Clinical Trials of Oil-adjuvant Influenza Vaccines, 1960–3

Report to the Medical Research Council by its Committee on Influenza and Other Respiratory Virus Vaccines*

Brit. med. J., 1964, 2, 267–271

Influenza vaccine prepared by emulsifying an aqueous suspension of inactivated virus in mineral oil is a far more potent antigen than the aqueous suspension alone. This fact was confirmed by the Committee in serological trials carried out in 1953 (M.R.C., 1955), but subsequent large-scale clinical trials in 1954–5 failed to show any protective effect, almost certainly because there was very little influenza during that winter (M.R.C., 1957). Unfortunately a small proportion (3.0 per thousand) of the volunteers who received oil-adjuvant vaccine developed persistent local reactions, similar to those described in the United States by Philip *et al.* (1954). These reactions were usually first noticed as fibrotic nodules several months after inoculation and took a year or more to resolve. In about half the cases (1.8 per thousand) the nodules became large and fluctuant and cleared only after incision.

In an attempt to overcome this problem, small-scale studies were made during 1956–8, using a different oil (Drakeol No. 6 instead of Bayol F), a more pure preparation of the emulsifier (Arlacel A) and vaccine of various viscosities (Himmelweit, 1960). The results of this work showed that vaccines of low viscosity gave as good an antibody response as those of higher viscosity, and it seemed possible that a low-viscosity vaccine might be less likely to produce a foreign-body reaction and subsequent necrosis. The number of subjects studied was too few, however, to allow any conclusions to be drawn on the risk of serious local reactions. Encouraged by the result of this work, a large field trial was begun in the autumn of 1960 in which over 6,000 adult volunteers were inoculated with low-viscosity vaccines by general practitioners, chest physicians, and industrial medical officers. The trial was continued for three winters, and its results are the subject of this report.

Material and Methods

Vaccines.—Two vaccines were used, one containing an egg-adapted influenza virus A2 strain (A2/Singapore/1/57) and the other an egg-adapted influenza virus B strain (B/England/939/59). Both vaccines were water-in-oil emulsions of low viscosity containing 2,000 haemagglutinating units of virus per dose (0.25 ml.); they were prepared by Dr. F. Himmelweit at the Wright-Fleming Institute of Microbiology in the manner described previously (Himmelweit, 1960). Supplies of mineral oil (Drakeol No. 6) and of emulsifier (purified Arlacel A) were obtained from the United States through the courtesy of Dr. Fred M. Davenport, of the University of Michigan. The vaccines were dispensed in disposable cartridges each containing a single dose of 0.25 ml. The cartridges were identical except that one vaccine was labelled "L" and the other "M." Most of the vaccine was used in 1960 within three months of manufacture, but some was stored at 4° C. and used for serological studies in the autumn of 1961.

Vaccination.—Supplies of the two vaccines, with special cartridge syringes and sufficient sterile disposable needles, were sent to the participating physicians during November 1960. Volunteers were asked to attend for inoculation before the end of the year. Their names were entered in order of attendance on inoculation registers which indicated by code letter the vaccine to be given. The doctors were asked to inject the vaccine deep into muscle—preferably into the left deltoid or triceps—and to take special care with thin or wasted patients.

At the end of December the inoculation registers were sent to Dr. W. J. Martin, at the London School of Hygiene and Tropical Medicine, and no record should then have remained with a doctor of the vaccine his patients had received.

Volunteers

Three groups of volunteers, comprising 6,123 in all, were inoculated as follows:

1. *Old People.*—Doctors in 29 practices, all members of the College of General Practitioners, prepared lists of all their patients who were aged 65 or more on 31 December 1960. Ten agreed to inoculate as many of these old people as possible, making it clear to them that two vaccines were under trial and that their participation was voluntary. The remaining 19 preferred to give no vaccine but undertook to observe their patients and to furnish similar records to the vaccinating doctors. A total of 1,619 old persons were vaccinated (647 males and 972 females).

2. *Patients with Chronic Bronchitis.*—The Research Committee of the British Tuberculosis Association enlisted the help of 68 chest physicians who sought volunteers among patients with chronic bronchitis attending their clinics. Only patients with dyspnoea of such severity as to cause them to walk on the level more slowly than other persons of the same age were eligible; those with paroxysmal dyspnoea apparently due to asthma who had no chronic cough or sputum were not accepted. A total of 2,341 patients agreed to take part, of whom 1,878 (80%) were males.

3. *Industrial Employees.*—Dr. J. D. Frame and Dr. C. P. Chivers, from the Medical Department of the Alkali Division of I.C.I., made an appeal for volunteers among employees of a large chemical plant at Northwich, Cheshire. The nature of the trial was fully publicized and 2,163 men and women of all ages were accepted. In this trial 1,927 (89%) of the volunteers were male.

The doctors in all three groups were reminded that, as the virus used for the vaccines was cultivated in egg, volunteers should be asked if they suffered from nausea or vomiting after eating eggs. Persons with this history or any other evidence of allergy to egg or chick protein were not to be vaccinated.

* Members of the Committee: Professor C. H. Stuart-Harris (chairman), Dr. B. E. Andrews, Professor G. Belyavin, Professor Sir Austin Bradford Hill, Dr. F. Himmelweit, Dr. F. O. MacCallum, Dr. W. J. Martin, Dr. H. G. Pereira, Dr. A. T. Roden, Dr. D. A. J. Tyrrell, Dr. J. C. McDonald (secretary).

Follow-up

The arrangements made for observation of the volunteers after vaccination differed somewhat between the three groups. The industrial workers were mainly young healthy persons in whom no mortality from influenza or any other disease was anticipated. This group was observed for three winters and every effort was made during this period to record absenteeism and its cause as accurately as possible. Any volunteer absent from work for whatever reason was interviewed by a senior nurse immediately on return. If she thought that a respiratory illness was the cause of absence the volunteer was seen by a doctor, who recorded particulars of the illness and his opinion of the diagnosis. In addition, during the first winter, volunteers absent from work were visited at home as soon as possible by a health visitor (public health nurse) and details of the illness were noted.

Among the old people and the patients with chronic bronchitis an appreciable mortality from various causes during the same follow-up period was anticipated ; the primary reason for having patients of this kind in the trial was to see whether influenza vaccine would reduce the death rate. In the event of death of an inoculated person, the physicians were asked to submit a copy of the death certificate and a description of events leading up to it. To ensure that no patient was lost sight of it was suggested that those in the bronchitis group should be seen at the clinic in January or February and again in May of each year and that the general practitioners should check their registers after each winter for any deaths or removals that might have been missed. It was realized that it would be most difficult to keep a precise record of acute respiratory illnesses in old or debilitated persons, but the practitioners were asked to record any cases of influenza and the chest physicians any respiratory illness which put their patients to bed.

The number of volunteers from the chest clinics and practices was much lower than had been intended, and after two winters it became clear that a much larger trial would be needed to test the effect of vaccine on mortality. As the old and bronchitic patients were also providing comparatively little information on morbidity, it was decided to terminate the trial in these two groups in May 1962 and to continue observations until April 1963 only on the industrial workers.

Serological Tests

Blood specimens were requested from up to four of the first patients to be inoculated at each chest clinic to test their serological response to the vaccines. A first specimen was taken immediately before inoculation in November 1960 and a second specimen when the patient was seen about six months later, in May 1961. The same patients were if possible vaccinated again in November 1961 and two further blood specimens then taken, the first immediately before the injection and the second about six months later, in May 1962. Half those bled in 1960 had received the influenza A2 vaccine and half the B vaccine, but only the A2 vaccine was used for revaccination in 1961. There were thus two groups available for study—one which had received two doses of the A2 vaccine and the other the B vaccine followed by the A2 vaccine. Complete sets of four blood specimens were obtained and tested from 67 patients in the first group and 63 patients in the second. The specimens were sent to Dr. F. Himmelweit, who tested them by a method he described (Himmelweit, 1960) for the presence of haemagglutination-inhibiting (H.I.) antibody against an A2 virus strain (A2/Pakistan/1/57) similar to that in the vaccine, and the homologous virus B strain (B/Eng./939/59).

More detailed serological studies over a three-year period were made in the trial at I.C.I., Northwich. The results from this inquiry are published separately (Hobson *et al.* 1964).

Epidemiological Background

As the experience of persons inoculated with an A2 vaccine was being compared with others given a B vaccine it was essential to have information on the type of influenza virus infections prevalent during the three winters of the trial. For the country as a whole, a sufficient indication was provided by statistics of mortality from the Registrar-General, of sickness-benefit claims from the Ministry of National Insurance, and of virological findings reported to the Public Health Laboratory Service. It can be seen in Fig. 1 that influenza A2 was probably epidemic in January and February 1961, influenza B in December 1961 and January 1962, and influenza A2 again to a lesser extent in February 1963. The figures for 1959–60, the year which preceded the trial, are shown for comparison as there was little evidence of influenza virus infections in that winter. During the whole period the antigenetic characteristics of virus A2 and B strains isolated in the general population were similar to those of the vaccine strains.

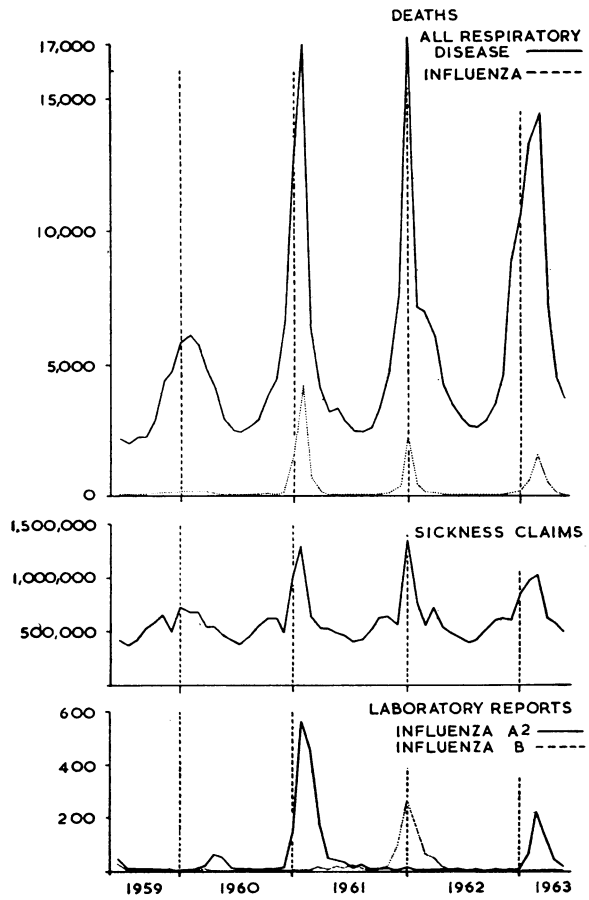

FIG. 1.—Respiratory disease mortality, sickness insurance claims, and laboratory reports—England and Wales, 1959–63.

It was felt that as the volunteers at I.C.I., Northwich, comprised a large localized group more detailed epidemiological information was needed for that population. This was obtained by the examination of specimens taken from a sample of employees sent home from work with an acute respiratory illness during each of the three winters of the trial. The specimens were tested in the Manchester Public Health Laboratory by Dr. B. E. Andrews and Dr. J. O'H. Tobin. Cases of influenza A2, but none of B, were confirmed between

1 January and the middle of February 1961 ; in the next winter cases of influenza B, but none of A2, were found between Christmas 1961 and the middle of January 1962 ; in 1963 very few patients were tested and only one case of influenza (A2) was found, with onset on 1 March. These results, together with sickness absence statistics in the factory, suggested that a fairly large outbreak of influenza A2 occurred early in 1961, a smaller outbreak of influenza B early in 1962, and very little virus influenza during the winter of 1962–3.

Results

Reactions to Inoculation

No attempt was made to record reactions other than those that were persistent or serious. At I.C.I., Northwich, the volunteers were inspected systematically at the end of May 1961 and kept under general observation until May 1963 ; nearly all the chest-clinic patients were seen periodically until May 1962, but their arms were not inspected routinely ; the old people were instructed to report any troubles from the vaccine but would have seen a doctor only at their request. Among the 6,123 vaccinated persons two delayed abscess-like reactions were reported, one four months and the other six months after inoculation. Both these reactions were in bronchitic patients who had received the influenza B vaccine and both cleared after incision. Induration or small fibrotic nodules were noted three to six months after inoculation in six volunteers and persistent soreness at the site of injection in another four ; 8 of the 10 had received the influenza B vaccine. No troublesome immediate reaction to inoculation was reported.

Serological Response

The serological results are summarized in Table I. At the second bleeding, six months after inoculation, the patients still had an antibody concentration appropriate to the antigen received about twentyfold greater than the starting figure. There was a slight fall between the second and third bleedings, and between the third and fourth in patients who were not given a second injection of the same vaccine. Those who were given the same vaccine again reached an even higher mean titre at the fourth bleeding, almost fortyfold greater than that at their first bleeding, and those who were given the influenza A2 vaccine for the first time in November 1961 responded almost as well as those given vaccine of the same batch a year earlier. Some evidence of a rise in antibody titres against virus A2 between the first and second bleedings and against virus B between the third and fourth, probably due to natural infection, can also be seen.

TABLE I.—*Serological Response in Patients with Chronic Bronchitis*

Vaccine History	Antigen	Geometric Mean Titre of H.I. Antibody			
		Nov., 1960*	May 1961*	Nov., 1961*	May 1962*
Influenza A2 vaccine in Nov., 1960, and Nov., 1961* (67 patients)	A2/Pak/1/57	22	515	410	853
	B/Eng/939/59	20	21	21	31
Influenza B vaccine in Nov., 1960, and influenza A2 vaccine in Nov., 1961* (63 patients)	A2/Pak/1/57	21	45	43	413
	B/Eng/939/59	29	537	370	340

* The dates of vaccination and bleeding are approximate, some volunteers were vaccinated a month later and bled up to two months later.

Clinical Protection

A summary of the sickness and mortality experience of the three groups of volunteers is presented in Tables II, III, and IV. The results were also studied in greater detail, taking into account age, sex, and, in the chest-clinic patients, severity of bronchitis. As the volunteers given the two vaccines in all three sections of the trial did not differ from each other with respect to the distribution of these variables, and as the findings in specific age, sex, and severity subgroups all followed the same general trends, the detailed information is not considered in this report.

The industrial volunteers had the highest attack rates from influenza, as expected, and in them could be seen the strongest evidence of vaccine-protection. The influenza A2 vaccine group had significantly less influenza in the first winter (Diff. ÷ S.E. = 4.1), and the influenza B vaccine group had just significantly less in the second (Diff. ÷ S.E. = 2.3). It is of interest that in 1961–2, when influenza B was prevalent,

TABLE III.—*Sickness and Death Rates in Old People in General Practice, 1960–2*

Year	Vaccine	No. Observed	Cases of Influenza		Deaths			
					All Causes		Initiated by Respiratory Infection	Initiated by Influenza
			No.	%	No.	%	No.	No.
1960–1	Influenza A2	789	9	1·1	28	3·5	4	0
	Influenza B	830	29	3·5	23	2·8	3	1
1961–2	Influenza A2	575	5	0·9	9	1·6	2	0
	Influenza B	597	8	1·3	21	3·5	2	0

TABLE IV.—*Sickness and Death Rates in Patients with Chronic Bronchitis, 1960–2*

Year	Vaccine	No. Observed	Respiratory Illness Putting Patient to Bed		Illness Initiated by Influenza		Deaths			
							All Causes		Initiated by Respiratory Infection	Initiated by Influenza
			No.	%	No.	%	No.	%	No.	No.
1960–1	Influenza A2	1,164	624	53·6	137	11·8	33	2·8	8	1
	Influenza B	1,177	648	55·1	177	15·0	38	3·2	16	3
1961–2	Influenza A2	876	396	45·2	116	13·2	25	2·9	9	1
	Influenza B	878	405	46·1	100	11·4	31	3·5	12	3

TABLE II.—*Sickness Rates in Volunteers at I.C.I., Northwich, 1960–3*

Year	No. Observed*	Vaccine	Influenza		Febrile Cold		Febrile Sore Throat		Other Respiratory Illness		Non-respiratory Illness	
			No.	%	No.	%	No.	%	No.	%	No.	%
1960–1	929	Influenza A2	110	11·9	98	10·6	34	3·7	41	4·4	164	17·7
	1,234	„ B	223	18·1	144	11·7	46	3·7	68	5·5	229	18·6
1961–2	860	„ A2	51	5·9	93	10·8	30	3·5	38	4·4	145	16·9
	1,219	„ B	45	3·7	81	6·6	21	1·7	60	4·9	199	16·3
1962–3	775	„ A2	31	4·0	38	4·9	5	0·6	27	3·5	63	8·1
	1,097	„ B	43	3·9	49	4·5	14	1·3	54	4·9	100	9·1

* The inoculation registers for this trial were modified so that 25% more volunteers received the influenza B vaccine than the influenza A vaccine as more of the former was available.

volunteers given the B vaccine had appreciably fewer illnesses diagnosed as febrile cold or febrile sore throat. Nothing comparable was seen in 1960–1, when influenza A2 was present, which suggests that virus B infections may have produced a wider range of clinical illnesses than those due to virus A2. In the third winter there was no difference between the two groups. Analysis of the influenza attack rate week by week showed that in 1960–1 the difference was present only in January and February, and in 1961–2 in January. During these periods, which coincided with laboratory evidence of virus influenza, the attack rate in the appropriate vaccine group was halved (Fig. 2).

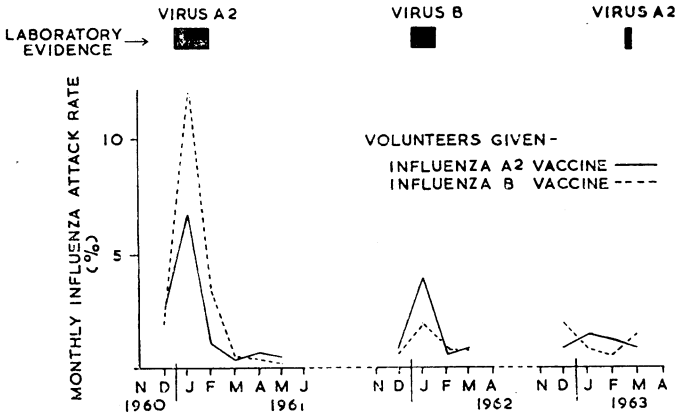

FIG. 2.—Influenza attack rates in trial at I.C.I., Northwich, 1960–3.

In 1960–1 the old people who had received the virus A2 vaccine also had significantly less influenza (Diff. ÷ S.E. = 2.3), as did the patients with chronic bronchitis Diff. ÷ S.E. = 2.3), but neither showed any important differences between the vaccine groups in the second winter. There was no evidence of an effect of vaccination on mortality in either winter, but the deaths initiated by a respiratory infection were few and by influenza even less.

The records of old people who were not vaccinated by the 10 vaccinating doctors or who were on the lists of the 19 doctors who preferred not to vaccinate were also analysed. Their sickness and mortality rates were generally similar to those for the patients who were vaccinated but tended to be a little lower; they were thus no substitute for a randomly selected control group.

Discussion

A high and persistent antibody response after oil-adjuvant vaccine has often been demonstrated and the serological findings reported in this paper and by Hobson *et al.* (1964) confirm it yet again. It was satisfactory that on this occasion the frequency of chronic cystic reactions was only 3.3 in 10,000 and that the emulsion was sufficiently stable for it to be used with equal effectiveness after storage for a year. Evidence of clinical efficacy has been reported only in three trials of oil-adjuvant influenza vaccines so far (Philip *et al.*, 1954; Davenport *et al.*, 1956; Meiklejohn, 1962). In all three protection was demonstrated up to about four months after vaccination and, in that described by Philip and his colleagues, after 14 months. The results presented in this paper suggest that in persons of working age protection is afforded equally against influenza A2 or B, probably for at least 18 months. The order of protection was difficult to assess because attack rates in both vaccinated and controls were certainly diluted with illnesses which were not influenza, but it was probably 50% or more.

This trial failed yet again to answer the important question of whether an effective influenza vaccine will reduce mortality in the elderly and debilitated in times when influenza is prevalent. Only a small proportion of the excess mortality that regularly occurs in patients of this type during epidemics is attributed to influenza on the death certificates. It seems probable, nevertheless, that influenza virus infection may be of critical importance and that, if prevented, mortality would fall. Though the excess mortality associated with influenza in old people is considerable the risk in absolute terms is small, and a larger trial than this one would be needed for any reduction to be demonstrable. During the first wave of Asian (A2) influenza in the autumn of 1957 it was estimated that in England and Wales there were about 10,000 excess deaths from all causes in persons over 65 years of age (McDonald, 1958); even so, this corresponded to an excess death rate in this age-group of only about 2 per 1,000. A trial involving only 1,619 old people and 2,341 patients with bronchitis could therefore not be expected to yield conclusive information on mortality even in two years.

Summary

In field trials of improved monovalent oil-adjuvant vaccines of low viscosity 6,123 volunteers, drawn about equally from general practitioners' patients aged 65 years or more, chest-clinic patients with severe chronic bronchitis, and employees in a chemical industry, were given either an A2 or a B vaccine in the late autumn of 1960. The old and debilitated patients were observed for two winters and the industrial group for three. A sample of the patients with bronchitis were bled for serological studies before inoculation and 6, 12, and 18 months later.

Reactions.—Troublesome immediate reactions were not reported and only two volunteers developed delayed abscess-like reactions at the site of injection. This frequency—3.3 per 10,000—may be compared with 17.9 per 10,000 observed by the Committee in trials of an earlier type of oil-adjuvant vaccine.

Serological Response.—The magnitude of response to both influenza A2 and influenza B vaccine was of the degree expected with oil-adjuvant and was well maintained for at least 18 months. Volunteers given vaccine stored for one year before use responded almost as well as with fresh vaccine; the mean antibody titre in other volunteers inoculated a second time with the same type of vaccine one year later rose to a higher figure than after the first injection.

Clinical Protection.—Statistically significant evidence of protection against influenza A2 was observed in all three groups of volunteers during the first winter of the trial and against influenza B in the industrial group during the second winter. The greatest reduction in the influenza attack rate was seen in the industrial group, especially during periods when influenza virus infections were shown to be prevalent; at these times the attack rates were halved. During the third year there was little influenza and no evidence of protection was seen.

Analysis of deaths in the old and bronchitic patients failed to reveal any effect of vaccination on mortality due to influenza, respiratory disease, or all causes. As the number of patients was small no conclusion from this was drawn.

The Committee is greatly indebted to general practitioners, chest physicians, industrial medical officers, and, to their assistants, for giving so much time to these trials. The help given by the College of General Practitioners and the Research Committee of the British Tuberculosis Association in finding doctors to collaborate is much appreciated. Special thanks are due to Dr. C. P. Chivers, who personally supervised the trial at I.C.I., Northwich, with all that this entailed. Grateful acknowledgment must also be made to the

volunteers, especially those who gave blood specimens, without whom the investigation would not have been possible.

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Serological Studies on Adult Volunteers Inoculated with Oil-adjuvant Asian Influenza Vaccine

Report to the M.R.C. Committee on Influenza and Other Respiratory Virus Vaccines

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In the autumn of 1960 a large-scale field trial into the efficiency of oil-adjuvant influenza vaccines was initiated by the Medical Research Council (1964). Although the main purpose was to evaluate the protective effect of this new form of vaccine against clinical influenza, it was felt that long-term serological studies were an integral part of the assessment. Accordingly the present investigation was undertaken at the request of the M.R.C. Committee in a group of industrial workers in the Alkali Division of Imperial Chemical Industries Ltd., at Northwich, Cheshire, who were vaccinated in the course of the main trial.

It seemed of particular interest to determine whether the serological response to Asian virus vaccine could be augmented by oil adjuvant, since earlier vaccine trials with these new strains—for example, Clarke *et al.* (1958), Himmelweit (1960), and Hobson and Pearson (1961)—gave variable results both with saline suspensions of inactivated virus and with virus adsorbed to aluminium phosphate. Antibody induction appeared to be slight, with considerable variation from person to person, and antibody production was not of long duration.

In the present survey serum samples were taken before and at various intervals after inoculation of a single intramuscular injection of oil-adjuvant vaccine. The particular points of study were (*a*) the duration and degree of serological response in the group as a whole in terms of haemagglutination-inhibiting (H.I.) antibody ; (*b*) the effect on individual responses of various factors such as age and prevaccination immune status, and (*c*) the effect of a second dose of vaccine in approximately half the group one year after the first injection, as compared with the antibody response obtained in fresh volunteers.

Materials and Methods

Vaccination Procedures.—The vaccine consisted of formalin-inactivated A/Singapore/1/57 virus blended with an equal volume of Drakeol No. 6 R containing 10% Arlacel A, to give a final concentration of 2,000 haemagglutination units (H.A.U.) per 0.25-ml. dose. Details of the vaccine preparation are described by Himmelweit (1960) and M.R.C. (1964). The vaccine was dispensed into single-dose containers. Sixty-two staff volunteers (with approximately equal numbers of men and women of under 30 or over 40 years of age) were selected for serological study from a clinical trial population of 2,163 industrial workers receiving adjuvant influenza vaccines in December 1960. Clinical evaluation on this group and the larger trial population is described in the main M.R.C. (1964) study. Blood samples were taken one month before, and at 1, 3, and 12 months after vaccination. At this time (December 1961) 25 of the original group received a second inoculation of the original vaccine which had been stored at 4° C. for one year, and 25 new volunteers not previously vaccinated were similarly inoculated. Further blood samples were taken 4, 11, and 16 months after the second injection—that is, 16, 23, and 28 months after the original inoculation.

Haemagglutination-inhibition Tests.—All sera were stored at −20° C., and so far as possible all samples from the same person were titrated in parallel in the same batch of tests. H.I. tests were performed by the plastic plate method (W.H.O., 1953), using 8 H.A. units of uninactivated virus. In tests with the A2/Pakistan/1/57 strain sera were inactivated at 56° C. for 30 minutes before testing. In subsequent titrations with an avid strain of A2/Sing/1/57 sera were pretreated with cholera filtrate (Philips-Duphar) prior to heat-inactivation.

Tissue-culture-neutralization Test.—Details of the method are as described by Pereira (1958) and Beare (1962). In the present tests an allantoic fluid pool of an avid strain of A2/Sing/1/57 was used. Infectivity titres were determined on monkey-kidney-tissue cultures, using a haemadsorption technique (Vogel and Shelokov, 1957). A fixed dosage of virus (final 100 haemadsorption doses) was incubated at 37° C. for one hour with a final 1/10 dilution of heat-inactivated serum, and the serum-virus mixture then inoculated into monkey-kidney tubes. After incubation at 37° C. for two days the tubes were examined for haemadsorption. Only complete inhibition of haemadsorption was accepted as evidence that the serum contained neutralizing antibody.

Degree and Duration of Antibody Response to Vaccination

The A2/Pak/1/57 strain of virus was selected for the H.I. tests discussed below, because its insensitivity to non-specific inhibitors of haemagglutination allowed the direct titration of

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