. These tests were done last and specimens were only tested when a complete set was available.

In spite of the fact that groups were matched as far as possible on clinical grounds, some differences in base-line antibody titres were found between the groups (Tables 3–8). Thus serum HI antibody titres and nasal antineuraminidase titres before vaccination were somewhat lower in the control group and higher in the saline split vaccine treated group than in others.

Table 2. *Clinical reactions to vaccination*

	Indicated result in subjects given vaccine			
	Live A2/HK	Split A2/HK+B	Adjuvant A2/HK	Unsplit B
No. vaccinated	11	13	12	13
No. without symptoms	3	3	3	4
Constitutional symptoms (headache, fever, anorexia, pain in back and legs)	37*	27	25	16
Respiratory symptoms (nasal discharge, obstruction and sore throat)	37	21	18	11
Local symptoms (pain and redness at injection site)	—	21	10	34
Total symptoms	89	75	53	57
Total symptoms per subject	8·1	5·0	4·4	4·0
Total symptom points†	134	112	86	71

* Total number of symptoms of all degrees of severity reported by volunteers at any time during observation for 10 days after vaccination.

† The scores given above have been devised by allotting 3 points for severe symptoms, 2 for moderate and 1 for mild; severe symptoms were rare.

RESULTS

Clinical reactions to vaccination

Symptoms were recorded against a check-list each day for 10 days after the vaccination and challenge procedures. Injection sites were examined clinically 24 hr. after vaccination. The results, presented in Table 2, show that rather more constitutional and respiratory symptoms were encountered in those given live vaccine. The number of volunteers in each group was small and, as they were not in isolation, respiratory symptoms due to the vaccine could be confused with those of intercurrent respiratory infections. Since a sensitive method was used to record reactions to vaccination, these background symptoms were doubtless collected. Thus, although the number of symptoms recorded in the post-vaccination period in all groups is large, they were not all due to the vaccines and were higher than might be expected in a general vaccination programme. In fact, no volunteer complained about the reactions to vaccination. Symptoms at the injection site occurred only in the parenterally inoculated subjects and seemed to be less frequent in those given oil-adjuvant vaccine. In particular, on examination, local redness did not occur in any subject given oil-adjuvant vaccine, while it was seen in five subjects given control influenza B vaccine and in a few who received split vaccine.

Serological response to vaccination

The results of vaccination were assessed by HI, AN and N antibody responses in serum and nasal washings for each volunteer. The titres for each group are shown in Tables 3–8 and summarized in Table 9.

There was some rise in titre of circulating HI in the control group, presumably due to some undiagnosed or asymptomatic natural A 2 influenza. In the vaccinated

Table 3. *Distribution of serum HI titres before and after vaccination and challenge*

Vaccine	Live A2				Split A2+B				Oil-adjuvant A2				Unsplit B			
Serum specimen ...	i	ii	iii	iv	i	ii	iii	iv	i	ii	iii	iv	i	ii	iii	iv
Titres																
8	—	—	—	—	—	—	—	—	1	—	—	—	2	—	—	—
16, 24	1	—	—	—	2	—	—	—	2	—	—	—	1	1	2	—
32, 40, 48	3	1	3	—	3	1	3	—	1	—	—	—	2	2	2	1
64, 96	3	3	1	2	2	3	2	1	5	—	—	1	2	4	1	2
128, 192, 240	1	3	2	5	2	2	2	2	1	3	1	—	1	1	2	4
256, 384	1	1	—	1	1	1	2	3	1	1	—	1	2	2	1	—
⩾ 512	1	2	3	2	2	5	3	6	1	8	11	9	—	—	2	3
Totals	10	10	9	10	12	12	12	12	12	12	12	11	10	10	10	10
Geometric means	76·6	148·8	170·9	188·5	90·9	248·6	177·5	404·2	69·1	547·8	856·5	770·8	47·1	74·4	102·2	188·5

In these and following tables serum specimen i was taken before vaccination, ii at 3 weeks and iii at 6 weeks after vaccination. Specimen iv was taken after challenge. The table shows the number of specimens with a titre in the indicated range.

Table 4. *Distribution of nasal HI titres before and after vaccination*

Vaccine	Live A2			Split A2+B			Oil adjuvant A2			Unsplit B		
Nasal specimen ...	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
Titres												
2, 3	2	1	—	—	1	—	2	1	—	—	—	—
4, 6	4	2	1	3	1	—	—	1	—	2	3	1
8, 12	1	2	—	7	5	2	8	2	1	7	3	4
16, 24	3	4	5	2	3	4	2	4	3	—	3	4
32, 48	—	—	2	—	—	3	—	2	3	1	—	—
64, 96	—	1	2	—	—	—	—	2	3	—	1	—
> 128	—	—	—	—	—	—	—	—	2	—	—	—
Totals	10	10	10	12	10	9	12	12	12	10	10	9
Geometric means	7·5	12·6	22·9	8·4	8·7	20·7	8·7	19·8	47·3	10·6	12·6	11·0

Nasal specimen i was a pool of three samples collected before vaccination. Specimen ii was a similar pool collected 3 weeks and iii collected 6 weeks after vaccination.

Table 5. *Distribution of serum AN antibody titres before and after vaccination*

Vaccine ...	Live A		Split A+B		Oil-adjuvant A		Unsplit B	
Nasal specimen ...	i	iii	i	iii	i	iii	i	iii
Titre								
< 5	2	—	—	—	3	—	3	—
5	2	2	4	1	1	—	2	1
10, 15	2	1	5	5	1	1	3	6
20, 30	2	2	1	3	4	—	1	2
45, 50, 60	1	2	1	—	—	2	1	1
80, 120, 150	—	2	—	1	1	6	—	—
160	—	—	1	2	1	—	—	—
320	1	1	—	—	—	2	—	—
640	—	—	—	—	—	—	—	—
⩾ 1280	—	—	—	—	—	—	—	—
Totals	10	10	12	12	11	11	10	10
Geometric means	14	34	12	26	14	98	8	17

groups the mean serum antibody titres did not rise further after 3 weeks although in several instances nasal titres rose more slowly. Maximal titres were found in all groups by 6 weeks and on these further studies are concentrated. The statistical significance of each increase in titre is shown in Table 9. There were small but significant rises in serum AN after live and split vaccines but no significant changes in nasal AN, while the rises in nasal HI were greater and were highly significant after live vaccine. Neutralizing antibody rises were greater in serum after split than after live vaccine and the small rises of nasal neutralizing activity did not reach statistical significance even after oil-adjuvant vaccine. The most striking general conclusion was that there were large and highly significant rises of antibodies measured by all methods after oil-adjuvant vaccine, ranging from 2·29 for nasal AN to 17·84 for serum N.

Multiple antibody rises were seen in a number of volunteers. For example, of 25 volunteers given vaccine, eight showed fourfold or greater rises by two or three tests in the serum and four by the same criteria in nasal washings. On the other hand, of five subjects who gave a history of influenza about the time of vaccination, four showed a rise in nasal washings and five in serum. Furthermore there were instances in which a rise was detected by only one test – for example, in the serum of the same 25 volunteers 5 by HI, 4 by AN and 3 by N alone.

Challenge of volunteers

A partly attenuated virus was used to estimate the degree of immunity induced by vaccination. Subjects were inoculated intranasally approximately 6 weeks after they were vaccinated and the result was assessed by virus recovery, circulating HI antibody titrations and clinical response. There was in this study a clear relationship between infection and the occurrence of respiratory symptoms, for infection was detected in 14 of 21 subjects with symptoms and in four of 24 without ($P < 0{\cdot}01$). It can be seen in Table 10 that a majority of the control subjects

https://doi.org/10.1017/S0022172400063117 Published online by Cambridge University Press

Table 6. *Distribution of nasal AN titres before and after vaccination*

Vaccine ...	Live A			Split A+B			Oil-adjuvant A			Unsplit B		
Nasal specimen ...	i	ii	iii	i	ii	iii	i	ii	iii	i	ii	iii
Titres												
< 2	7	6	5	5	6	5	7	4	3	8	8	7
2	—	—	—	—	—	—	—	—	—	—	—	—
5	—	1	—	—	—	—	2	3	3	1	—	1
10	1	2	2	3	4	3	1	2	—	—	—	—
15	—	—	—	1	—	—	—	2	2	—	—	—
20	—	—	1	—	—	—	—	—	—	—	—	—
25	1	1	1	—	—	1	—	1	1	—	1	1
50	—	—	—	—	—	—	—	—	1	—	—	—
Totals	9	10	9	9	10	9	10	12	10	9	9	9
Geometric means	1·8	2·6	3·3	2·9	2·5	3·1	1·7	4·5	5·7	1·2	1·4	1·7

Table 7. *Distribution of serum N titres before and after vaccination*

Vaccine ...	Live A		Split A+B		Oil adjuvant A		Unsplit B	
Nasal specimen ...	i	iii	i	iii	i	iii	i	iii
Titres								
4, 6	—	—	—	—	1	—	2	—
8	1	—	2	—	—	—	—	1
16	2	1	—	—	1	—	1	2
32	1	2	2	—	1	—	—	—
64, 96	—	—	1	—	—	—	1	—
128	1	1	3	7	1	—	1	1
256	—	—	—	1	—	—	1	2
512	1	1	—	—	2	1	1	1
⩾ 1024	1	2	—	—	—	5	1	1
Totals	7	7	8	8	6	6	8	8
Geometric means	70·7	141·3	41·5	139·6	76·9	1290·2	67·3	98·7

Table 8. *Distribution of nasal N titres before and after vaccination*

Vaccine ...	Live A		Split A+B		Oil-adjuvant A		Unsplit B	
Nasal specimen ...	i	iii	i	iii	i	iii	i	iii
Titres								
< 1	2	2	3	2	1	—	2	2
1	3	1	1	1	—	—	3	2
2	1	2	2	2	—	1	1	1
4	1	2	1	1	2	1	1	3
8	—	—	1	1	1	1	—	—
⩾ 16	—	—	—	1	2	3	1	—
Totals	7	7	8	8	6	6	8	8
Geometric means	< 1	< 1	< 1	1·5	4·3	8·0	1·0	1·0

Table 9. *Rises in antibody titres during the first 6 weeks after vaccination, expressed as a proportion of the initial value*

Vaccine	Live A	Split A+B	Oil adjuvant A	Unsplit B
Nasal HI	1·93***	1·36**	3·83***	0·05
Serum HI	0·89*	0·74	6·50***	1·02*
Nasal AN	0·80	0·06	2·27***	0·43
Serum AN	1·39*	0·97*	6·10***	1·05
Nasal N antibody	0·35	0·89	0·96	0·00
Serum N antibody	1·21	2·36**	17·84***	0·47

Each figure in the table is the geometric mean of the ratio of rise in titre to the initial titre for the subjects in the appropriate group. *, **, *** denote that the rises were significantly different from zero at 5%, 1% and 0·1% levels, respectively.

The analyses of variance (using log titres) showed differences between the four groups in rises of titres of nasal and serum HI, and serum N antibodies (all at 1% significance level) and of serum AN (at 5% significance level); the heterogeneity is almost entirely due to the larger antibody rises in the subjects given oil adjuvant vaccine.

Any slight disparity between the results as shown above and in the previous tables is due to a difference in the approximations used.

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Table 10. *Results of challenge*

	Wellcome Laboratories			Oil refinery	
Vaccine given	Antibody rise	Virus isolated	Clinical response	Antibody rise	Virus isolated
A. Live influenza A	2/10	1/10	3/10	—	—
B. Split A+B	3/9	6/9	7/9	3/38	5/33
C. Oil-adjuvant A	1/12	2/12	3/12	—	—
D. Unsplit B	3/10	7/10	7/10	9/24	6/23

Five volunteers at Wellcome Laboratories had natural influenza at the beginning of the study. Of the one receiving live influenza A vaccine and three receiving unsplit B vaccine none was infected or developed symptoms on challenge. One who received split A+B was not challenged. These have not been included in the above table.

and rather fewer of those given split vaccine were infected; on the other hand, most of those given either live or oil-adjuvant vaccine resisted challenge. With the small numbers in this study, the difference between the results in the split vaccine and control group are not significant. However, in a larger supplementary study, carried out at an oil refinery, it is clear that the split vaccine provided protection against a similar artificial challenge procedure. At Beckenham four subjects who had clinical influenza at the start of the trial followed by one or other vaccine were resistant when challenged.

Comparing these with the serological results, it is not surprising to find that the oil-adjuvant vaccine protected well, in view of the high titres of antibody it produced. However, it is surprising that the live influenza vaccine protected to a similar extent. The relation between antibody titres and resistance to infection was therefore studied in the whole group of volunteers.

The relationship between antibody titre and resistance to infection

The absolute and relative contributions of local antibody and circulating antibody to resistance to infection is still uncertain. To examine the evidence provided by the results of the serum titrations after vaccination and the outcome of the challenge, linear discriminant analysis was used, as in a previous study (Slepushkin *et al.* 1971).

The challenge result was quantified, no infection being taken as 0 and infection as 1, and regressed on each of the antibody titres, giving six linear predictors of the challenge result. A good predictor, or discriminant function, is one giving good separation between the two groups of subjects (i.e. those who contracted influenza and those who did not). As a measure of this separation, d/s, the ratio of the difference between the means of the predicted values for the two groups (d) to the estimated standard deviation within the groups (s), was calculated for each discriminant function; results are shown in Table 11, together with the number of observations on which the calculation was based. The value of d/s is approximately 4·0 for a discriminant function that correctly classifies 95% of the subjects. Thus none of the antibody titres was particularly good at predicting the outcome of the challenge, but the values of d/s show that serum titres of HI, AN and N

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Table 11. *Prediction of outcome of challenge from antibody titres*

	Circulating			Nasal			All six antibody titres
	HI	AN	N	HI	AN	N	
No. with antibody titre measures	45	44	31	43	32	31	45
d/s	0·97	0·97	1·01	0·67	0·68	0·32	1·18
No. correctly classified	32	31	21	26	24	17	37

antibody were all of approximately equal value, and better than nasal HI and AN Nasal N antibody was of no value as there was no significant difference between its titres for the two groups.

Similar results were obtained when estimates of the missing observations, calculated from those titres which had been measured, were used in the analyses. Using these estimated observations, multiple regression of the challenge result on all six titres gave a linear discriminant function for which *d/s* was found to be 1·18. The residual sum of squares was not significantly smaller than that for the linear regression on circulating N antibody titres alone, so that no combination of titres was a better predictor than the single titres mentioned above.

The predictors can also be roughly evaluated by classifying each subject as susceptible or resistant to infection, according to whether his predicted value is greater or less than 0·5. The predictions can be compared with the results of the challenge; the number of subjects correctly classified by each predictor is also shown in Table 11. A worthless linear predictor ($d/s = 0$) will classify about half the subjects correctly.

The results indicate that some factor other than those measured is involved in resistance to infection by artificial challenge. There were too few sera to examine the question of whether the predictive value of the antibodies was the same whether induced as a result of natural or vaccine infection or of either form of parenteral vaccination.

DISCUSSION

There is, as far as we know, no published record of a comparison of live attenuated, detergent-split and oil-adjuvant influenza vaccines in which reactions to vaccination, local and circulating HI, AN and N antibody responses and also resistance to challenge have been examined. The amount of laboratory work involved in this study limited the number of subjects, but the intensive monitoring of response to vaccination yielded results which would be difficult to achieve in a large-scale field trial. It is unfortunate that at the start of the trial natural influenza occurred in some subjects and it is impossible to be certain that no other subjects were infected. However, the clear differences between the vaccination groups and the results in the group given influenza B vaccine indicate that this did not disturb the trial results to an important degree. Large-scale field trials are necessary, for they provide a better assessment of the general acceptability and reactivity of vaccines than can the present type of investigation. Live vaccine strains which

produced readily detectable symptoms in volunteers at the Common Cold Unit produced no detectable symptoms when administered in offices and factories (unpublished data). It is difficult to compare the discomfort of one sort of symptom with another, but the respiratory symptoms produced by the live vaccine were numerically roughly equivalent to the local symptoms which followed the injected vaccines. However, this trial population was partially immune, and there might have been relatively more symptoms in those given live vaccine if a higher proportion had been susceptible.

In this study the protection afforded against artificial challenge forms the most important index of the efficacy of vaccination. It is clear that the live and oil-adjuvant vaccines gave the most satisfactory level of protection and, although the latter have been out of favour recently, they merit further consideration, and it would be worth confirming the degree of protection against natural infection in a field study.

The rises in titre of circulating AN after live and killed vaccines confirm those reported in previous studies (Slepushkin *et al.* 1971; Schild & Newman, 1969; Downie, 1970; Kasel *et al.* 1969). We were surprised to find such small increases in nasal N antibody titre even when good protection was produced, though our earlier studies had shown that circulating antibodies were more important than local in resistance to infection; this and the results of the statistical analysis make it clear that we cannot at the moment predict satisfactorily from antibody measurements the resistance to infection induced by vaccines. As in our early studies with influenza B, we cannot explain why the live vaccine protected better than saline-killed vaccine (Beare *et al.* 1968). Other immunological mechanisms must be involved and consideration is being given to cell-mediated immunity, and to IgE antibody attached to cells in the respiratory tract.

We would like to thank the volunteers for their willing and conscientious help and Mr R. Newman for technical assistance. We thank Dr A. Ward Gardner and Dr A. S. Beare for conducting the trial at the oil-refinery and making their results available to us.

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