vaccine was, moreover, matched against the best saline vaccine from the standpoint of antigenic content which could be made, and its superiority was thereby abundantly demonstrated.

Salk's observations concerning the breadth of antibody response could not, however, be substantiated so far as one other virus A strain was concerned. Use of adjuvants in influenza vaccines is probably not, therefore, a solution to the problem of the immunological specificity of the inactivated influenza virus vaccine.

The rate of reactions recorded in this trial was considered to be satisfactory and to be low enough to permit exploration of a field trial using oily vaccines. At the same time, the occasional chronic reaction, often discovered only on clinical examination and not causing any grave inconvenience, appears likely to be encountered, but even the alum-containing saline vaccine is not free from such chronic effects.

The antibody response to the emulsified vaccines appears relatively less persistent than had been anticipated. This may have been due to a deficiency of the adjuvant principle contained in the emulsions which were used. The arlacel provided by Dr. Salk was of exceptional purity, but the efficiency of emulsions may be related in part to the content of impurities in the emulsifier. It is therefore possible that an emulsion containing more of these impurities might give a more durable antibody response. The reaction rate with such preparations might, however, be greater than with purer and therefore more easily assimilated materials. More work is clearly desirable on this and other matters connected with adjuvant vaccines.

The comparison of the antibody titres obtained with egg- and mouse-adapted virus vaccines favours the latter. It is, however, difficult to be sure of the significance of the higher antibody levels with mouse-adapted virus. The differences with the two lines are not so striking as in experiments with mice. Since a number of factors such as small antigenic differences and differences in the avidity for antibody may be concerned, it seems wisest to delay interpreting these results and to compare instead the protective value of the two lines of virus. The results of trials of this nature are awaited at the moment.

Summary

A trial of approximately one year's duration was carried out to compare the antibody responses and clinical reactions to three virus A influenza vaccines, the strain A/Eng/1/51 being used as the antigen in each case. Two of the vaccines were of the emulsified type—from mouse-adapted and egg-adapted viruses respectively—and the third—from a mouse-adapted virus—was of the saline type with aluminium phosphate. A total of 399 volunteers in eleven volunteer centres were involved. The emulsified vaccines gave a greater and more sustained antibody response than the saline vaccine in spite of the fact that they contained only one-tenth of the amount of virus antigen. A decrease in the antibody levels found one year after inoculation occurred in all vaccinated groups. No indication of an increased breadth in antibody response to a heterologous virus strain in those given oily vaccines was demonstrated by a small series of tests. The local reactions were more numerous in those given the saline than in those given the oily vaccines, but with the latter vaccines unusually extensive and persistent local reactions occurred in two volunteers.

The Committee wishes to acknowledge the very generous support and co-operation it has received in this trial from many sources. In particular it is anxious to record its special thanks to all the students at the eleven centres who at much personal inconvenience took part in the trial; to the student health officers and their staffs at University College, London, and at Sheffield and Manchester Universities, whose help was invaluable; and, finally, to the medical staffs concerned at St. Bartholomew's, St. George's, Guy's, King's College, London, St. Mary's, Middlesex, and University College Hospitals, who never failed in affording all possible assistance in the organization and carrying out of the trial.

REFERENCES

Freund, J., Jefferson Thomson, K., Hough, H. B., Sommer, H. E., and Pisani, T. M. (1948a). *J. Immunol.*, 60 383.

—— —— Sommer, H. E., Walter, A. W., and Pisani, T. M. (1948b). *Amer. J. trop. Med.*, 28, 1.

Holt, L. B. (1950). *Developments in Diphtheria Prophylaxis*, p. 60. Heinemann, London.

Isaacs, A., and Sampaio, A. A. de C. (1953). *J. Path. Bact.*, 65, 613.

Medical Research Council Committee on Clinical Trials of Influenza Vaccine (1953). *British Medical Journal*, 2, 1173.

Philip, R. N., Bell, J. A., Davis, D. J., Beem, M. O., and Beigelman, P. M. (1954). *Amer. J. Publ. Hlth.*, 44, 34.

Salk, J. E. (1953). *J. Amer. med. Ass.*, 151, 1169.

—— Bailey, M. L., and Laurent, A. M. (1952). *Amer. J. Hyg.*, 55, 439.

—— and Laurent, A. M. (1952). *J. exp. Med.*, 95, 429.

Stanley, W. M. (1944). Ibid., 79, 255.

World Health Organization (1953). Technical Report Series, No. 64.

DIAGNOSTIC METHODS IN AN INFLUENZA VACCINE TRIAL*

BY

J. C. McDONALD, M.D., D.P.H., D.I.H.

AND

B. E. ANDREWS, M.R.C.S., L.R.C.P., Dip.Bact.

From the Epidemiological Research and Virus Reference Laboratories, Central Public Health Laboratory, Colindale, London, N.W.9

Large-scale field trials of a polyvalent influenza virus A vaccine were conducted by the Medical Research Council Committee on Clinical Trials of Influenza Vaccine during the winter of 1952–3 (Report, 1953). In these trials the attack rate of clinical influenza was 3% in 6,340 volunteers who received the virus vaccine and 4.9% in 6,370 who received a control bacterial vaccine. It therefore seemed clear that the vaccine had a protective effect. The method of diagnosis used in these trials was entirely clinical, unaided by laboratory tests. The Committee chose the method deliberately because of the practical difficulty in obtaining and testing blood samples and throat washings from sick volunteers in trial centres scattered throughout the United Kingdom, and because it was doubted whether serological methods were valid for comparing the influenza attack rates in two groups of persons, one of which had been vaccinated with influenza virus. The Committee agreed that a small-scale trial using the same vaccines should be carried out simultaneously within the main scheme to study the practicability of basing diagnoses on both clinical and laboratory methods, and to compare results of serological tests in the vaccinated and control groups. This report describes a trial carried out in Vauxhall Motor Works, Luton, with these aims in view.

Organization of the Trial

Early in October, 1952, the firm was asked whether facilities could be given for an influenza vaccine trial which would include the home visiting of the vaccinated volunteers when they were away from work, and the taking of specimens where indicated for laboratory examination. The management and chief medical officer agreed, but thought that the works council, representing the employees, should be consulted about the home inquiry. The proposed scheme was therefore brought before the works council. It was explained that the home visits would not be made by one of the firm's doctors and that the findings on any individual person would not be disclosed to the employers. The works council approved the scheme, and a circular

*A report to the Medical Research Council Committee on Clinical Trials of Influenza Vaccine.

letter was sent to all employees asking for volunteers. The scheme was discussed with the medical officer of health of Luton and with general practitioners at a meeting of the local branch of the British Medical Association. As soon as the names of volunteers were known, an explanatory letter was sent to all doctors with patients who had offered to take part.

Five hundred and five persons came forward, rather fewer than had been hoped for. All were interviewed, and 16 excluded because of a past history of tuberculosis or sensitivity to eggs, or at the request of their own doctor. Thirty-five volunteers failed to attend for inoculation, and six of those who attended were not injected because they did not appear to be fit. In all, 458 were inoculated, but as three left the firm during the follow-up period complete records are available for only 455, 226 of whom received the trial vaccine and 229 the control.

Vaccines and Inoculation Procedure

Precisely the same vaccines and inoculation procedure were used as in the main trial. The trial vaccine contained equal proportions of threefold concentrations of the F.M.1 strain and the egg-adapted Liverpool (1951) strain. Each dose of 1 ml. contained 9,600 haemagglutinating units of the F.M.1 strain and 12,000 units of the Liverpool (1951) strain adsorbed on to 10 mg. of aluminium phosphate. The control vaccine was a low-potency bacterial vaccine of the anticatarrhal type. Each dose of 1 ml. contained 12.5 million *H. influenzae*, 25 million pneumococci, and 2.5 million streptococci, in addition to 10 mg. of aluminium phosphate and merthiolate to a final concentration of 1 in 10,000.

The vaccines were given on December 8, 9, and 10 by a single deep subcutaneous injection into the left upper arm. The vaccine chosen for each volunteer was determined by use of inoculation registers, one for each sex, in which the random sequence in which the two vaccines were to be given had been set out.

Reactions

All those inoculated were seen 48 hours later by one of us and were asked about local and general reactions. These were assessed by their severity and recorded as nil, slight, and insufficient to interfere with normal activities, moderate and interfering with normal activity, or severe and causing absence from work. Most of the inoculated had a slight local reaction, but no local or general reaction was severe enough to cause absence from work. The incidence and severity of reactions still present 48 hours after inoculation are shown in Table I.

TABLE I.—*Percentage of Persons in Whom Reactions were Observed*

Severity	Local		General	
	Trial Vaccine	Control Vaccine	Trial Vaccine	Control Vaccine
Nil	33%	25%	89%	88%
Slight	66%	73%	10%	11%
Moderate ..	0%	2%	1%	1%
Severe	0%	0%	0%	0%

At an interview during the second week in March—that is, three months after inoculation—six persons mentioned that they still had a lump at the site of injection. In each case inspection showed a round indurated subcutaneous swelling about an inch (2.5 cm.) in diameter, and in one the swelling was acutely inflamed. As this information was volunteered and not obtained by inquiry it seems possible that others may have had similar reactions.

The Follow-up

Those inoculated were observed for 12 weeks, from December 15 to March 6. Each day the records department prepared a list of persons in the study who were absent from work, excluding those known to be absent for non-medical reasons. All those on the list were visited by one of us the same day and the cause of absence with details of any illness was recorded. Of 217 persons so visited 59 were not found at home, but a satisfactory explanation for 36 of these was given by another member of the household. A blood sample and throat washings were taken from any person who was thought possibly to have influenza, and from every fourth person with any other respiratory illness. A second specimen of blood was taken between 19 and 24 days later.

At the end of the trial the volunteers' attendance record cards were examined and a note was made of the date and duration of all absences of half a day or more. It was discovered that in about a quarter of the absences the volunteer's name had not appeared on a daily list and as a result they had not been visited during their illness.

Paired samples of serum were tested for complement-fixing antibody to the soluble antigens of influenza viruses A and B, and also for haemagglutination-inhibiting (H.I.) antibody to the two virus strains incorporated in the vaccine (Liverpool (1951) egg-adapted, and F.M.1), a 1953 (A/Eng/1/53) virus strain, and a strain of influenza virus C. A fourfold or greater rise in antibody was regarded as positive evidence of infection with influenza virus. Virus isolation from garglings was attempted by the amniotic inoculation of fertile hens' eggs.

The Epidemic

Cases of clinical influenza in volunteers were seen between the weeks ending January 3 and February 28, 1953. All but one of the illnesses in which serological evidence of influenza was obtained had their onset between weeks ending January 24 and February 28. The single exception was in a volunteer who was inoculated with the trial vaccine on December 9 and who developed symptoms of a common cold six days later, on December 15. Blood specimens were taken from him on December 16 and 30, and it seems probable that the rise in antibody observed was due to the vaccine rather than to natural infection. All cases diagnosed as influenza serologically were due to virus A, except one which was positive for virus C infection. After the usual fluctuations around Christmas and the New Year the number of claims for sickness benefit received by the Luton office of the Ministry of Pensions and National Insurance began to rise in the week ended January 27, reached a peak in mid-February, and then fell away. There is thus general agreement that the epidemic period extended from the middle of January to the end of February—that is, 7 to 12 weeks after inoculation—and this is illustrated in the accompanying Chart.

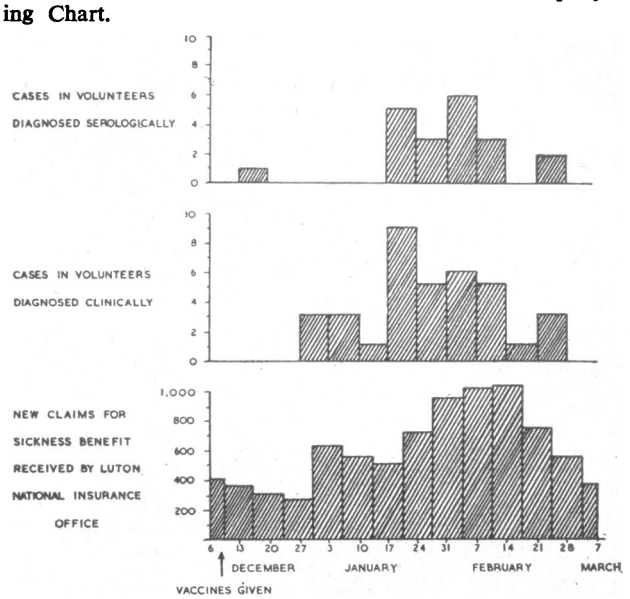

Chart showing evidence of influenza during follow-up period.

Results

Eighty-two cases of acute respiratory illness causing absence from work were found in the course of the home-visiting. A clinical diagnosis of influenza was made in 36 of these, and throat washings were obtained from all and paired sera from all but one. Paired sera and throat washings were also obtained from 21 persons in whom the diagnosis of influenza was considered possible but improbable and from 6 out of 25 thought to be suffering from a cold or sore throat. Thus paired sera were obtained from 62 persons and throat washings from 63. Seventeen pairs of sera were positive when tested for haemagglutination-inhibition with a current 1953 influenza virus A strain, and 12 pairs with each of the strains of virus used in the vaccine. Twelve positive results were also obtained by complement-fixation test, using virus A soluble antigen. In all, 20 pairs of sera gave a positive result in one or more tests for virus A infection; five were positive in all four tests, eight in three tests, two in two tests, and five in one test only. All serum pairs were negative in tests for influenza virus B infection (soluble antigen C.F.T.), but one pair from a clinically typical case of influenza was positive for influenza virus C infection (H.I. test). Influenza virus A was isolated from four cases diagnosed clinically and serologically as influenza. One of the four had received the trial vaccine, and it is of interest that the only serological test positive in this case was the H.I. test using the current strain.

Of the 36 clinical cases of influenza 15 had received the trial vaccine and 21 the control vaccine, absence rates being 6.6% of 226 and 9.2% of 229 respectively. Though the difference between these rates might have been due to chance (difference ÷ standard error = 2.6/2.5 = 1.0), it is compatible with that observed in the main trial, where, because of the much larger numbers involved, the corresponding rates, 3% and 4.9%, were found to be statistically significant. In the main trial these figures amounted to a 40% reduction in absences due to influenza in the trial group, and in the Luton trial to a 30% reduction.

Serological evidence of influenza virus A infection was obtained in 16 of the 35 clinical cases tested, but in Table II it may be seen that, whereas 16 out of 34 pairs (47%) taken from persons who received the control vaccine were positive, only 4 out of 28 (14%) who had received the trial vaccine were positive. Serological evidence of influenza was thus found in 4 out of 226 persons in the trial group, and in 16 out of 229 persons in the control group, rates of 1.8% and 7% respectively. The difference between these rates is appreciably greater than that observed between the absence rates due to clinical influenza, and is statistically significant at the 5% level (difference ÷ standard error = 5.2/1.4 = 3.7). However, as pointed out below, the validity of this estimate of the protective value of the vaccine depends on the interpretation of serological results in vaccinated persons.

TABLE II.—*Serological Results in Relation to Clinical Diagnosis*

Clinical Diagnosis	Vaccine	Absences	No. Tested	No. Positive*	% Positive
Probably influenza	Trial	15	15	3	20%
	Control	21	20	13	65%
Probably not influenza	Trial	26	13	1	8%
	Control	20	14	3	21%
Total respiratory illness	Trial	41	28	4	14%
	Control	41	34	16	47%

* Number of persons showing a fourfold or greater rise in antibody in at least one serological test.

Discussion

Experience showed that, on the scale employed in this field trial, the method was practicable. It could be improved by taking steps to ensure more complete notification of absences from work, and to learn of short illnesses which occur during week-ends and so do not cause absence on a working day. Its application on a scale large enough for evaluation of a vaccine adequately would be expensive in the number of field workers required and would entail a great deal of laboratory work. The cost might be justified if greater precision was thereby obtained than in the main studies in which reliance was placed on clinical diagnosis. As the figures presented are small the difference observed between the clinical and the serological assessment cannot be accepted without further evidence, but, if confirmed, there would appear to be two possible explanations for it. If the serological method was accurate and unbiased a clinical diagnosis of influenza must have been applied to many illnesses which were not influenza. Dilution of the group of clinical influenzas with illness due to other causes probably took place, because, even in the control group, only two-thirds of illnesses so diagnosed were confirmed serologically, whereas the methods used might be expected to confirm 80 to 90% of cases of virus influenza. Alternatively, serological tests may be less able to detect evidence of influenza virus infection in vaccinated than in unvaccinated persons.

The antibody rise resulting from a single injection of influenza virus vaccine into adults is probably not a primary response, since most adults would previously have encountered a closely related antigen as the result of natural infection with the virus. The raised level of H.I. antibody observed after immunization is generally accepted as an indication of reduced susceptibility to infection; but if infection does occur in persons exhibiting this secondary response it may not be capable of provoking a further fourfold rise in antibody titre.

The level of H.I. antibody in acute-stage sera was considerably higher in the trial group than in the control group, the difference between geometric mean titres being approximately fivefold when the current 1953 virus strain was used as antigen and tenfold when the strains present in the vaccine were used. Further evidence of this difference is shown in Table III, where it may be seen that 33 out of 34 acute-stage sera from controls had a H.I. titre to the 1953 virus strain of less than 1/10, whereas 20 out of 28 acute stage sera from those who had received the trial vaccine were 1/10 or higher. Sixteen out of 17 volunteers found positive by this test had an initial titre of less than 1/10, and that of the remaining positive case was 1/10. H.I. tests using the other two virus strains as antigen gave very similar results. Comparison of findings on acute- and convalescent-stage sera by the same tests showed that the acute-stage antibody levels in the trial group were approximately equal to those found in the convalescent stage in the controls. Thus the vaccine provoked antibody levels of the same order as those resulting from natural infection. Though one conclusion is that persons with an antibody titre above a given level are not susceptible to infection, it may also be that serological tests are less able to detect it when it occurs.

TABLE III.—*Serological Diagnoses of Influenza in Relation to Antibody Level in Acute-stage Sera (Based on Haemagglutination-inhibition Tests Using a 1953 Virus A Strain as Antigen)*

Antibody Level in Acute-Stage Sera	Trial Group		Control Group	
	No. of Illnesses	No. Positive	No. of Illnesses	No. Positive
Titre less than 1/10	8	3	33	13
,, 1/10 or higher	20	1	1	0
Total No. tested ..	28	4	34	13

Wiener, Henle, and Henle (1946) observed that a rise in antibody to soluble antigen was detected only occasionally with the batches of vaccine they were using. Kirber and Henle (1950) later suggested that the soluble antigen complement-fixation test should therefore be of value for the diagnosis of influenza in a recently vaccinated population. We were unable to confirm that injection with the vaccine used failed to cause an increase in antibody to soluble antigen. Table IV shows antibody levels to soluble antigen in acute-stage sera taken from volunteers in control and trial groups, and in convalescent sera from the 16 cases

TABLE IV.—*Distribution of Antibody Titres to Influenza Virus A Soluble Antigen*

	No. of Sera having Titres of:					Total Sera	Percentage 1/2 or Less	Percentage 1/8 or More
	1/2 or Less	1/4	1/8	1/16	1/32			
Trial group: Acute-stage sera	0	8	15	5	0	28	*0*	*71*
Control group: Acute-stage sera	19	7	6	1	1	34	*56*	*24*
Convalescent sera from confirmed cases of influenza A ..	0	4	5	7	0	16	*0*	*75*

confirmed as influenza A by at least one serological test in the control group. In the control group 56% of acute-stage sera had titres of 1/2 or less, whereas in the trial group all had a titre of at least 1/4, and 71% had a titre of 1/8 or higher. Furthermore, as was found with the H.I. tests, the distribution of titres of acute-stage sera in the trial group was very similar to that for convalescent sera from the confirmed cases of influenza A in the control group. Titres to influenza B soluble antigen did not differ in the control and trial groups, which suggests that the differences with influenza A soluble antigen were due to specific antibody. It seems improbable, therefore, that the soluble antigen complement-fixation test has any advantage over the H.I. test for the diagnosis of influenza in persons recently vaccinated with vaccine of the type used in this trial.

The main disadvantage of using clinical methods unaided by laboratory tests in the assessment of an influenza vaccine is that the proportion of illnesses correctly diagnosed as influenza is unknown, and so the degree of protection conferred cannot be determined. If the proportion of cases of true influenza to the total number of clinically diagnosed cases is low the resulting dilution may obscure the protective effect even of a good vaccine. This is particularly undesirable in the experimental stages of the development of a vaccine, when quite small differences may have considerable significance. However, it is essential that a trial method should be free from observer bias, and the clinical method in a well-designed trial has this virtue, whereas the serological method is open to question. Unless it can be shown that the chance of obtaining a detectable fourfold rise in antibody following natural infection is unrelated to the initial antibody level it would appear unwise to rely on serological methods only in any estimate of protection.

Perhaps the most important contribution which the laboratory can make to the precision of a large-scale field trial at the present time is in determining the duration and type of any influenza outbreak in communities in which the inoculated volunteers live or work. Periods in which there is no detectable virus influenza can then be excluded from the final analysis. Furthermore, knowledge of the proportion of influenzal to non-influenzal illnesses in the control group during the epidemic period would help in the interpretation of protection rates calculated from clinical diagnoses alone.

Summary

In a small-scale influenza vaccine trial undertaken mainly to determine the practicability of a trial method using both clinical and laboratory tests in the diagnosis of influenza, results led to the conclusion that the assessment of a vaccine on purely clinical grounds might understate the degree of protection, whereas one based on serological findings might exaggerate it. Possible reasons for this difference are discussed. It is concluded that, until it is shown that serological methods are able to detect cases of influenza equally well in persons who have received an influenza vaccine and in those who have not, serological diagnosis should not be the only method of diagnosis used in a vaccine field trial. In a clinical assessment the fullest use should be made of laboratory evidence to define the epidemic period in the communities concerned and to estimate the proportion of illness in the control group due to influenza virus infection. This information would enable dilution of the figures by non-influenzal illness to be reduced and should allow a more accurate assessment of the vaccine to be made.

We should like to thank the management of Vauxhall Motors Ltd. for giving facilities for this trial, and their employees who volunteered to be immunized and who tolerated blood and other tests with such good humour. We are especially grateful to the firm's medical officers, Dr. A. R. Thomson and Dr. P. M. Bennett, for their great help in finding and interviewing volunteers and giving inoculations; to Mr. Humby, of the factory records department, for his daily list of absentees; and to Dr. J. H. C. Walker, Director of the Luton Public Health Laboratory, for separating sera and storing specimens. We are indebted to Dr. R. M. Dykes, Medical Officer of Health, Borough of Luton, for his help and encouragement in the organization of this investigation.

REFERENCES

Kirber, M. W., and Henle, W. (1950). *J. Immunol.*, 65, 229.
Report to the Medical Research Council by its Committee on Clinical Trials of Influenza Vaccine (1953). *British Medical Journal*, 2, 1173.
Wiener, M., Henle, W., and Henle, G. (1946). *J. exp. Med.*, 83, 259.

A FOLLOW-UP TUBERCULIN SURVEY IN THE RHONDDA FACH

BY

T. FRANCIS JARMAN, M.D.
Medical Director, Mass Radiography Service, Welsh Regional Hospital Board

Tuberculin-testing of the school population of the Rhondda Fach was first undertaken in 1950–1 as an integral part of the total community *x*-ray survey carried out in this mining valley during that period (Cochrane *et al.*, 1952). The object of the first survey was to ascertain the prevalence of cases of pulmonary tuberculosis in the valley, whether already known or as yet undiscovered, and to isolate and treat all active and infectious cases. The object of the second survey was to determine the effect of the first survey on the tuberculosis situation in the valley (Cochrane *et al.*, 1955). It was thought that tuberculin-testing of the school population should provide evidence on the existing degree of total tuberculous infectivity in 1950–1 and that retesting in 1953 might reflect changes in this situation resulting from the efforts made during the first *x*-ray survey of the valley. A sample of schoolchildren in another "control" valley were also tested in 1951, and a similar sample of children from the same schools were retested in 1954. This retesting has been combined with a follow-up study of children who were tuberculin-tested on both occasions, in the hope that a determination of the conversion and reversion rates in the two valleys might be compared.

Method of Testing

In order that the results of tuberculin-testing in 1953–4 should be comparable with those obtained in 1950–1, the same Mantoux technique was employed on both occasions—namely, 0.1 ml. of a 1/10,000 dilution of standardized O.T.—that is, 1 T.U.—was injected intradermally, and all negative reactors were retested with 0.1 ml. of a 1/100 dilution—that is, 100 T.U. The criterion adopted for the reading of these tests was that any reaction producing less than 5 mm. of induration, read at 48 hours after the injection, was regarded as negative. I administered and read the tests, as