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Live influenza B vaccine in volunteers

A report to the Medical Research Council by their Committee on Influenza and Other Respiratory Virus Vaccines*

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In this paper we describe our experience with a live vaccine made from an influenza type B virus. Type B was chosen for a number of reasons. We had had no previous experience with it, an attenuated virus with an acceptable passage history was available, and the low incidence of haemagglutination-inhibiting (HI) antibodies in the population was likely to make it easier to find susceptible volunteers than when an A virus was used. In addition, because different antigenic subtypes of influenza B circulate at the same time (Communicable Disease Center Report, 1965) we wished to look at the evidence for cross-protection. We also wished to assess the importance of repeated vaccinations, on which some Russian workers lay considerable stress (Smorodintsev *et al.*, 1965).

Previous trials of live influenza vaccines in Great Britain (in which repeated vaccinations had not been given) had used vaccines made from a 1957 Russian A 2 virus (McDonald *et al.* 1962; Andrews *et al.* 1966; Beare *et al.* 1967). The results showed that attenuated live viruses infected fairly readily when serum antibody was low or absent, that infection was followed by resistance to challenge with the same virus, and that there was a significant rise of antibody in a proportion of people.

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MATERIALS AND METHODS

Virus

The influenza B virus was derived from throat swabs from which the virus had previously been isolated and which had been stored at $-40°$ C. Fluid from bottles containing the swabs was inoculated into the allantoic and the amniotic cavities of fertile hens' eggs, which were guaranteed free of avian leucosis and of mycoplasma and which had been incubated for 10 or 11 days. An agent known as B/England/13/65 was later chosen for serial passage. Haemagglutinin was detected both in the allantoic and in the amniotic fluids on first isolation, and passages at 10^{-4} and 10^{-5} dilutions were made from the allantoic fluid in groups of leucosis free eggs. Pools of virus were dispensed in 1·0 ml. amounts and stored in sealed glass ampoules at $-65°$ C.

The virus used in the trial was taken from a pool which had had six egg passages and which contained $10^{8 \cdot 7}$ fifty-per-cent-egg-infecting doses (EID 50) in 0·1 ml. It agglutinated 0·5 % fowl red cells to a titre of 1/320.

Testing of the vaccine for safety

Safety tests were performed to exclude the possibility of accidental contamination with bacteria and other viruses (McDonald *et al.* 1962).

Volunteers for trial.

One hundred and sixty-four employees of Sankey Ltd., Wolverhampton, were enrolled for vaccination. They were questioned by one of us about recent and chronic respiratory disease and about their general health. Apart from the elimination of a few after questioning they were unselected, were of both sexes and ranged in age from 17 to 65 years. It had been hoped to arrange for an unvaccinated control group, which would be observed for cases of any infectious respiratory disease that might break out in the vicinity, but this proved impracticable because additional volunteers were not available.

In the trial later held at the Common Cold Research Unit nine volunteers were inoculated, of whom five were male and four female. They were all healthy adults under 40.

Administration of the vaccine

Before each vaccination session the virus was diluted to $10^{-4 \cdot 0}$ in Hanks's balanced salt solution (BSS) with 0·2 % bovine plasma albumin (BPA) and adjusted to a pH of about 7·2. A coarse hand-spray (Fig. 1) was used to administer the vaccine, which was inoculated into both nostrils. The dose of virus given to each individual was 0·5–0·6 ml.—that is, about $10^{5 \cdot 4}$ EID 50. Ampoules from a single pool of virus were used throughout the trial.

Three vaccinations were given, at intervals of 3 weeks, in the autumn of 1966. Most volunteers attended for all the vaccinations, but some for only one or two. In June 1967, 7 months after the third vaccinations, as many of the original

volunteers as were still available were challenged with the vaccine virus in an attempt to assess their long-term resistance to infection.

At the Common Cold Research Unit a single dose of the virus was given intranasally from a measured dropper. The same pool of virus was used as in Wolverhampton.

Collection of blood for antibody estimations

Volunteers were bled six times—immediately before each of the first three vaccinations, 3 weeks after the third vaccination, immediately before the challenge dose and about 3 weeks after that. At Salisbury, volunteers were bled before the trial and 2–3 weeks afterwards.

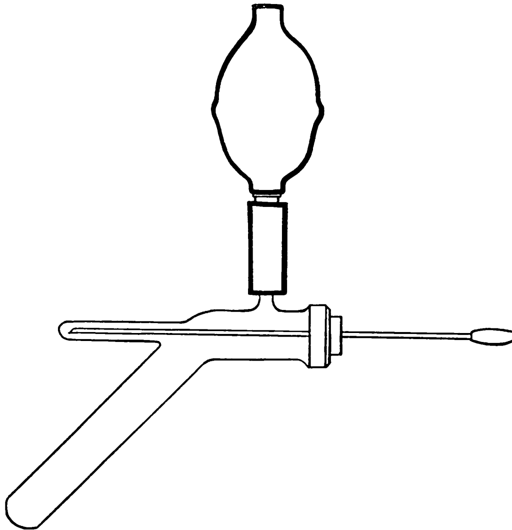

Fig. 1. Spray used in the vaccination of volunteers. It was copied from a model provided by Prof. A. A. Smorodintsev of the U.S.S.R. Academy of Medical Sciences, Leningrad.

Virus isolation after vaccination

In Wolverhampton, throat swabs were taken 72 hr. after vaccination, and in the Common Cold Research Unit nasal washings were taken 2, 3 and 4 days after virus inoculation. The specimens were at both places inoculated into primary cultures of monkey kidney which were tested for haemadsorption after 5 days incubation in a rolling drum at 33° C.

Measurement of antibodies

Serum antibodies were measured by haemagglutination-inhibition (World Health Organization, 1953) and by the strain-specific complement fixation test (VCF) (Lief & Henle, 1956; Pereira *et al.* 1967).

Neutralization tests at Salisbury were performed as described previously (Beare, 1962). The microtitre method for the estimation of HI antibody titres against other subtypes of influenza B was that of Sever (1962). Tests for antibody on the surface of the nasal mucosa were as described by Smith *et al.* (1966).

Recording of clinical symptoms

Volunteers at Wolverhampton reported their symptoms to the sick bay at the factory and were questioned when they attended for the next vaccination. Symptoms were easily recognizable and histories, although subjective, were probably mainly reliable. A degree of error was, however, probably inherent in the system and it seems likely that the incidence of reactions tended to be overestimated. At the time of the fourth vaccination each volunteer was given a simple questionnaire in place of the interview that followed the earlier vaccination sessions.

RESULTS

The effects of vaccination were judged by virus isolation, rises of circulating antibody and clinical reactions. Table 1 provides a general summary of results. Virus recoveries were almost certainly never complete but the same technique

Table 1. *Frequency of virus isolations, antibody rises and clinical reactions after repeated vaccination with B/England/*13/65

(Numerators denote responses, denominators numbers tested.)

Vaccination	Virus isolations		Antibody rises: HI		Antibody rises: VCF		Incidence of clinical reactions	
First	29/135	21 %	43/128	34 %	18/66	27 %	85/121	70 %
Second	8/113	7 %	10/103	10 %	3/65	5 %	38/101	38 %
Third	0/70	0 %	1/76	1 %	2/65	3 %	5/61	8 %
Challenge	0/29	0 %	0/39	0 %	Not done		10/39	26 %

HI = haemagglutination-inhibition, VCF = strain specific complement-fixation.

Table 2. *Virus isolation in relation to serum antibody before each vaccination*

(Numerators denote responses, denominators numbers tested.)

Antibody (and range of titre)	Virus isolations: After the 1st vaccination		After the 2nd vaccination		After the 3rd vaccination		After the challenge	
HI < 6–24	25/113	22 %	4/59	7 %	0/34	0 %	0/20	0 %
> 24	4/22	18 %	4/54	7 %	0/36	0 %	0/9	0 %
VCF < 8–16	9/60	15 %	3/39	8 %	0/36	0 %	Not done	
> 16	2/5	40 %	1/27	4 %	0/24	0 %	—	

HI = haemagglutination-inhibition, VCF = strain-specific complement-fixation.
Sera for VCF were available from only a proportion of volunteers.

was adopted after each vaccination and it is probably fair to compare them. The reduction in virus isolations with successive vaccinations was notable. They dropped from 21 % after the first administration to nil after the third. When the challenge was made 7 months later there were again no isolations; five volunteers

Table 3. *Antibody formation after vaccination, measured by haemagglutination-inhibition and by strain specific complement-fixation*

(Numerators are numbers who responded, denominators numbers of specimens tested.)

Range of antibody titres before each vaccination	Frequency of antibody rises after each vaccination							
	First		Second		Third		Challenge	
HI								
<6–24	42/102	41 %	7/59	12 %	1/37	3 %	0/26	0 %
>24	1/26	4 %	3/44	6 %	0/39	0 %	0/13	0 %
VCF								
<8–16	18/61	30 %	3/40	7 %	2/37	5 %	Not tested	
>16	0/5	0 %	0/25	0 %	0/28	0 %		

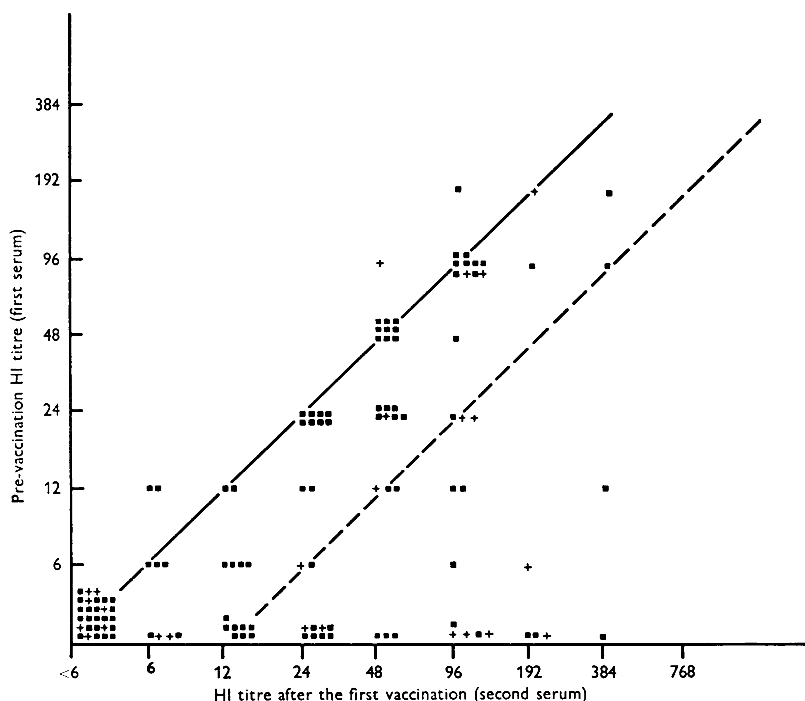

Fig. 2. The effect on HI antibody of the first dose of the vaccine. The ordinate represents HI titres before the trial and the abscissa the HI titres 3 weeks after vaccination. +, A volunteer from whom virus was recovered. The continuous line marks the line of no change in titre, the dotted line that of a fourfold rise in titre.

who were vaccinated for the first time on that occasion yielded one isolation. Antibody rises occurred mostly in those who had had little or no antibody at the beginning of the trial and the two tests measured them with differing sensitivity. The commonest clinical reaction was afebrile coryza lasting about 3 days; a very small percentage of volunteers had a mild influenza-like illness and some had no symptoms at all. Reactions were recorded for 70 % of the volunteers receiving the first virus dose; they fell to 8 % after the third and rose again to 26 % after the

challenge. It seems likely that the incidence of reactions reflected the development of immunity and its waning with the passage of time, but it is also clear that our estimates of reactions nearly always included some false positives.

The relationship between initial titres of HI and VCF antibody and virus isolation is shown in Table 2. Those with low titres were not conspicuously more susceptible to infection than those with high titres; and after the second and third vaccinations both groups were resistant.

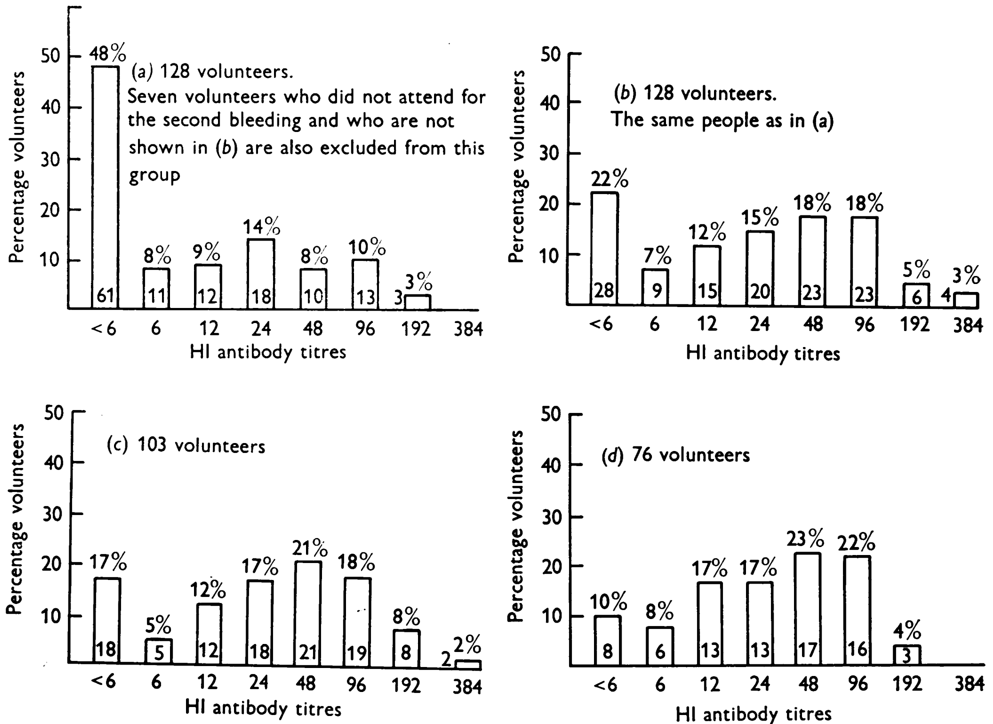

Fig. 3. Volunteers according to serum HI antibodies at different stages of the trial. (*a*) Immediately before the first vaccination (first serum). (*b*) Three weeks after the first vaccination (second serum) and immediately before the second vaccination. (*c*) Three weeks after the second vaccination (third serum) and immediately before the third vaccination. (*d*) Three weeks after the third vaccination (fourth serum).

Rises of antibody after the vaccinations are illustrated in Figs. 2–4 and in Table 3. The highest rises of titre came after the first vaccination and were most notable in people with low initial titres. But the scale of the rises (Fig. 2) rarely matched those stimulated by a killed vaccine. A minority of people did not respond at all and some of these had been shown to be excreting virus.

The modifying effect of serum antibody on clinical reactions is shown in Table 4. Seventy-four per cent of people with low HI antibody titres experienced a reaction after the first vaccination, but only 15 % after the third. This trend is reproduced in the VCF results and suggests a protective influence independent of measurable circulating antibody.

HI antibody estimations were performed on sixty of the original volunteers

7 months after the third vaccination. Fifty-two (87 %) had maintained their antibody titres while eight (13 %) showed a significant fall. It is noteworthy that there were no antibody rises after the challenge.

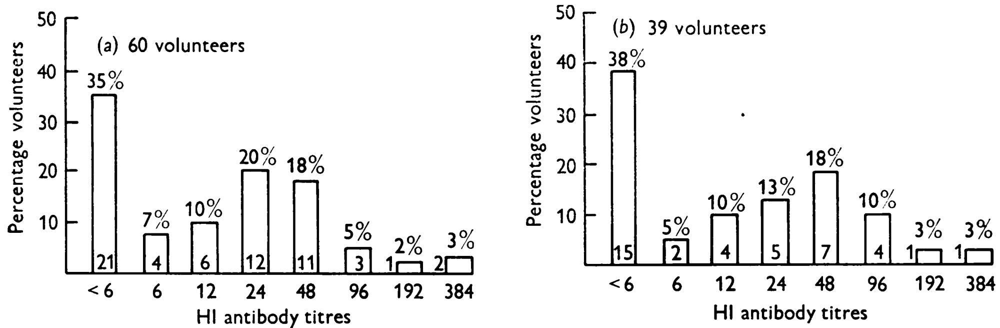

Fig. 4. The serological state of the volunteers in the follow-up period. (*a*) Seven months after the third vaccination and immediately before the challenge. (*b*) Twenty days after the challenge.

Table 4. *Clinical reactions in relation to serum antibody titres before each vaccination*

(The reactions were classified as follows: 0, no symptoms; I, coryza; II, coryza with malaise; III, mild influenza-like reactions.)

Range of titre before each vaccination	Incidence of different grades of clinical reaction after the vaccinations (%)				Numbers providing data
	0	I	II	III	
	(*a*) HI antibodies				
< 6–24					
Before:					
1st vaccination	26	35	36	3	98
2nd vaccination	63	26	11	0	57
3rd vaccination	85	12	3	0	34
Challenge	75	21	4	0	24
> 24					
Before:					
1st vaccination	65	13	18	4	23
2nd vaccination	61	23	16	0	44
3rd vaccination	96	4	0	0	27
Challenge	73	20	7	0	15
	(*b*) VCF antibodies				
< 8–16					
Before:					
1st vaccination	27	33	35	5	57
2nd vaccination	75	19	6	0	31
3rd vaccination	92	4	4	0	26
> 16					
Before:					
1st vaccination	40	20	40	0	5
2nd vaccination	46	31	23	0	26
3rd vaccination	100	0	0	0	18

VCF tests were not performed on sera collected before or after the challenge.

It was thought desirable to investigate the likelihood of heterotypic responses after infection and especially after multiple vaccinations. The antigenic position of the virus was established by Dr H. G. Pereira of the World Influenza Centre

Table 5. *Relationship of clinical reactions (all grades) and laboratory evidence of infection (virus isolation or antibody increase or both)*

(Numerators show clinical reactions, denominators numbers who provided data.)

Vaccination	Frequency of reactions							
	Virus isolated		Virus not isolated		Antibody rise		No antibody rise	
First	21/25	84 %	64/96	67 %	38/43	88 %	42/71	59 %
Second	3/5	60 %	34/94	36 %	6/13	46 %	23/76	30 %
Third	0/0	—	5/60	8 %	0/4	0 %	5/53	9 %
Challenge	0/0	—	9/36	25 %	0/0	—	6/25	24 %

Table 6. *Antigenic relationship of the vaccine to other influenza type B viruses*

(Table provided by Dr H. G. Pereira of the World Influenza Centre.)

Strain	Haemagglutination-inhibition test (Ferret Sera)				
	B/Johannesburg/33/58	B/Amakusa/1/64	B/Singapore/3/64	B/Eng/13/65	B/Rome/1/67
B/Johannesburg/33/58	**960**	60	120	40	< 10
B/Amakusa/1/64	80	**480**	160	120	60
B/Singapore/3/64	80	20	**960**	480	150
B/England/13/65	60	60	480	**960**	480
B/Rome/1/67	15	20	160	240	**480**

Table 7. *Antibody responses to related and rare influenza B viruses after repeated vaccination with B/England/*13/65 (*see Table* 6)

(The incidence of heterotypic responses in twelve volunteers who had developed antibody rises to the vaccine virus (*a*) is compared with a similar number who had not responded (*b*). Antibodies were measured by HI by the microtitre method of Sever (1962). There was a fourfold rise of HI antibody against the viruses shown.)

	B/England/13/65		B/Johannesburg/33/58		B/Amakusa/1/64		B/Singapore/3/64	
(*a*)	12	100 %	8	67 %	9	75 %	6	50 %
(*b*)	0	0 %	2	17 %	0	0 %	0	0 %

	B/Rome/1/67		B/Taiwan/2/62		B/India/363/64	
(*a*)	3	25 %	6	50 %	4	33 %
(*b*)	0	0 %	1	8 %	0	0 %

(Table 6) and the incidence of fourfold antibody rises in a few available sera is illustrated in Table 7. All the volunteers who had responded to the vaccine showed rises in antibody against some other influenza type B viruses, even against those

distantly related and even against B/Taiwan/2/62 and B/India/363/64 which have made only rare and circumscribed appearances as agents of natural infection. This small series did not reveal a broadening of the antibody spectrum after the later vaccinations such as was shown by Henle & Lief (1963) with the influenza A viruses in animals.

Finally, the many pointers to protective influences other than those mediated by circulating antibodies prompted a limited investigation on nine volunteers at the Common Cold Research Unit, into the possible role of local antibody. Five of these had no circulating antibody when tested by HI and by neutralization, and had no neutralizing antibody in their nasal secretions. All were infected with the virus. Two of the remainder, one with a high titre of circulating antibody and the other with a low titre, did have nasal antibody and neither was infected. Of the remaining two, one had a high titre of circulating antibody and no nasal antibody and was shown to be infected both by virus isolation and subsequent antibody rise; the other had an insignificant titre of circulating antibody and no nasal antibody and apparently could not be infected. The overriding influence of local antibody is amply indicated in this small series except in the case of the last volunteer, and it is possible that in his case the test was not sensitive enough to detect it.

DISCUSSION

The effects of the experimental live vaccine made from an influenza type B virus that was given repeatedly to a group of factory workers in the Midlands can be briefly summarized as follows: (1) a high rate of infection initially (Table 1), (2) considerable antibody formation in those infected (Table 1, Fig. 2), and (3) a high incidence of clinical reactions (especially after the first vaccination) which appeared to be a reflexion of residual pathogenicity in the virus (Table 4). All the effects were sharply reduced after the second vaccination and were almost wholly absent after the third (Tables 1–4). Most of the people without antibodies at the beginning of the trial developed them later, but about 8 % failed to do so and some of these actually excreted virus. Seven months after vaccination a challenge with the same virus showed that immunity had persisted, but there was a suggestion that it was then beginning to wane. Finally the vaccine gave promise of a protective effect against related serotypes (Table 7).

The difficulty of achieving infections and immunological responses with live influenza vaccines without clinical reactions has often been commented on. The high rate of recorded reactions in our trial (Table 5) could have been due in part to suggestion and to intercurrent infection, and evaluation of the results would have been easier if we had been able to vaccinate a control group with placebo. A much lower rate of clinical reactions was observed in the nine Salisbury volunteers and this may have been due to a better standard of clinical surveillance or to the different conditions that prevailed there. Methods of administering the virus may also have affected the clinical response: a spray which was used at Wolverhampton could certainly be expected to produce a greater and more widespread effect on susceptible cells than a simple dropper.

In the main it was the people without initial antibodies who were most readily infected (Tables 2, 4) and it was they who provided the bulk of antibody rises later (Table 3). In the former respect the results of this trial were at variance with those of the trial of live vaccine prepared from a Russian A_2 virus a few years before (McDonald *et al.* 1962). On that occasion it appeared that nearly all the volunteers with antibodies before vaccination were infected by the virus since they produced a boost of antibodies later. However, there were certain people in the present trial who were similarly proved to be infected in spite of their previous high antibody titres. Another curious feature of the earlier trial was that, although the volunteers without initial antibodies were readily infected and excreted virus for several days after vaccination, they formed no antibodies; nevertheless, when challenged with vaccine 1 month after the vaccination they were evidently immune, since the virus could not then be isolated. Thus low antibody titres did not denote susceptibility to infection at the time of revaccination. In the present trial, although antibodies were produced after the first vaccination, something of the same trend was seen in that a low level of antibody was compatible with protection against the second and third virus dose (Table 4). This feature was also exemplified in antibody rises; a quite small rise after the first vaccination was rarely followed by another rise after the other vaccinations (Table 3; Figs. 3–4).

Although the reason why some people failed to respond serologically to vaccination and the mechanism by which they were later protected is not precisely known, it seems clear that circulating antibody is only one factor in protection against influenza. Smith *et al.* (1966) found poor correlation between resistance to infection against parainfluenza type 1 virus and serum antibody. But it agreed well with the presence of nasal antibody. In our limited investigation of this aspect of the problem we found the same and it seems likely that it is the induction of local antibody by live influenza vaccines which makes them immunologically effective.

SUMMARY

A trial of an experimental live influenza B vaccine has been described.

The virus it contained was active and produced infections, antibody rises and clinical reactions.

Second and third vaccinations had much less effect than the first. Resistance to revaccination was only partially reflected in the serological response.

It seems that another factor, probably local antibody, exerts a considerable influence on resistance to infection with influenza viruses.

We are greatly indebted to Dr P. G. Higgins of the Public Health Laboratory, Cirencester, who went to much trouble to provide us with specimens from patients with influenza; to Dr H. G. Pereira of the National Institute for Medical Research for the antigenic analysis of the vaccine virus; to Messrs Sankey Ltd., Bilston, Wolverhampton, for their unfailing courtesy and forbearance throughout the trial; to the volunteers for their enthusiastic co-operation in the face of some discomforts; and to Messrs Pfizer Ltd., Sandwich, for originally providing facilities for the preparation of the vaccine.

In particular we wish to record our gratitude for the invaluable technical help of Miss Pamela Ball of the Common Cold Research Unit, Mrs Maria Gregory of the Bacteriology Department of Liverpool University, Mrs L. Johnson of the Virus Laboratory of New Cross Hospital, Wolverhampton, and Mr A. Westoby, an assistant in the practice of Dr Tyler.

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