evidence of enlargement of the main pulmonary artery were assessed in all.

We attempted to assess changes in the peripheral pulmonary arterial branches as recommended by Davies, Goodwin, Steiner, and Van Leuven (1953), but found in many cases this was too difficult to determine with sufficient certainty.

The three criteria we have used (pulmonary hypertension, E.C.G. signs of right ventricular hypertrophy, and enlargement of the pulmonary artery) as a reflection of the pulmonary vascular resistance are not of course exact, but, accepting the inevitable arbitrary grading, we have divided our patients into four grades of presumptive severity: nil, mild, moderate, and severe (Table XXIX).

TABLE XXIX.—*Pulmonary Vascular Resistance and Results at Six Years*

Raised Pulmonary Vascular Resistance*	No. of Cases	Long-term Results					
		Good		Fair		Poor	
		No.	%	No.	%	No.	%
Nil	35	21	60	10	29	4	11
Mild	26	17	65	7	26	2	8
Moderate ..	81	55	68	20	25	6	7
Severe	43	33	70	5	12	8	19

* See text.

Pulmonary hypertension was graded into four degrees depending on the mean pressure at rest and on exercise. Enlargement of the pulmonary artery was graded into four degrees as judged on a postero-anterior radiograph. Electrocardiographic criteria for the diagnosis of right ventricular hypertrophy in rheumatic heart disease and the difficulties of grading in degrees of severity have previously been fully discussed (Fraser and Turner, 1955a). It will be seen that there is no significant difference between the four groups.

Associated Pulmonary Disease

Bronchitis and sometimes chronic bronchitis and emphysema are not infrequently associated with mitral valvular disease, and the differentiation of the parts played by each as regards dyspnoea is not always easy to distinguish.

Bronchitis in the form of cough productive of mucoid sputum may be a manifestation of pulmonary oedema, and in any case by itself appears often to be improved by valvotomy. The real problem is that of emphysema. The diagnosis of emphysema by clinical means and radiography is often difficult or impossible and in the presence of rheumatic heart disease cannot be suspected from the history as might otherwise be the case. Pulmonary function tests are often useful and the results taken in conjunction with those of cardiac catheterization will usually indicate when symptoms are due to severe mitral stenosis.

[The conclusion of this article, together with a list of references, will appear in our next issue.]

The American Physiological Society has acquired ownership of the *Journal of Neurophysiology*, which was owned and operated from 1937 by the late Dr. J. F. Fulton and Charles C. Thomas, publisher, until the former's death in 1960. Dr. Fulton's one-half interest was left to Yale University, and the transfer has been effected by agreement between the society and the organizations concerned. (*J. Neurophysiol.*, January, 1962.)

TRIALS OF LIVE INFLUENZA VACCINE IN THE ROYAL AIR FORCE

A REPORT TO THE MEDICAL RESEARCH COUNCIL COMMITTEE ON INFLUENZA AND OTHER RESPIRATORY VIRUS VACCINES

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Formalinized influenza virus vaccine administered by subcutaneous injection is generally used in this country and the U.S.A. to immunize against influenza. Live influenza virus inoculated direct into the upper respiratory tract has been used extensively in the U.S.S.R. for this purpose (Smorodintsev, Drobyshevskaya, Ostrovskaya, and Shishkina, 1937; Smorodintsev, Tushinsky, Drobyshevskaya, Korovin, and Osetroff, 1937; Chalkina, 1938; and others). A number of trials of live vaccine were conducted by Burnet (1942, 1943), Mawson and Swan (1943), and Bull and Burnet (1943), but their vaccinated volunteers could still be infected experimentally, and they felt that the method was unsatisfactory.

Russian workers have used vaccine strains capable of multiplying in the nose and upper respiratory tract but causing few or no symptoms; they have selected virus strains obtained either by serial passage in the chick allantois or by passage through human embryonic lung tissue followed by culture in chick embryos. This vaccine in a strength of 10^6 50% egg-infective doses (EID_{50}) or more per ml. was introduced into the nose either by spraying or as drops, but they found that spraying gave better results.

Zhdanov and Solov'ev (1952) and Smorodintsev and Zhdanov (1957) stated what they believed to be the necessary conditions for the successful use of live attenuated influenza virus vaccine. These were: (1) that the vaccine should contain $10^6 EID_{50}$ or more per ml.; (2) that the virus could be recovered for two to four days from 50% or more of vaccinated subjects who were without antibody to the vaccine strain at the time of inoculation; (3) that there should be a fourfold or greater rise in haemagglutination-inhibiting (H.I.) antibodies in 50% or more of susceptible vaccinated subjects; and (4) that vaccine reinoculated two to three weeks later should fail to survive. It was stated that, up to 1960, vaccine meeting these requirements led to a reduction in morbidity rate to between a third and a half that in unvaccinated populations; furthermore, protection was obtained in the face of epidemic influenza (Smorodintsev, 1960).

In this country Isaacs and Roden (1956) and Isaacs, Negroni, and Tyrrell (1957) inoculated volunteers intranasally or by throat-swabbing with small doses ($10^3 EID_{50}$) of live influenza virus grown in eggs. In the first study, using A1 strains, they were unable to

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infect the volunteers and no antibody was produced. In the second series of experiments with A2 strains virus was recovered from a small proportion of the volunteers inoculated. Those who were successfully infected had symptoms of influenza and showed a rise in complement-fixing antibody. Meiklejohn (1960) reported his observations on the use of larger doses of a vaccine prepared from the Russian Iksha influenza A2 strain on a small group of volunteers in the United States. He concluded that the Russian vaccine, given intranasally, was innocuous but not very effective in producing H.I. antibody, but he remarked that he had not complied with all the criteria set out by Smorodintsev and Zhdanov.

As the properties of live vaccine still did not seem to have been fully examined in this country, it was decided to investigate in a small number of volunteers the immunological and clinical effects of the nasal instillation of a vaccine prepared from the Russian Iksha strain. A small R.A.F. unit in Lincolnshire was chosen, the nature of the investigation was explained to members of the unit, and volunteers were sought in September, 1960, for inoculation in the following month.

In January, 1961, after completion of the preliminary trial, there was evidence of an epidemic of influenza A at an R.A.F. recruit camp, and small outbreaks were reported from two other units. The opportunity was taken to conduct a field trial with the same vaccine in these three stations. The outbreaks proved abortive and no information on the protective effect of the vaccine was obtained, but we were able to observe the frequency of reactions.

Preparation of Vaccine and Preliminary Tests

The Iksha strain of influenza A2 virus was obtained from Professor V. M. Zhdanov, of the Ivanovsky Institute, Moscow, as freeze-dried allantoic fluid of the fourth passage in eggs. It was passed twice at high dilution in the allantoic cavity of 10-day chick embryos, and the fluids of the sixth passage were pooled and stored in ampoules at −65° C. The identity of the virus was confirmed by H.I. tests and neutralization tests with ferret immune serum. The fluid was tested by aerobic and anaerobic bacterial culture and by inoculation into mice, rabbits, and guinea-pigs; no evidence was found of contaminating viruses or bacteria. The haemagglutinating titre of the fluid was 1/1,280 and the egg-infectivity titre 10^9/ml. This live virus vaccine was administered as nasal drops to nine human volunteers living in strict isolation at the Common Cold Research Unit, Salisbury; three received 10^6 and six received 10^7EID_{50}. None showed symptoms during the six days following inoculation. Virus was recovered from the throat of one of the six who had received 10^7EID_{50}.

Trial 1

Blood specimens from 128 volunteers from R.A.F. Digby were screened for neutralizing antibody to influenza A2 (see below). Twenty-one (16%) were found without antibody at a serum dilution of 1 in 10; 20 of these, together with 31 men with antibody at this level, were included in the trial. The volunteers were divided into three groups each containing some men with antibody and some without. Vaccine was given to each volunteer on two occasions at intervals of four weeks, with the exception of five men who were for a variety of reasons not available for the second inoculation. At the time of the second inoculation 13 further volunteers—11 with antibody and two without, selected by screening another 20 men—were given vaccine for the first time to act as a control on its potency. In all, therefore, 64 men were given vaccine and 22 (34%) of these were without neutralizing antibody. Subsequent tests showed that 42 (66%) of the 64 men had H.I. antibody at a titre of less than 1 in 10. The vaccination programme is shown in Table I.

The vaccine was stored in an electric refrigerator at −70° C. until transported to the station on solid carbon dioxide. The vaccine was diluted with 0.5% gelatin in Hanks's solution adjusted to about *p*H 7.2 with sodium bicarbonate immediately before use and kept at 4° C. until used; this period was never longer than 30 minutes. The dose employed for the first inoculation of the 12 men in group 1 was 10^6EID_{50} in 1 ml., but a higher dose, 10^7EID_{50}, was used both for the second inoculation of the first group and for all other inoculations. The vaccine was instilled into the nose with a dropper—0.5 ml into each nostril. The volunteer lay with his head overhanging the edge of the examination couch during the instillation, and remained there for one minute afterwards.

The volunteers were seen daily, at the same time each morning, for four days after both inoculation and reinoculation, and a record was kept of any symptoms and signs. During this period of observation they remained within the confines of the station. Throat swabs, taken daily for four days from each man, were broken off into 5-ml. bottles containing 0.5% lact-albumin in Hanks's balanced salt solution with 0.02% sodium bicarbonate added. The bottles were stored on solid carbon dioxide before and during transportation to the laboratory at the end of each week. Blood samples were obtained two weeks after the first instillation and immediately before the second instillation; a final specimen was collected two weeks later. As the 13 controls were not given a second inoculation they were bled only two and four weeks after the first. The blood specimens were stored at 4° C. until taken to the laboratory for separation of the serum.

Throat swabs from contacts were obtained from men sleeping in the billet in the beds on either side of the inoculated volunteers. These specimens were taken four days after the first and second instillations of the vaccine, and at the same time the contacts were asked if they had any symptoms. Finally, from October, 1960, until the middle of January, 1961, a clinical record was kept of all other personnel on the station reporting a febrile respiratory illness—five in all—and specimens were taken from each of them.

TABLE I.—*Trial I. Vaccination Programme*

Group	Date Inoculated	Dose (EID_{50}/ml.)	No. Inoculated: With Neutralizing Antibody	No. Inoculated: Without Neutralizing Antibody	Controls Inoculated: With Neutralizing Antibody	Controls Inoculated: Without Neutralizing Antibody	Contacts
1	Oct. 24	10^6	6	6	—	—	15
	Nov. 21	10^7	6	4	2	1	11
2	Oct. 31	10^7	8	8	—	—	19
	Nov. 28	10^7	8	8	4	0	19
3	Nov. 7	10^7	17	6	—	—	25
	Dec. 5	10^7	15	5	5	1	9
Total	First inoculation	All	31	20	—	—	59
	Reinoculation	,,	29	17	11	2	39

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Trial 2

In January, 1961, with the appearance of outbreaks of influenza A, volunteers for a controlled trial were sought at three R.A.F. units—Bridgnorth, Wroughton Hospital, and Northolt. Large quantities of the same batch of vaccine used at R.A.F. Digby had been stored in ampoules at −70° C. at Colindale, and were ready for use. The dose of the vaccine administered was $10^7 EID_{50}$, given intranasally with a dropper in the same manner as at Digby. Controls received 1 ml. of the diluent—that is, 0.5% gelatin in Hanks's solution adjusted at about *p*H 7.2 with sodium bicarbonate—but in other respects the same method of inoculation was used.

The names of the volunteers were entered in their order of attendance on an inoculation register, showing the type of vaccine that was to be given and a serial number for entry on the personal record card employed for the follow-up. The recipients were not told which preparation had been given, and those responsible for the follow-up knew only the register serial number.

A total of 1,026 persons were inoculated, 513 with the virus vaccine and 513 with the diluent only. At Bridgnorth 392 recruits were inoculated, 266 within two hours of their arrival on January 20 and 27, and the remaining 126 48 hours after their arrival one week later. At Wroughton Hospital 437 men and women of all ages were inoculated in two sessions on February 2 and 10, and at Northolt a further 197 volunteers, including some women, were inoculated on February 7.

A personal record card was prepared for each volunteer, entering identifying information, reactions, and illnesses. Arrangements were made to inspect all volunteers 48 hours after inoculation, when any symptoms and signs that had occurred were noted. It was impossible to inspect a few members of the night nursing and auxiliary staff at Wroughton Hospital who were off duty at the time, but they were all interviewed later. In addition, an illness record card was completed for any person with respiratory illness who required admission to sick quarters. Each station was visited approximately once every 10 days until the end of March, to see the record cards and to collect specimens for laboratory examination from any patients who had recently been admitted to sick quarters because of a respiratory illness.

Laboratory Procedures

The techniques used for isolating influenza virus and for estimating antibody in serum specimens were as follows.

Virus Isolation.—Fluid from bottles containing throat swabs was treated with antibiotics and inoculated in amounts of 0.1 ml. into the amniotic cavity of 10-day fertile hens' eggs. After 72 hours' incubation at 35° C. the eggs were kept overnight at 4° C. and the amniotic fluids were harvested, tested, and stored. On completion of the first passage of throat swabs all the serum specimens from each man were tested simultaneously, first for H.I. antibody and then for neutralizing and complement-fixing (C.F.) antibodies. If a serum showed a fourfold or greater rise in titre in any test and virus had not been isolated, the relevant amniotic fluids were given a second passage in eggs, but no further strain was isolated in this way.

Neutralization Test.—This test (Pereira, 1958) was based on the haemadsorption phenomenon of Vogel and Shelokov (1957) A standard strain of A2 virus, A/Singapore/1/57, was titrated in duplicate in tubes containing monkey-kidney-tissue culture maintained in medium 199 without serum. After 48 hours' incubation the tubes were aspirated and the cultures washed with fresh medium 199; 0.2 ml. of a 0.4% solution of guinea-pig erythrocytes was inoculated into each tube, which was then sloped for 20 minutes at room temperature. The highest dilution with microscopically detectable haemadsorption was recorded as one haem-adsorption dose (1 H.D.).

The serum specimens were inactivated at 56° C. for 30 minutes and doubling dilutions prepared in tissue-culture medium. To 1 ml. of each dilution was added an equal quantity of medium 199 containing 200 H.D. of virus per ml., giving a final dilution of 100 H.D. per test. The mixtures were allowed to stand on a bench for one hour and were inoculated in 1 ml. quantities into each of two monkey-kidney tubes, previously emptied of culture medium. After rolling at 37° C. for a further 60 minutes, the fluid was poured off and replaced by fresh medium. Tubes were then incubated on rollers for 48 hours and tested for haemadsorption as already described. The neutralizing titre was the highest dilution with complete absence of microscopic haemadsorption. In recording titres the diluting factor of the virus suspension was taken into account.

The virus strain A2/Singapore/1/57 was considered to be preferable to Iksha as the testing agent, as the latter is a "Q"-phase or non-avid virus and is unsuitable for the standard H.I. test. It was thought desirable to use the same virus for H.I. and neutralization tests.

Haemagglutination-inhibition (H.I.) Test.—This was performed by the method described in the W.H.O. Technical Report Series (1959). Sera were treated with *Vibrio cholerae* filtrate (Van der Veen and Mulder, 1950). Doubling dilutions of treated serum were prepared in 0.25 ml. volumes in plastic plates, to which were added equal volumes of 0.5% fowl cells standardized colorimetrically. After the addition of a like volume of eight haemagglutinating doses of virus in saline, the plates were allowed to stand at room temperature for 50 minutes. The dilution of serum giving 50% haemagglutination was recorded as the H.I. titre.

Complement-fixation (C.F.) Test.—Doubling dilutions of serum in saline in 0.1 ml. quantities were prepared in W.H.O. plastic plates. Equal volumes of complement (2 M.H.D.) and influenza virus A soluble antigen (optimum dilution) were added. After overnight fixation at 4° C. the plates were removed to the incubator and two volumes of 1% sensitized sheep cells were added. These were kept at 37° C. for 30 minutes with occasional shaking and then at 4° C. in a refrigerator for two hours before being read. The highest dilution with no trace of haemolysis was recorded as the complement-fixing titre.

All the serological tests were performed and the findings recorded without reference to the results of attempted virus isolation.

Results in Trial 1

Virus Isolation

The results of tests on swabs taken up to four days after the first instillation of vaccine are shown in Table II. Virus was isolated from one or more specimens in 5/42 (12%) men with neutralizing antibody and from

TABLE II.—*Virus Isolation Test Results After First Inoculation*

Days after Inoculation	Volunteers with Neutralizing Antibody			Volunteers without Neutralizing Antibody		
	$10^6 EID_{50}$	$10^7 EID_{50}$	Both Doses	$10^6 EID_{50}$	$10^7 EID_{50}$	Both Doses
1	0/6	1/36	1/42	1/6	7/16	8/22
2	0/6	1/36	1 42	1/6	6/16	7/22
3	1/6	3/36	4 42	1/6	5/16	6/22
4	0/6	0/36	0/42	0/6	3/16	3/22
At any time	1/6	4/36	5/42 (12%)	3/6	9/16	12/22 (55%)

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12/22 (55%) men without. Included in these figures are the two controls without antibody from one of whom virus was isolated, and the 11 controls with antibody, in all of whom the tests were negative. The difference between these proportions is statistically significant at the 5% level $\left(\frac{\text{difference}}{\text{standard error}} = \frac{43}{11.6} = 3.7\right)$. Though the numbers are small there also appeared to be a difference between the two groups in the time after inoculation when virus strains were most likely to be recovered; those without antibody showed falling proportions from day 1 to day 4, whereas those with antibody had the highest proportion on day 3. Virus was isolated from the same proportion of volunteers whether high or low dosage was given, but when the higher dose was used virus strains were recovered from more specimens per man. But once again numbers were too small to be sure of this.

The virus isolation results may also be related to the H.I. antibody status of the volunteers before inoculation. There were 42 men with an H.I. antibody titre of less than 1 in 10, and virus was isolated from 15 (36%) of them; there were 22 men with antibody above this level, and virus was isolated from two (9%) of them. This difference was also statistically significant $\left(\frac{\text{difference}}{\text{standard error}} = \frac{27}{9.6} = 2.8\right)$.

Of the 51 volunteers, not counting controls, who were given a first inoculation 46 were reinoculated four weeks later (Table I). No virus strain was recovered from the 184 throat swabs taken during the next four days from these men.

Serology

The main findings are presented in Tables III and IV and comparison of results with the two doses of vaccine in Table V. In all these tables the controls are included, hence the much smaller denominators at six weeks after inoculation.

The serological findings in controls and original volunteers at two and four weeks after inoculation were virtually identical, which seemed to justify their being added together. This and the fact that virus was isolated from one of the two controls without antibody indicated that the vaccine used for reinoculation was still active. There are a few other minor differences between the various denominators shown in the tables and the number of persons inoculated, mainly because a few volunteers could not be bled at the appropriate times, especially at the sixth week. In addition a few specimens proved to be anticomplementary and some tests could not be done because there was not enough serum.

About three-quarters of those inoculated had a fourfold or greater rise in titre in at least one of the three serological tests whether or not they had neutralizing antibody beforehand. However, the pattern of the response was quite different in the two groups and was also related to whether or not virus was recovered after inoculation. In those with neutralizing antibody before inoculation, whether or not virus was isolated, and in those without antibody from whom virus was not recovered, the response was almost confined to neutralizing and H.I. antibody. Only five of 46 persons in these groups had a rise in C.F. antibody. By contrast, almost all those without neutralizing antibody from whom virus was isolated had a rise in antibody; only two of the 12 showed any rise in H.I. antibody and only one in neutralizing antibody. The proportion of volunteers with antibody of any kind was higher at six weeks than at four weeks, but it is impossible to say whether this was related to the second instillation of vaccine.

The serological results showed a greater difference between the effect of vaccines containing 10^6 and those containing $10^7 EID_{50}$ per ml. (Table V) than was apparent from the virus isolation tests. Four times as many men had been given the higher than the lower

TABLE III.—*Proportion of Volunteers Showing a Fourfold or Greater Rise in Antibody Titre in Various Tests*

Weeks after First Inoculation		With Neutralizing Antibody				Without Neutralizing Antibody			
		Neut. Test	H.I Test	C.F. Test	Any Test	Neut. Test	H.I. Test	C.F. Test	Any Test
Virus isolated	2	3/4	3/4	1/4		1/12	0/12	6/12	
	4	3/5	3/5	0/5		1/12	1/12	7/12	
	6	4/4	3/4	0/4		1/10	2/10	9/10	
	At any time	4/5	4/5	1/5		1/12	2/12	10/12	
Virus not isolated	2	10/35	12/35	2/32		3/10	1/10	1/7	
	4	17/34	13/34	1/30		3/10	1/10	1/7	
	6	18/28	13/28	1/26		4/7	2/7	2/6	
	At any time	23/37	20/37	2/34		4/10	2/10	2/7	
All volunteers	2	13/39	15/39	3/36	19/39 (49%)	4/22	1/22	7/19	11/22 (50%)
	4	20/39	16/39	1/35	21/39 (54%)	4/22	2/22	8/19	11/22 (50%)
	6	22/32	16/32	1/30	25/33 (76%)	5/17	4/17	11/16	16/17 (94%)
	At any time	27/42 (64%)	24/42 (57%)	3/39 (8%)	32/42 (76%)	5/22 (23%)	4/22 (18%)	12/19 (63%)	17/22 (77%)

"At any time" = between first and any subsequent bleeding.

TABLE IV.—*Proportion of Volunteers Showing a Four fold or Greater Rise in Antibody Titre in Various Tests*

Weeks after First Inoculation		With H.I. Antibody				Without H.I. Antibody			
		Neut. Test	H.I. Test	C.F. Test	Any Test	Neut. Test	H.I. Test	C.F. Test	Any Test
Virus isolated	2	2/2	2/2	1/2		2/14	1/14	6/14	
	4	2/2	2/2	0/2		2/15	2/15	7/15	
	6	2/2	1/2	0/2		3/12	4/12	9/12	
	At any time	2/2	2/2	1/2		3/15	4/15	10/15	
Virus not isolated	2	6/20	6/20	1/19		7/25	7/25	2/20	
	4	8/20	5/20	1/19		12/24	9/24	1/18	
	6	10/17	6/17	1/16		12/18	9/18	2/16	
	At any time	12/20	9/20	1/19		15/27	13/27	3/22	
All volunteers	2	8/22	8/22	2/21	10/22 (45%)	9/39	8/39	8/34	20/39 (51%)
	4	10/22	7/22	1/21	10/22 (45%)	14/39	11/39	8/33	22/39 (56%)
	6	12/19	7/19	1/18	13/19 (68%)	15/30	13/30	11/28	28/31 (90%)
	At any time	14/22 (64%)	11/22 (50%)	2/21 (10%)	16/22 (73%)	18/42 (43%)	17/42 (40%)	13/37 (35%)	33/42 (79%)

"At any time" = between first and any subsequent bleeding.

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TABLE V.—*Proportion of Volunteers Showing a Fourfold or Greater Rise in Titre Four Weeks After Inoculation in Relation to Vaccine Dose*

Dose	With Neutralizing Antibody			Without Neutralizing Antibody		
	Neut. Test	H.I. Test	C.F. Test	Neut. Test	H.I. Test	C.F. Test
$10^6 EID_{50}$	1/6	0/6	0/6	1/6	0/6	1/4
$10^7 EID_{50}$	19/33	16/33	1/29	3/16	2/16	7/15

dose, but all except three of the fourfold rises in antibody were in those who received the larger amount of virus.

The magnitude of the response in those with a rise in antibody ranged from 4- to 128-fold. In the C.F. and neutralization tests almost two-thirds were eightfold or more, compared with one-third in the H.I. tests.

Clinical Reactions

Sixty-four men were questioned during the four days after they received a first inoculation of vaccine; 61 had no symptoms whatever and three had very mild febrile illnesses. In addition one man with a large furuncle on the tip of his nose, but no other symptoms, was found to have a temperature of 99.4° F. (37.4° C.) on the fourth day, and another man without any symptoms had a temperature of 99° F. (37.2° C.) on the second day. The three with mild febrile illness were all without neutralizing antibody before inoculation, all received $10^7 EID_{50}$ of virus, all had a fourfold or greater rise in C.F. antibody, and virus was recovered from two of them. It therefore seems probable that their symptoms, though trivial, were caused by inoculation, so they are given below in detail:

H. W. G.—Complained of sneezing, blocked nose, dry cough, and pain on deep breathing on the day after inoculation and had a temperature of 98.6° F. (37° C.). Next day his temperature was 98° F. (36.7° C.) and his voice was hoarse; he had no symptoms thereafter.

P. H. T.—Began feeling unwell on the evening of the first day after inoculation. When seen on the second morning he was complaining of aches and pains, and was sweating. His temperature was then 100° F. (37.8° C.) and occasional rhonchi were heard in the chest. Apart from a slight cough he was well again on the next day. This man had received a T.A.B.T. injection three days before the influenza vaccine, which might conceivably have been a contributing factor.

J. H.—Complained of a slight headache and dryness of the throat two days after inoculation, and had a temperature of 99.2° F. (37.3° C.). Apart from dryness of the throat he had completely recovered by the next day.

Only one of the 46 men who were given a second dose of vaccine had any symptoms during the four days that followed. This man had a clear nasal discharge, blocked nose, and temperature of 99° F. (37.2° C.) two days after inoculation. Next day he was afebrile, and two days later he was well. Neutralizing antibody was present in his serum before the first inoculation; no antibody response was detected subsequently at any stage and virus was not recovered from him. It therefore seems probable that he had a coincidental common cold.

Contacts

Throat swabs were taken from 98 bedroom contacts of the inoculated men—59 four days after the first dose and 39 four days after the second. Influenza virus A2 was isolated from three of the 59 and from none of the 39. All three were contacts of volunteers initially without neutralizing antibody; virus was recovered and serological evidence of infection was obtained from two of the volunteers in question (G. T. C. and S. W.), but neither virus nor serological evidence from the third (D. H.). Before vaccination began blood had been taken for screening purposes from two of the three contacts. They were bled again as soon as it was known that virus had been isolated from them, though by this time it was six to eight weeks after contact. The serological findings are shown in Table VI.

TABLE VI

	Serum	Neutralization Test	Titres in	
			H.I. Test	C.F. Test
Contact of D. H.	1	>1/640	1/40	Anticomplementary
	2	>1/640	1/80	,,
Contact of S. W.	1	1/10	1/5	<1/2
	2	1/20	1/10	1/16

From all the available evidence it seems certain that the contact of S. W. was infected and that the contact of G. T. C. probably was, though this could not be tested serologically; whether the contact of D. H. was infected remains in doubt. Neither these three nor any of the other 95 bedroom contacts had any symptoms.

Febrile Respiratory Illness in Other Personnel

In the last three months of 1960 five men not included in the trial reported sick with a febrile respiratory illness—two with tonsillitis at the end of October, and one with tonsillitis and two with influenza during November. A throat swab was taken from all five and tested for the presence of influenza virus with negative results. Acute-stage blood specimens were also taken from all five, but convalescent specimens could be obtained from only three of them. None showed serological evidence of influenza virus A infection. The two patients from whom there were no second specimens became ill on November 1—one with influenza, the other with tonsillitis; neither had at that time any known contact with any of the 12 volunteers inoculated on October 24.

Results in Trial 2

During the follow-up period, which lasted approximately two months, only 25 of the 1,026 inoculated volunteers were admitted to sick quarters with a febrile respiratory illness; 16 had received the vaccine and nine the diluent only. The excess in the vaccinated occurred more than five days after inoculation, so it can be attributed more probably to chance than to illness produced by the vaccine. Paired sera and throat swabs were obtained from eight of the 25 patients admitted and none showed evidence of influenza A infection. In these circumstances nothing was learnt of the protective effect of the vaccine, but the analysis in Table VII of reactions noted 48 hours after inoculation gave some useful information. It can be seen that the symptoms and signs were almost all trivial and that there was little difference in their frequency in the vaccinated and the control groups.

Discussion

In the first trial at R.A.F. Digby three of the four criteria of Smorodintsev and Zhdanov (1957) for a satisfactory live influenza vaccine were met; the dose used was $10^6 EID_{50}$ per ml. or more, virus was recovered from over half those initially without neutralizing antibody, and virus could not be recovered from those reinoculated four weeks later. We failed, however, to

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TABLE VII.—*Reactions Noted 48 Hours After Inoculation in 1961 Field Trial*

	Live Vaccine	Diluent Only
Number inoculated:	513	513
Number reporting reactions	39 (7·6%)	41 (8·0%)
Severity:		
Mild	27	28
Moderate	12	13
Severe	0	0
Duration:		
Under 24 hours	30	31
24–27 ,,	5	4
48 hours or more	3	3
Not known	1	3
Fever:		
Afebrile	33	33
Febrile (none more than 100° F.)	6	8
Main symptoms:		
Running nose	9	14
Blocked ,,	9	19
Headache	10	8
Cough	1	4
Malaise	7	2
Sweating	4	1
Shivering	4	2

satisfy the fourth criterion that there should be a fourfold rise in H.I. antibody in 50% or more of susceptible subjects. Using the neutralization test to define susceptibility, the response in our susceptible volunteers was mainly in C.F. antibody, and only 18% had a rise in H.I. antibody (Table III). An H.I. response occurred most frequently (57%) in volunteers who on the basis of the neutralization test were not considered susceptible. Using the H.I. test to define susceptibility (Table IV), a higher proportion of susceptibles (40%) showed a rise in titre, but this is clearly due to the inclusion of men with neutralizing antibody among the susceptibles. The neutralization test (Pereira, 1958) appears to have had considerable advantages over the more generally used H.I. test both for the detection of susceptible individuals and for the measurement of antibody response. It is hoped to discuss this in greater detail in a separate paper.

It would be interesting to know why some volunteers who were apparently without antibody showed no evidence of infection after being given 10^7EID_{50} of virus. Assuming that those without antibody had not been previously infected with influenza A2, then it is reasonable that they would develop antibody to the soluble C.F. group antigen rather than to the specific antigen of the A2 virus. We should also like to know why viruses were not isolated from many volunteers with antibody although they showed an antibody response. Perhaps antibody in the respiratory secretions inactivated the virus. It is, however, just possible that the response was caused by antigen contained in the original inoculations and that there was no virus multiplication. The poor response to the soluble C.F. antigen would support this explanation.

The larger field trial in 1961 unfortunately did not answer the main question of how much protection live vaccines give against natural infection. The absence of reactions to inoculation was very satisfactory, but it must be remembered that probably some 90% of the vaccinated subjects had neutralizing antibody against influenza A2 virus before the trial began, and, from experience at Digby, no reactions would be expected in them. Nevertheless there must have been 50 or more without antibody, and there was no evidence of any excess incidence of febrile reactions in them.

The results of these trials seem promising enough to warrant further investigation of live influenza vaccines. The selection of suitable strains, dosage, methods of administration, and clinical efficacy are all questions that deserve study.

Summary

A trial of live influenza vaccine prepared from the Russian Iksha A2 strain was carried out in R.A.F. volunteers in the autumn of 1960. Virus in the vaccine had been passed six times in eggs and the dose of 1 ml. containing either 10^6 or 10^7EID_{50} was administered by intranasal drops to 64 men selected to include 22 without neutralizing antibody. A second dose of vaccine containing 10^7EID_{50} was given by the same route four weeks later to 46 of them. The volunteers were bled before the first inoculation and at two, four, and six weeks after it; throat swabs for virus isolation tests were taken daily for four days after each inoculation.

Virus was recovered after the first inoculation from 5 (12%) of those with neutralizing antibody and from 12 (55%) of those without; it was not isolated from any volunteers after the second dose.

About three-quarters of the volunteers had a fourfold or greater rise in antibody titre in at least one of the three types of serological tests used. There was a rise in C.F. antibody in almost all those initially without neutralizing antibody and from whom virus was recovered; in other volunteers the rises were mainly in neutralizing and H.I. antibody.

A better serological response was obtained with 10^7EID_{50} than with 10^6EID_{50}, and virus was recovered more frequently, though not from more volunteers, after the higher dose.

The neutralization test appeared to have considerable advantages over the H.I. test both for the detection of susceptibles and for the measurement of antibody response.

Reactions possibly attributable to the vaccine occurred after the first inoculation in three of the 64 volunteers. All three were initially without neutralizing antibody, and they had very mild febrile and respiratory symptoms lasting about 24 hours. There were no reactions after reinoculation.

Spread of virus from the inoculated volunteers to two, or possibly three, bedroom contacts was detected, but there was no evidence of any febrile respiratory illness due to influenza virus A in the unit during the trial.

In a clinical field trial with the same vaccine in three R.A.F. units early in 1961 513 men and women were given 10^7EID_{50} in 1 ml. by intranasal drops and the same number were given 1 ml. of diluent only. No information on the protective effect of the vaccine was obtained because of the absence of influenza A, but the incidence of reactions in the vaccine and control groups was virtually identical.

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INACTIVATED INFLUENZA VACCINE IN AN INDUSTRIAL UNDERTAKING

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Influenza must be regarded as a serious disease. The mortality it occasions is difficult to determine exactly, because a diagnosis of influenza can never be made with certainty on the basis of clinical observation alone.

Figures for excess mortality during pandemics and epidemics reveal that, though greatly reduced by progress in methods of treatment, the number of deaths caused by the disease is still fairly large.[1-3] During the 1957 pandemic the death rate in the Netherlands, Britain, and the U.S.A. was 1 per 2,000 to 4,000 head of population.[4-6] These high death rates are by no means confined to pandemic years but also occur during ordinary epidemics, as will be clear from Table I.[7] The drastic effect of influenza on sick-absence figures is evident from Fig. 1.

TABLE I.—*Deaths from Influenza in England and Wales. Uncorrected Notifications for the First Quarters of the Last Five Years*

	1957	1958*	1959*	1960	1961*
Number of deaths ..	361	2,093	7,098	504	6,377

* In these years an influenza A2 epidemic occurred in the first quarter.

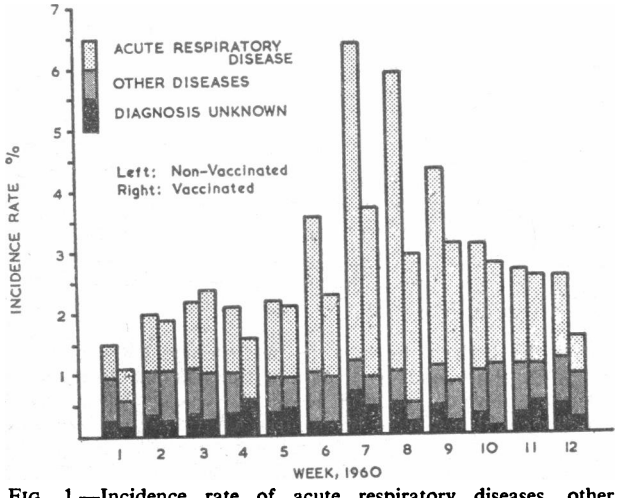

FIG. 1.—Incidence rate of acute respiratory diseases, other diseases with recorded diagnoses, and absences with diagnoses unknown in the group 1-1, males (1,779 vaccinated, 8,222 non-vaccinated) during weeks 1-12 of 1960 (see below, p. 1044).

In view of its high mortality and morbidity rates, determined action against influenza is called for. Vaccination is so far the only form of prevention the effectiveness of which has been demonstrated.

In practice there is a great deal of difficulty in proving that influenza vaccinations have actually yielded positive results: the clinical picture may be so similar to those of other acute diseases of the respiratory passages that differential diagnosis is possible only with the aid of complicated and costly laboratory tests, such as those whereby the virus is shown to be present in garglings or whereby antibodies are demonstrated in the blood. It has been possible, in investigations in which these methods were employed, to demonstrate whether vaccination has or has not been effective in a given case. Vaccination against influenza is at present thought to be effective in 65 to 75% of cases.[8-22]

In circumstances where vaccination of industrial employees is undertaken as a routine, elaborate laboratory methods are impracticable and an attempt must be made to ascertain the effect of the vaccinations in some other manner. One obvious yardstick is sickness absence. We have acquired some experience in the use of it since 1949, in which year workers in the Dutch Philips factories were vaccinated against influenza for the first time.

It has been found that a number of requirements must be satisfied if the sick-absence method is to provide reliable results.

1. The recording of the sick-absence data must be absolutely accurate.

2. Absence due to influenza cannot itself be made the yardstick, as it is practically impossible to diagnose the disease with a sufficient degree of certainty.

3. Absence due to acute respiratory diseases cannot be taken as a yardstick either, unless the number of spells recorded under the heading "diagnosis unknown" is sufficiently small.

4. The spells of sick absence must be recorded for males and females separately, as the absence rates for the two sexes may differ considerably (see Table II).

5. Absences must be recorded by age-groups, because young people tend to be absent more often than older

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