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Reactions and antibody response to live influenza vaccine prepared from the Iksha (A2) strain. (A report to the Medical Research Council by their Committee on Influenza and other Respiratory Virus Vaccines*)

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INTRODUCTION

Live influenza vaccine has been used extensively in the U.S.S.R. (Ritova, Zhukovsky & Yevstigneyeva, 1963; Smorodintsev, Alexandrova, Chalkina & Selivanov, 1965), in eastern Europe (Starke, Siebelist & Hiller, 1964) and occasionally in other countries (Okuno & Nakamura, 1966). In England five trials of this vaccine have been made since 1960; the first two during 1960–61 (McDonald, Zuckerman, Beare & Tyrrell, 1962) and the third in 1962 (Andrews *et al.* 1966).

The findings from these three trials were encouraging in that serological evidence of symptomless infection was produced by the vaccine in most recipients and the volunteers were apparently resistant to re-infection with a challenging dose of live virus given intranasally 1 month later.

Accordingly, in 1963 a field trial was made to try to assess the protection conferred against influenza. Approximately 1500 personnel of the Royal Air Force were vaccinated and a similar number who acted as controls received an inert fluid.

The antibody response to the batch of vaccine used in this trial was much less satisfactory than in the previous investigations, and did not suggest that adequate protection had been induced. However, there was no outbreak of influenza during the follow up, and although a few cases of influenza were observed in both the

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vaccinated and control groups their numbers were too small to permit valid conclusions about efficacy. The aspects of this trial concerned with the clinical evidence of protective efficacy have therefore not been reported. However, the antibody estimations which were made in some participants are described below.

A fifth trial designed to assess response and reactions in children and adults was made in 1965, since vaccine which was satisfactory for adults might nevertheless produce more severe reactions in children. This investigation provided an opportunity to check the results of the previous (1963) trial on a small scale. The findings from this trial in children and adults are also described here, and the vaccination reactions and antibody responses observed in all five trials are reviewed.

MATERIALS AND METHODS

Virus isolation

In the fourth trial, throat swabs were broken off into transport medium consisting of Hanks's balanced salt solution (BSS), containing 0·5 % lactalbumin hydrolysate (LAH) and 0·02 % $NaHCO_3$; this was replaced in the fifth trial by BSS and 2 % bovine plasma albumin (BPA). Specimens were taken to the laboratory in thermos flasks containing water ice. In trial 4, virus isolation was attempted by amniotic inoculation in five 10-day embryonated eggs followed by a blind passage in three eggs; in trial 5, the culture system consisted of two tubes of monkey kidney tissue culture which were tested for the presence of a haemadsorbing agent after 5 days incubation in a rolling drum at 33° C.

Antibody titrations

Haemagglutination-inhibiting (HI) antibodies were titrated by methods described by the WHO Expert Committee on Respiratory Virus Diseases (1959) and by Beare (1962). Receptor Destroying Enzyme (RDE) was obtained from Burroughs Wellcome and Company. All tests were performed in WHO plastic plates. Viruses used in the HI tests were: (1) a strain of A2/Singapore/1/57 which had received many egg passages, (2) a fifth egg passage of the 1964 A2 virus known as A2/England/12/64, and (3) a strain called A2/England/7/65 which had had an unspecified number of egg passes. The viruses were diluted in saline to contain eight 50 % haemagglutinating doses per 0·25 ml. volume.

Complement-fixation tests were performed with soluble A antigen by overnight fixation at 4° C.

The technique of the neutralization test has been described (Pereira, 1958; Beare, 1962). In the fifth trial, neutralization tests and preliminary virus titrations were done at 33° C. instead of the 35° C. temperature originally used. This was for convenience only and was considered unlikely to make any practical difference. A2/Singapore/1/57 was at all times the test virus.

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TRIAL 4: IN ROYAL AIR FORCE

General plan

During November and December, 1963, 2950 apprentices or boy entrants aged 15–18 years received live vaccine or control fluid intranasally. Ninety-two from the 1468 given live vaccine were bled at the time of vaccination and again 14 days later. Throat swabs taken from 77 participants 48 and 72 hr. after vaccination were examined for the presence of the vaccine virus.

The vaccine

A sixth allantoic pass of the Russian A2 Iksha virus used in the previous trials was inoculated intranasally into a human volunteer. Virus recovered from throat washings was grown allantoically in leucosis-free eggs by Dr F. Himmelweit of the Wright-Fleming Institute of Microbiology.

The pool of vaccine was titrated by Dr H. G. Pereira of the World Influenza Centre who reported a haemagglutination titre of 640 and an infectivity titre of 10^9 50% egg infective doses (EID 50) per ml. The vaccine strain grew readily in embryonated eggs and was antigenically closely related to A2/Singapore/1/57; but, as before, it differed from the latter in that it was inhibitor resistant.

The vaccine pool was tested for safety by the procedures described by Andrews *et al.* (1966). Paired blood specimens from the volunteer from whom the vaccine virus was recovered were examined by Dr R. J. C. Harris for antibody against Resistance Inducing Factor (RIF) with negative results.

In October 1963, immediately before the trial began, two ampoules of vaccine selected at random were re-tested by Dr Pereira for virus content; one of these ampoules had a titre of $10^{9.1}$ EID 50 per ml. and the other $10^{8.8}$ EID 50 per ml. In using the vaccine for the trial the lower estimate was taken and a dilution of 1/50 was assumed to contain about 10^7 EID 50/ml.

Methods of storage and dilution of vaccine were as previously described (Andrews *et al.* 1966).

Dose

Forty of the participants tested serologically received a dose of 10^6 EID 50 of virus and the remainder a dose of 10^7 EID 50. Each dose was contained in a volume of 0·5 ml., half of which was given into each nostril; after instillation of the vaccine, participants were asked to sniff gently and not to blow their nose for at least 15 min.

RESULTS

Virus isolation

No influenza virus was isolated from the 77 throat swabs examined.

Antibody response to vaccination in volunteers for special tests

As in the previous trials the results were considered according to whether the participants had or had not neutralizing antibody before vaccination.

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The antibody response to vaccination after each dose of vaccine in recruits with and without neutralizing antibody is shown in Table 1. It is evident that this result is poor. The response was slightly better with the larger dose; but less than half of the participants without pre-vaccination neutralizing antibody produced an HI rise against the 1957 virus, while the proportion of those *with* prevaccination antibody who responded similarly is less than one-fifth. When A2/England/12/64 is used as the indicator strain, the result is equally disappointing.

In no group was there a significant production of antibody to soluble antigen.

Table 1. *Antibody response in subjects with and without pre-vaccination neutralizing antibody given different strengths of vaccine*

Pre-vaccination neutralizing antibody	Dose EID 50	Antibody response according to three tests: HI* A 2/Sing/1/57	HI* A 2/Eng/12/64	CF*
Absent	10^6	2/13	1/11	0/12
	10^7	8/21	4/19	2/20
Present	10^6	1/25	2/21	0/21
	10^7	5/28	8/28	0/27

* Fourfold or greater rise.

TRIAL 5: IN CHILDREN AND ADULTS

General plan

This trial was made in March 1965 among 31 children resident in Wednesfield Cottage Homes, Staffordshire, and in adult members of the staff. The children were placed in two groups according to age. Group 1 contained children aged from 11 to 15 years and group 2 those from 7 to 10 years. The adults were immunized on the first day of the trial, the group 1 children on the third day and the group 2 children on the fifth day. In this way it was possible to observe any reactions in older participants before giving the vaccine to the younger ones.

A nasal and a throat swab were taken from each participant before vaccination and examined in monkey kidney cell cultures for haemadsorbing agents. Forty-eight hours after vaccination a second throat swab was taken and an attempt made to isolate virus. Serum samples were withdrawn before vaccination and approximately 3 weeks later. Oral temperature readings were recorded before vaccination and each night and morning for the next forty-eight hours.

The vaccine

A new vaccine pool was prepared by propagating the virus of trial 4 in leucosis-free eggs supplied by the Poultry Research Station, Houghton Grange. This virus had now had six allantoic passages, one passage in humans and two further allantoic passages. In general, the virus was very similar to that of the previous vaccine: the haemagglutinating titre (reciprocal dilution) was 640 and the in-

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fectivity titre $10^{8\cdot8}$ EID 50 per ml. Identification tests by cross-haemagglutination-inhibition indicated that it was similar to the original and later Iksha vaccines except that the virus had undergone a slight increase of inhibitor-sensitivity. Safety tests for bacterial sterility and absence of other adventitious agents were uniformly negative.

Storage and transport of vaccine

The vaccine was stored at $-70°$ C. and was kept in a mixture of solid carbon dioxide and alcohol during transport. At each vaccination session an ampoule was thawed immediately before use. Part of the contents was then diluted in Hanks's BSS plus 0·5% gelatin adjusted to pH 7·2 and without antibiotics. Diluted and undiluted vaccine from the same ampoule was returned to Colindale on ice and inoculated into eggs.

Vaccination procedure

Each participant lay with the head hanging back over the side of a bed. Three drops (each 0·02 ml.) of vaccine diluted 1/7·6 (total dose 10^7 EID 50) were then instilled into each nostril.

Participants remained in this position for 1 min. after vaccination and were instructed to sniff after getting up.

Testing of potency of vaccine used at Wednesfield

Samples of the actual vaccine dilutions used on days 1, 3 and 5 of the trial were titrated in eggs and indicated that the calculated number of EID 50 of live vaccine had in fact been administered throughout.

Results

There were 16 adults and 31 children of whom 12 were aged 7–10 years and 19 aged 11–15 years. In three children, all in the group aged 7–10 years, a slight *rise in temperature to 99° F. was found in the 24 hr. after vaccination. No* constitutional reactions occurred in any participant.

No viruses were isolated in monkey kidney cells from swabs taken before or after vaccination either at Colindale or at Wolverhampton.

An attempt was made to isolate virus in eggs from post-vaccination swabs without success.

Antibody titres: before vaccination

The pre-vaccination antibody titres in children and adults in all the tests are shown in Table 2. It is clear that a substantial proportion of both adults and children possessed neutralizing, HI, and CF antibodies before vaccination. It is also evident that, apart from those demonstrated by complement-fixation, antibodies in children were found more frequently than in adults.

Antibody: response to vaccination

The antibody response to vaccination is shown in Table 3. The proportion responding with a fourfold rise of antibody was low both in those with and in those

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without pre-vaccination antibody. There was no apparent difference in the response of children and adults.

The analysis shown in the table includes participants with all levels of antibody, and the participants with high pre-vaccination antibody might not be expected to show a fourfold response to vaccination. Thus, for those who responded with a fourfold or greater rise in neutralizing antibody the geometric mean titre before vaccination was 26·9, whereas the initial geometric mean titre of those who failed to respond was much greater—140·7. However, this was not so for HI antibody. The initial geometric mean titres HI antibody (A2/Singapore/57) were almost identical for those who did and did not respond—50·4 and 50·1 respectively.

Table 2. *Wednesfield Cottage Homes: antibody titres before vaccination in children and adults*

Type of antibody	Participants	Antibody present
Neutralizing A2/Sing/57	Adults	14/16
	Children	31/31
HI A2/Sing/57	Adults	10/16
	Children	30/31
HI A2/Eng/65	Adults	7/16
	Children	21/31
CFT	Adults	15/16
	Children	20/31

Table 3. *Fourfold or greater antibody rise to vaccination in participants with and without each type of antibody before vaccination*

	Post-vaccination antibody response			
Pre-vaccination antibody	Neutralizing A2/Sing/57	HI A2/Sing/57	HI A2/Eng/65	CFT
Absent	1/2	3/7	3/19	3/12
Present	7/45	3/40	0/28	1/35

DISCUSSION

The two trials of live influenza vaccine described above are the last of a series of five undertaken in England since 1960. Though the purpose and complexity of the five trials varied, observations on reaction and antibody response to the Iksha strain were common to them all. The findings of the trials showed some variation and the differences encountered have accordingly been discussed below.

Some relevant characteristics of each trial are shown in Table 4. There are basic similarities in all the trials; adults or adolescents were included in each, and in each at least some participants were given the same large dose of vaccine, 10^7 EID 50, by the same method—nasal drops. Three batches of vaccine were used: batch 1 for trials 1, 2 and 3; batch 2 for trial 4 and batch 3 for trial 5.

In each trial, with the exception of trial 2, serum antibodies in some participants were estimated before and after vaccination. The trials thus provide an indication

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of the antibody response to each of the three batches of vaccine used. The trials also give some indication of the frequency and character of reactions produced by three batches of vaccine.

Table 4. *Trials of live influenza vaccine in England: participants, preparation and dose of vaccine*

Trial	Year	Participants	Preparation of vaccine	Dose EID 50	No. of doses
1	1960	Adolescents and adults	Batch 1. Six passes in eggs	10^6, 10^7	Usually 2
2	1961	Adults		10^7	1
3	1962	Adults		10^7	1
4	1963	Adults	Batch 2—batch 1 plus one pass in man and one further egg pass	10^6 10^7 10^7	1
5	1965	Adults, and children 7–15 years	Batch 3—batch 2 plus one further pass in eggs	10^7	1

Table 5. *Reactions to vaccination in five trials*

	Neutralizing antibody present or not known before vaccination			Neutralizing antibody absent before vaccination		
Trial	No. vaccinated	Febrile reactions	Afebrile reactions	No. vaccinated	Febrile reactions	Afebrile reactions
1. R.A.F. 1960–61 (A)	42	0	0	22	3	0
2. R.A.F. 1960–61 (B)	513 (513 controls)	6 (8)	33 (33)	—	—	—
3. R.A.F. 1962	53	0	5	66	0	30
4. R.A.F. 1964	1468	0	Not known			
5. Wednesfield 1965	45	0	0	2	0	0

Vaccination reactions

The only reaction encountered was coryza, occasionally with fever (Table 5). Afebrile coryzal symptoms were recorded most frequently in trial 3, mainly in volunteers without neutralizing antibody, but since there was no unvaccinated group available for comparison, reactions cannot necessarily all be attributed to the vaccine. There were no febrile reactions with batch 2 of vaccine used in trial 4 or reactions of any kind with batch 3 in children or adults in trial 5. These two batches also gave a poorer antibody response than batch 1.

Antibody response

In Table 6 a comparison has been made of the frequency of virus recovery and antibody response in the participants in each of the four trials who were given

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10^7 EID 50 and for whom pre- and post-vaccination serum samples were available. It is evident that only a small proportion of participants without pre-vaccination antibody responded. The proportion of participants *with* pre-vaccination antibody who responded to batch 2 and to batch 3 is also small. The better results with batch 1 suggest that the virus underwent some change during its passage in man and its subsequent recovery in leucosis-free eggs. This process may have selected a virus which was too attenuated to stimulate an antibody response in a partially immune subject. However, an *increase* of virulence following human passage would usually be expected.

Table 6. *HI antibody response (fourfold or greater rise) and virus recovery in each of four trials among participants with and without pre-vaccination neutralizing antibody*

	Trial no.	Trial	No. of doses	No. tested and responding	Virus isolated
With pre-vaccination neutralizing antibody	1	R.A.F. 1960	2	24/42 (57 %)	5/42
	3	R.A.F. 1962	1	17/27 (63 %)	1/27
	4	R.A.F. 1963	1	5/28 (18 %)	0/28
	5	Wednesfield	1	5/45 (11 %)	0/45
Without pre-vaccination neutralizing antibody	1	R.A.F. 1960	2	4/22 (18 %)	12/22
	3	R.A.F. 1962	1	5/29 (17 %)	10/31
	4	R.A.F. 1963	1	8/21 (38 %)	0/21
	5	Wednesfield	1	1/2	0/2

It is also possible that variations in response were occasioned by changes in the immunity of the population since the start of the Asian epidemic. However, Hobson, Gould & Flockton (1967) found certain differences between the laboratory characteristics of the virus contained in batch 1 and of that contained in batches 2 and 3; and it seems likely that the infectivity or antigenicity of the Iksha strain has become modified by successive passage.

The better response to batch 1 in participants *with* pre-vaccination antibody suggests that a response may be more likely in the presence of previous sensitization by a related antigen. Persons without pre-vaccination antibody may, therefore, require several doses of live influenza vaccine for effective immunization, and it is now the Russian practice to use three doses of vaccine. Multiple inoculations may also be an advantage because of the possession by some volunteers of a temporary resistance induced by natural infection with other viruses such as rhinoviruses (Cate, Couch & Johnson, 1964).

Finally, as a result of these trials, it appears that the Iksha strain of influenza A 2 is not now suitable for general use. The strain was initially reasonably satisfactory but it has probably changed its properties with passage in man and eggs. In future work, attempts should be made to avoid shifts in the genetic constitution of otherwise suitable viruses by the use of such techniques as limit dilution passages. There is also a need to extend the studies such as those of Soloviev, Orlova, Porubel & Vasilieva (1961), Kolchurina (1966) and Hobson *et al.* (1967) to enable a selection to be made of immunogenic and non-virulent viruses on the basis of laboratory

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studies. For this reason new strains of influenza A 2 and B are being studied in man and in the laboratory and will be given limited trials in small groups of antibody-free adults and children when indicated.

SUMMARY

Two field trials of A 2 live influenza vaccine (Iksha) are described; the clinical reactions and antibody response in three earlier trials are compared.

The antibody response in the last two trials was unsatisfactory and was inferior to that observed in the earlier investigations. This reduction in response was probably due to an alteration in the virus during further passage.

In trial 4 we are greatly indebted to Technical Training Command, Royal Air Force (P.M.O. Air Vice-Marshal T. C. Macdonald) for granting facilities for this trial. Much of the work fell on the medical officers in the six Stations where the trial was conducted and upon the staff of their departments; special thanks are due to Group-Captain H. W. Whittingham, Wing-Commanders J. A. Cooney and G. Gilchrist, and Squadron-Leaders A. R. Bone, J. D. MacAllister, J. G. Robertson and K. E. A. Underwood-Ground.

The trial was supervised by Dr R. V. Peters, and Dr A. J. Zuckerman assisted in the vaccination of the volunteers. The virological tests were performed at the Central Public Health Laboratory, Colindale; valuable assistance in this work was given by Mrs J. Coetzee.

In trial 5 we are greatly indebted to the Management Committee, to Mr F. Macho, Superintendent, and to the staff of the Cottage Homes. Our thanks are also due to Pfizer Ltd. who provided facilities for the preparation and testing of the vaccine. The trial was greatly assisted by Mr David Smith who undertook the dilution of the vaccine at the clinic and the antibody tests at Colindale.

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