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An assessment of oil adjuvant and aqueous influenza vaccines

II. Antibody responses to the vaccines*

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INTRODUCTION

Over the winters of 1962–3 and 1963–4 trials were carried out to ascertain the reactions to contemporary aqueous and adjuvant vaccines which contained strains of both influenza A and influenza B (Forsyth, 1967). It was felt that an assay of the antigenic efficacy of these vaccines was an essential part of this investigation. In addition, several of the recent trials of British-made influenza vaccines, evaluated in terms of haemagglutinating units (HAU), were done with special material with only one antigenic variant (M.R.C. 1955, 1957, 1958; Himmelweit, 1960, Hobson, *et al.* 1964) and it seemed that further information relevant to commercially available vaccines could be gained by serological tests on the volunteers.

This paper describes the results of these tests.

MATERIALS AND METHODS

Trials and vaccines

The vaccines used and the nature and organization of the trials have been described in the previous paper (Forsyth, 1967).

Virus strains

An avid strain of A/Singapore/1/57 was kindly supplied by Dr A. S. Beare (Colindale), and Dr D. Hobson (Evans Medical Ltd.) sent B/England/939/59. This latter was the most avid of a number of antigenically similar strains.

Antibody titrations

Haemagglutination-inhibition (H.I.) tests were performed in a standard manner using plastic plates, 0·2 ml. volumes and 8 HAU of virus antigen. The test plates were incubated at 4° C. and the agglutination patterns read and scored in the usual manner.

All sera were inactivated with cholera filtrate (N. V. Philips-Duphar) and made up to a 1/10 dilution with 0·01 M phosphate-buffered saline, pH 7·0.

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Neutralization tests were carried out and the results read as described by Beare (1962). The same strains of virus were used as in the H.I. tests.

In all series compared, sera from any one person were tested together against each antigen.

RESULTS

First trial

During the period of the first trial no evidence of widespread influenza in the community was received by the Virus Reference Laboratory nor was there any significant change in the levels of antibody of those who were only inoculated with saline placebo.

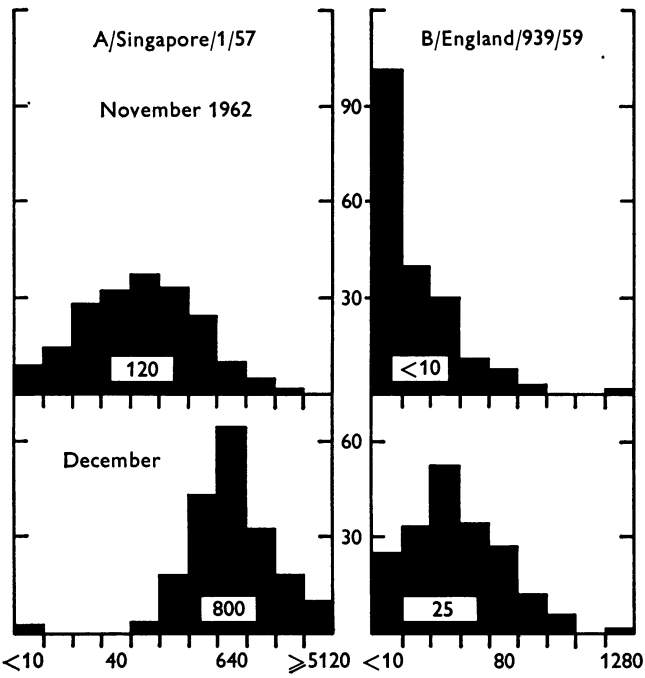

Fig. 1. The effect of an inoculation of 0·5 ml. *Invirin* on H.I. antibodies. Median titres inserted in each histogram.

One hundred and ninety volunteers were given 0·5 ml. of *Invirin*. This was one-half the dose of vaccine recommended by the manufacturer.

Before inoculation there was a striking difference in the distribution of titres of antibody to the two principal antigens. Very few persons (8) lacked detectable antibody to A/Singapore/1/57, but the majority (101) did not have antibody to B/England/939/59. Three weeks after inoculation even this small dose of vaccine had stimulated high levels of antibody to the influenza A strain. Twenty-five per cent of those without antibody to A/Singapore/1/57 or B/England/939/59 failed to convert serologically. However, in the case of influenza B not only were the antibody titres elicited poor, less than 25 % being 80 or more, but the numbers of subjects left without detectable antibody were large.

From individuals in the second part of the trial, sera taken before the second inoculation and 4 weeks and 9 months after inoculation were tested together. Therefore, the 1·0 ml. *Invirin* (aqueous vaccine) and the adjuvant vaccine were compared in, effectively, two populations; one without previous vaccine experience and the other having had the 0·5 ml. dose of *Invirin* previously.

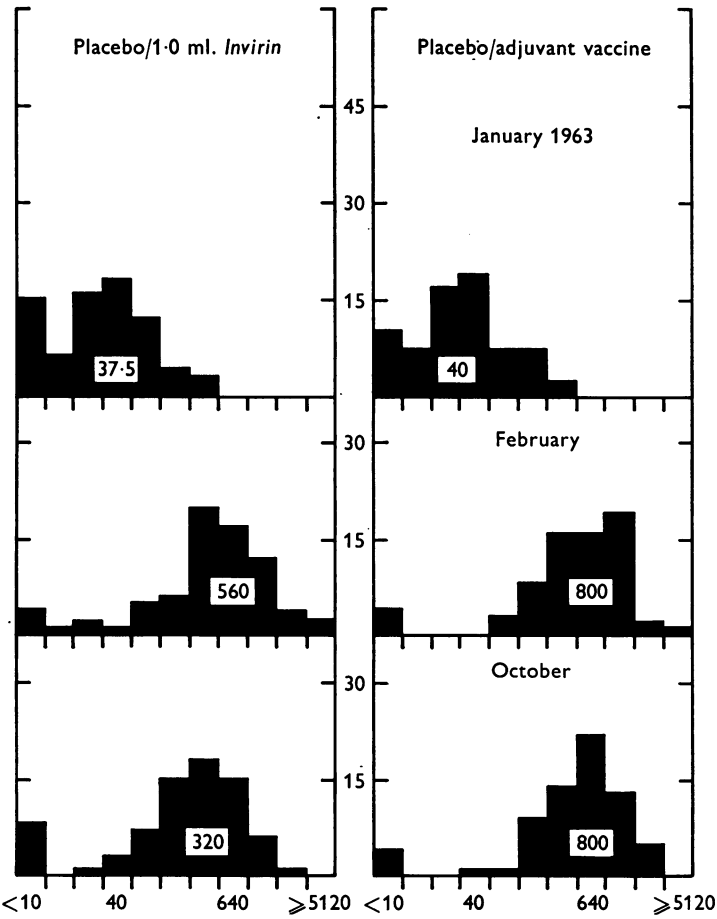

Fig. 2. The distribution of antibodies to A/Singapore/1/57 before, and at intervals after the inoculation of either 1·0 ml. *Invirin* or adjuvant vaccine, in subjects previously given saline placebo.

In those who had had no vaccine previously

Antibody to influenza A. The 1·0 ml. dose of *Invirin* given to 74 volunteers was associated with a rise of the median antibody titre from 37·5 before inoculation to 560 a month later. Once again, about a quarter (4 of 15) of those without antibody before failed to acquire it afterwards. However, 9 months after inoculation the median antibody level had fallen to 320 and now eight people showed no antibody.

Following the adjuvant vaccine, the median titre of the 69 volunteers rose from 40 to 800. While the distribution of titres associated with the two vaccines was

not significantly different at 1 month ($0{\cdot}2 > P > 0{\cdot}7$), at 9 months the advantage of the adjuvant vaccine was clear ($0{\cdot}01 > P > 0{\cdot}005$). Although the adjuvant vaccine failed to stimulate antibody in four of the 10 volunteers without it, after 9 months no others lacked detectable antibody.

Antibody to influenza B. For this antigen the aqueous vaccine, *Invirin*, gave a better over-all result than the adjuvant vaccine. The former not only caused a

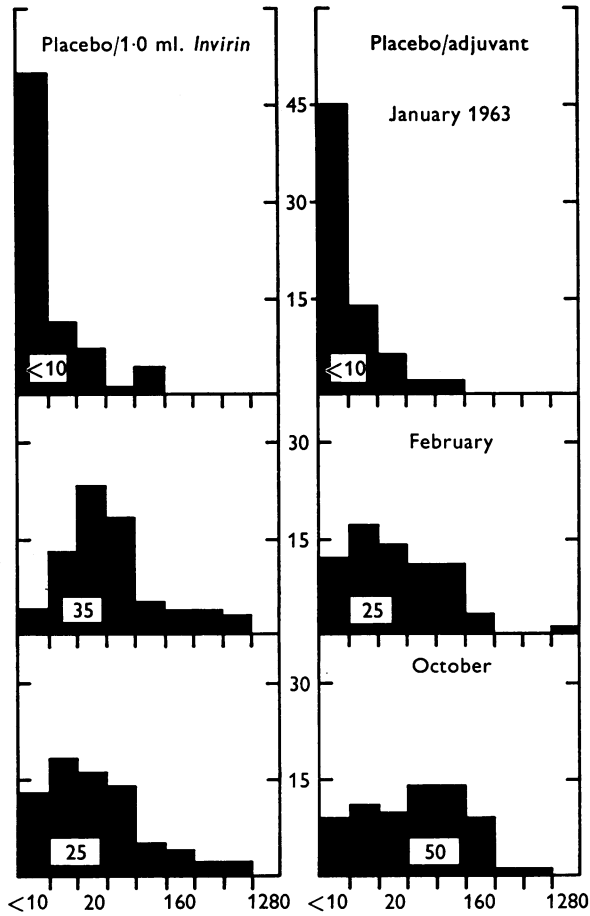

Fig. 3. The distribution of antibodies to B/England/939/59 before and after the administration of *Invirin* or adjuvant vaccine to subjects previously given saline placebo

significantly ($0{\cdot}005 > P > 0{\cdot}001$) better antibody response at 1 month but also elicited antibody in a greater proportion of those previously negative (47 of 51, as against 33 of 45). Nine months after inoculation the adjuvant vaccine showed to advantage but the differences in antibody titre between the groups were not significant ($0{\cdot}2 > P > 0{\cdot}1$).

In those who had had 0·5 *ml.* Invirin *previously*

Antibody to influenza A. A further dose of aqueous vaccine caused virtually no alteration in antibody level a month later and at 9 months the median titre was the same as before the inoculation and was no better than the group which had received a single 1·0 ml. dose of *Invirin.* These results were much inferior ($P < 0{\cdot}0005$) to those after adjuvant vaccine both at 1 month and 9 months. Adjuvant vaccine caused a considerable rise in median titre—from 320 to 800—and this was essentially maintained 9 months after immunization.

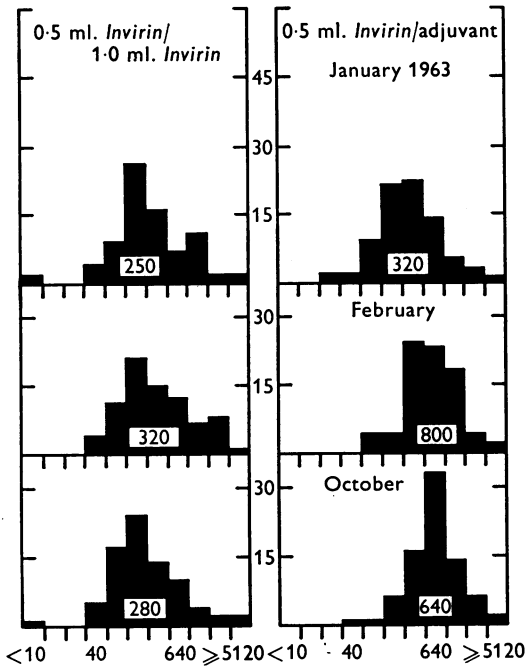

Fig. 4

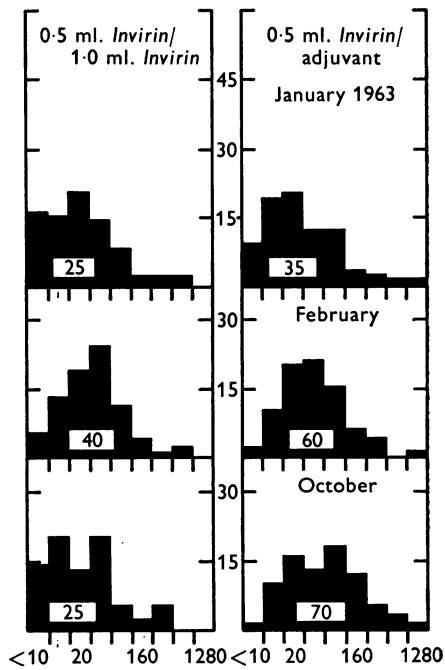

Fig. 5

Fig. 4. The distribution of antibodies to A/Singapore/1/57 before and after the administration of *Invirin* or adjuvant vaccine to subjects previously given 0·5 ml. *Invirin.*

Fig. 5. The distribution of antibodies to B/England/939/59 before and after the inoculation of either 1·0 ml. *Invirin* or adjuvant vaccine in subjects previously given 0·5 ml. *Invirin.*

Antibody to influenza B. In contrast to the results in the volunteers who received placebo for the first injection (see Fig. 3), the adjuvant vaccine here gave better results than *Invirin.* While the titres achieved at a month were no better ($0{\cdot}7 > P > 0{\cdot}6$), at 9 months the difference was marked ($P < 0{\cdot}0005$). At the same time, of the 16 who had no detectable antibody before 1·0 ml. of *Invirin,* 14 lacked antibody at 9 months but after the adjuvant only one of nine failed to have antibody at this time.

Second trial

In the second trial (1963–4) 97 volunteers were given the adjuvant vaccine, *Admune*. They were bled at the time of inoculation and 1 and 3 months later. Once more there was no evidence of widespread influenza in the community

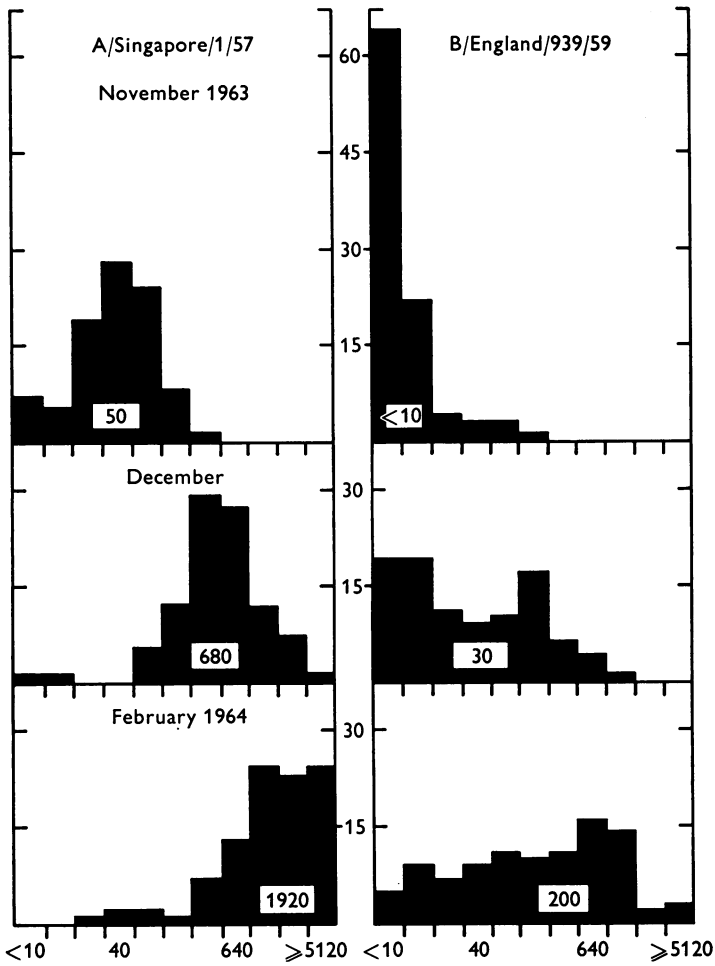

Fig. 6. The distribution of antibodies to two strains of influenza before and at intervals after a single inoculation of *Admune* adjuvant vaccine.

during the period of the trial. The new group of volunteers exhibited the same low incidence of antibody to influenza B as noted in the previous trial.

Antibody to influenza A. Four weeks after inoculation the antibody titres had risen to the levels similar to those achieved in the earlier trial. At 3 months there had been a further substantial rise to a median titre of 1920. As only a small sample of those in the first trial had been bled at a similar interval no direct comparison can be made.

Antibody to Influenza B. Here again the results at 4 weeks were similar to those

receiving adjuvant vaccine in the first trial, both in regard to median titre and in the proportion failing to acquire antibody. At 3 months, however, the median titre had risen to 200 and now only five out of the initial 64 remained without antibody.

Neutralization tests

The low levels of H.I. antibody to influenza B could have been an artifact caused by poor avidity of the virus strain used. This possibility was investigated by performing neutralization tests on a sample of 419 sera from the first trial. The same strain of virus was used as in the H.I. tests.

Of 137 that showed no antibody (< 10) with H.I., with the neutralization test, 97 gave the same result, 12 showed no antibody at the 20 level and 28 showed antibody. In 26 sera found to be positive by H.I., no antibody was shown by the neutralization test.

DISCUSSION

Salk & Laurent (1952) showed that influenza virus antigen in a water-in-oil emulsion with oily adjuvant elicited higher and more persistent levels of antibody than antigen in aqueous suspension. Also, after the adjuvant vaccine, antibody continued increasing a month after inoculation. Hobson *et al.* (1964) disputed this last finding but it is confirmed in the present study.

With influenza A the adjuvant vaccine tested gave results equal to those of the aqueous vaccine, *Invirin*, at a month and far superior at 9 months despite containing only a fifth as much antigen. It has been shown that individuals appear to have a 'ceiling' level of antibody to influenza not affected by further antigenic stimulation (McDonald & Andrews, 1955). This is shown here with *Invirin* but the adjuvant vaccine seems to be able to overcome this, possibly to establish a 'ceiling' at a higher level (Hobson *et al.* 1964).

Low initial levels of antibody to influenza B were a constant feature in the volunteers tested and this was, in the main, confirmed by the neutralization tests. Thus, despite the prevalence of influenza B in Northern Ireland in 1961–2 (Forsyth, 1962) it seems that the vaccine strains represented a primary antigenic stimulus for many of the volunteers. This explains the poor response to influenza B. It is consistent with previous reports on the poor antibody response which occurs against new antigenic variants of influenza virus (Meiklejohn & Bruyn, 1949; Appleby, Himmelweit & Stuart-Harris, 1951; McCarroll & Kilbourne, 1958).

In contrast to the findings with influenza A neither the half dose of *Invirin* nor the adjuvant vaccine gave as good a response of influenza B antibody as the 1·0 ml. dose of *Invirin*. Even in the second trial with *Admune* the median response of antibody to B/England/939/59 was no better a month after inoculation than with the earlier adjuvant vaccine. However, although the amount of influenza B antigen in *Admune* had been increased substantially this was by means of the relatively dissimilar B/Taiwan/4/62 variant.

The poor effect of adjuvant relative to aqueous vaccine at 1 month can be attributed to a delayed response by those volunteers lacking pre-inoculation antibody. On analysis it is seen that the antibody levels elicited by the two vaccines

were similar in volunteers with antibody before inoculation ($0{\cdot}99 > P > 0{\cdot}975$) but different in those without ($0{\cdot}01 > P > 0{\cdot}005$). This difference had disappeared at 9 months. The delayed response did not occur in people given *Invirin* previously.

Throughout these trials the experience with antibody to B/England/939/59 confirms the overwhelming importance of previous experience with the antigen. None of the vaccines used provided high average titres rapidly in people without preinoculation antibody.

SUMMARY

In trials with polyvalent commercial influenza vaccines the antibody responses to oil-adjuvant vaccine persisted longer and were often higher. Antibody conversion was poor after all vaccines and delayed after adjuvant.

We wish to thank Drs A. S. Beare, D. Hobson and H. G. Pereira for supplying strains of virus and for their advice on technical problems.

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