

ment is inadequate. As a rough guide for non-surgical cases treatment given over a period equal to the length of the history would in most cases have been adequate.

Summary

A group of 350 children with urinary-tract infection have been kept under continued surveyance. The infections were not controlled in 28 cases, all of which had major renal-tract abnormalities. The remaining 322 responded to treatment but had a high relapse rate, particularly during the first 18 months after initial control.

During treatment relapses might occur at any time, and many were associated with resistant organisms or the child's failure to take the prescribed medicine or pills. Relapses after treatment had stopped were usually unexplained, but were most likely to occur within the first three months.

Thirteen per cent. of all relapses were associated with upper respiratory infections. Relapses were significantly more frequent during the winter half-year, November to April, than in the summer.

There was some suggestion that for non-surgical cases with a history of over two years' duration anything less than three months' treatment was inadequate.

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Further Trials of Live Influenza Vaccine*

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Small-scale trials of a live influenza A₂ vaccine in 1960-1 showed that symptomless infections were induced in most subjects, and that despite a rather poor antibody response the volunteers could not be reinfected a month later (McDonald *et al.*, 1962). The vaccine used in these trials was prepared from the Russian A₂ strain Iksha attenuated by serial passage in eggs, and was given by drops intranasally. It was therefore pertinent to investigate whether other A₂ strains would have similar properties if treated in the same way and to investigate whether the vaccine could be given equally well by spray. At the same time we thought it important to determine whether inactivated virus, given intranasally, has any effects (Przesmycki *et al.*, 1959). Vaccines were prepared from the Iksha strain and from a Scottish strain of A₂ virus, A/Scot/49/57, and their efficacy compared in volunteers.

Technical Procedures

Preparation of Vaccines and Preliminary Safety Tests

The live vaccines were prepared in a similar way. The virus was inoculated into the allantoic cavity of 10-day-old chick embryos and these were incubated at 33° C. for two days. The fluids were harvested and stored at -70° C. in glass ampoules. The fluids were tested for the presence of contaminating bacteria by aerobic and anaerobic culture and for viruses (a) by inoculating tissue cultures after mixture with ferret immune serum against A/Singapore/1/57, and (b) by inoculating into mice by the intracerebral and intranasal route, into guinea-pigs by the

intracerebral route, and into rabbits by the subcutaneous route. All the animals remained well. The viruses showed specific haemagglutination-inhibition with ferret immune serum that had been treated with cholera filtrate to remove non-specific inhibitors.

The Iksha strain had been passed six times *in ovo* and had a titre of 10^{8.2} 50% egg-infectious doses (EID₅₀/ml.), while the A/Scot/49/57 strain had received about five such passages and had a titre of 10⁹ EID₅₀/ml. A portion of the Iksha virus was inactivated by dialysing it against distilled water and then adding 0.025% formalin and storing at 4° C. The virulence for man of the A/Scot/49/57 virus was roughly gauged by administering it in doses of 10⁶ and 10⁷ EID₅₀ to two groups of four volunteers living in isolation. Three of those given the larger dose developed symptoms, but only one of those given the smaller; it was considered safe to give doses of less than 10⁶ EID₅₀ to larger numbers of subjects.

As both the strains gave low titres of inhibition with horse serum, a further strain, A/Eng/443/57, which is very sensitive to inhibitors, was also tested. This produced such marked symptoms in a small group of isolated volunteers that it was not studied further.

The live vaccines were stored in sealed glass ampoules in an electric refrigerator at -70° C. until transported to the station on solid carbon dioxide. The vaccine was diluted with Hanks's balanced salt solution containing 0.5% gelatin (pH about 7.2) immediately before use and kept at 4° C. until used; this period never exceeded 30 minutes. The dose of vaccine intended for nasal drops was made up to 1 ml. with diluent, whereas for spray it was suspended in 0.1 ml.

Virus Isolation

Specimens from nose and throat swabs, prepared as described below, were transported to the laboratory on melting ice, and on the same day 0.2 ml. was inoculated into the amniotic cavity

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of three 10-day fertile hens' eggs. Penicillin and streptomycin were added to the inocula in the proportion of 100 units or micrograms respectively per egg. The eggs were incubated at 35° C. for three days, then after chilling overnight at 4° C. the amniotic fluids were harvested and tested for agglutination of human and fowl red-blood cells. Positive amniotic fluids were passed as necessary, and the virus was identified by means of a complement-fixation (C.F.) test for influenza-A soluble antigen. Negative fluids were inoculated into a further set of eggs. At one stage, when the supply of eggs broke down, specimens were stored at -70° C. until eggs were again available.

Laboratory Methods

When the trial was first projected it was expected that a pool of approximately 80 volunteers without neutralizing antibody to A₂ influenza would be required. Previous experience had suggested that the selection of this number would entail the testing of 700-800 sera; the number finally used was in fact 764. While the neutralization technique is appreciably more sensitive than that of haemagglutination-inhibition (Beare, 1962), it is very cumbersome. It was therefore decided to screen all sera at a dilution of 1:5 for haemagglutination-inhibiting antibodies and to test only negative sera by neutralization.

Neutralization and Haemagglutination-inhibition Tests.—These were performed as previously described (McDonald *et al.*, 1962).

Complement-fixation Tests.—These were performed by the method described by Bradstreet and Taylor (1962), but the conditions for fixation of complement were modified to one and a half hours at 37° C.

Trial Procedure

Blood specimens from 764 R.A.F. personnel and 71 civilian employees were screened for neutralizing antibody to A₂ influenza in September 1962. One hundred and seven (13%) were without antibody at a serum dilution of 1 in 10 (Table I), and 96 of these volunteered to take part in the trial; in addition, 98 of those with antibody at this titre agreed to do so.

TABLE I.—Distribution of Influenza A₂ Neutralizing Antibody

Age-group (Years)	R.A.F.		Civilians		Both	
	No. Tested	No. without Antibody at 1/10	No. Tested	No. without Antibody at 1/10	No. Tested	No. without Antibody at 1/10
16-25	448	22 (5%)	8	3 (26%)	456	25 (5%)
26-44	257	44 (17%)	39	9 (18%)	296	53 (18%)
45+	59	20 (34%)	24	9 (38%)	83	29 (35%)
Totals	764	86 (11%)	71	21 (30%)	835	107 (13%)

The volunteers were divided into groups, and during October and November were given differing doses of the Iksha or A/Scot/49/57 vaccines, live or inactivated, by drops or by spray. Four weeks later as many as possible of the volunteers initially without antibody were challenged with a further 10⁴ EID₅₀ of the A/Scot/49/57 vaccine given by drops. As a control on the potency of the challenging vaccine, 11 volunteers without antibody were held back and given their first dose of vaccine at the same time as the others were given their second dose.

The vaccines were instilled into the nose with a dropper—0.5 ml. into each nostril. The volunteer lay with his head overhanging the edge of an examination couch during instillation, and remained there for one minute afterwards. For spraying, the vaccine was loaded into an insufflator and 0.05 ml.

administered into each nostril with the volunteer sitting up after taking a deep breath and retaining it in maximum inspiration. This latter manoeuvre was employed to ensure that the sprayed vaccine was not inhaled deeply during the inoculation.

All volunteers were examined about 36 hours after vaccination and after the challenge dose. Their temperatures were recorded and throat swabs were taken; nasal swabs were also obtained from those given the spray. The swabs were broken off into 5-ml. bottles containing 0.5% lactalbumin in Hanks's balanced salt solution with 0.02% sodium bicarbonate added. The bottles were stored and transported to the laboratory on melting ice.

Blood samples were taken from volunteers with antibody two weeks after vaccination. Volunteers initially without antibody had blood taken immediately before the second dose of vaccine, and a final blood sample two weeks later. The blood specimens were stored at 4° C. until taken to the laboratory for separation of the serum.

A careful record was kept of any respiratory illnesses on the station and among the family contacts of the volunteers. A throat swab and paired serum specimens were taken for laboratory examination from all patients admitted to sick quarters with acute respiratory illness.

Results

The number of volunteers who received each type of vaccine and the symptoms of which they complained after vaccination are shown in Table II. Fever was observed in only one person, a man without neutralizing antibody who developed a mild influenza-like illness. He had received 10⁵ EID₅₀ of the A/Scot/49/57 vaccine, and his symptoms lasted 48 hours. Mild afebrile coryzal symptoms were recorded in 38 of the 96 (40%) volunteers without antibody and 5 of the 55 (9%) with antibody. There was little evidence that these rates were related to the type of vaccine, the dose, or the method of administration. After the challenging dose 66 of the 75 (8%) had coryzal symptoms.

TABLE II.—Vaccination Programme and Reactions

Vaccine	Dose (EID ₅₀)	Method	First dose of Vaccine			Challenge Dose (10 ⁴ EID ₅₀ of A/Scot/49/57)		
			No. Vaccinated	Reactions		No. Vaccinated	Reactions	
				Coryzal	Influenzal		Coryzal	Influenzal
<i>Volunteers Without Antibody</i>								
Iksha	10 ⁶	Drops	10	5	—	9	2	—
			Spray	8	4	—	8	2
" "	10 ⁷	Drops	21	10	—	18	2	—
			Spray	27	11	—	22	—
A/Scot/49/57	10 ³	Drops	4	—	—	4	—	—
			10 ⁴	21*	7	—	9	—
			10 ⁵	4	—	1	4	—
			10 ⁶	1	—	—	1	—
			10 ⁸	1	—	—	—	—
Total			96	38	1	75	6	0
<i>Volunteers With Antibody</i>								
Iksha	10 ⁷	Drops	27	3	—			
			Spray	28	2	—		
Iksha (inactivated)	10 ⁷	Drops	20	{ Not observed				
			Spray	23				
Total			98	5/55				

* Including 11 who were vaccinated at the same time as the others were given the challenge dose and who were not themselves challenged.

Virus Isolation

The results of virus isolation tests are shown in Table III. Influenza virus was isolated from 1 of the 27 (4%) men with neutralizing antibody given the Iksha vaccine by drops at 10⁷ EID₅₀, and from none of the 28 men who were sprayed.

Virus was isolated from 9 of the 35 (26%) men without antibody who were sprayed, from 10 of the 31 (32%) men who received Iksha vaccine by drops, and from 5 of the 30 (17%) who were given various doses of A/Scot/49/57 vaccine by drops. After the challenge dose of A/Scot/49/57 vaccine virus was recovered from 4 of the 75 (5%) men. There was little evidence to show that the route of administration influenced virus isolation results, and the same was true of vaccine dose. The recovery rate of virus after challenge was much lower than after the primary dose; but as different vaccines were used this finding must be interpreted with caution.

TABLE III.—Virus Isolation Results About 36 Hours After Live Vaccine

Vaccine	Dose	Volunteers with Antibody After First Dose	Volunteers without Antibody		
			After First Dose	After Challenge Dose (10 ⁴ EID ₅₀ of A/Scot/49/57)	
Iksha spray	10 ⁶	—	2/8	1/8 } 1/30	
	10 ⁷	0/28	7/27 } 9/35		0/22
,, drops	10 ⁶	—	3/10	2/9 } 2/27	
	10 ⁷	1/27	7/21 } 10/31		0/18
A/Scot/49/57 drops	10 ⁸	—	0/4	0/4 } 1/18	
	10 ⁴	—	2/21*		1/9
	10 ⁶	—	2/4		0/4
	10 ⁸	—	1/1		0/1

* Including 1/11 volunteers who were vaccinated at the same time as others were given the challenge dose.

Serological Tests

The findings of the serological investigations are summarized in Tables IV and V. The majority of volunteers without antibody showed no response to the Iksha vaccine by complement-fixation or haemagglutination-inhibition tests, and there was little difference between drops and spray. The Scottish vaccine strain produced antibody in a considerably higher proportion of subjects, but the number in this group was not large.

In volunteers with antibody there was a sharp contrast between the results after drops or spray and those after live or inactivated vaccine. A high proportion (63%) who received live vaccine by drops had a fourfold or greater rise by haemagglutination-inhibition, whereas only one volunteer from the other three groups showed a response. A further contrast lay between the haemagglutination-inhibition and complement-fixation test findings; not a single rise of complement-fixing antibodies was observed.

Of all volunteers receiving Iksha drops 28 out of 84 (33%) showed a fourfold or greater rise of antibody titre. This com-

TABLE IV.—Proportions of Volunteers Without Neutralizing Antibody Showing a Fourfold or Greater Rise in Antibody Titre Between First and Subsequent Bleedings

Weeks after First Dose	Iksha Spray (10 ⁶ or 10 ⁷ EID ₅₀)		Iksha Drops (10 ⁶ or 10 ⁷ EID ₅₀)		A/Scot/49/57 (10 ³ –10 ⁸ EID ₅₀)		
	H.I.*	C.F.†	H.I.*	C.F.†	H.I.*	C.F.†	
Virus isolated	4	1/7	0/7	3/10	1/10	2/5	3/5
	6	0/7	2/7	3/9	1/9	4/4	2/4
Virus not isolated	4	3/26	5/26	2/19	3/19	8/25	7/25
	6	6/24	6/24	3/19	1/19	6/14	3/14
Total	4	4/33 (12%)	5/33 (15%)	5/29 (17%)	4/29 (14%)	10/30 (33%)	10/30 (33%)
	6	6/31 (19%)	8/31 (26%)	6/28 (21%)	2/28 (7%)	10/18 (56%)	5/18 (28%)

* Haemagglutination-inhibition. † Complement-fixation.

TABLE V.—Proportion of Volunteers with Neutralizing Antibody Showing a Fourfold or Greater Rise in Antibody Titre Two Weeks After Vaccination

Vaccine	Iksha Drops (10 ⁷ EID ₅₀ in 1 ml.)		Iksha Spray (10 ⁷ EID ₅₀ in 0.1 ml.)	
	H.I.*	C.F.†	H.I.*	C.F.†
Live vaccine ..	17/27 (63%)	0/27	0/28	0/28
Inactivated vaccine ..	1/20 (5%)	0/20	0/23	0/23

* Haemagglutination-inhibition. † Complement-fixation.

pared poorly with a similar population given inactivated vaccine (Hobson *et al.*, 1964), when 44 out of 54 (81.5%) produced a significant response.

Respiratory Illness in Other Personnel

There was very little respiratory illness on the station during the period of the trial. Between the time that vaccine was first given at the end of October and the end of the year only eight R.A.F. personnel were admitted to sick quarters with a febrile respiratory illness. Paired sera from these patients were examined by complement-fixation tests for evidence of infection with influenza viruses A and B, adenovirus, psittacosis virus, and *Coxiella burnetii*. Three showed a fourfold or greater rise in titre against influenza virus B and the remainder were negative.

Discussion

Fairly clear answers were obtained to most of the questions for which the investigation was designed. Vaccine prepared from the Scottish strain apparently immunized volunteers as effectively as the widely used Iksha vaccine strain, and the reactions were no greater. Moreover, a dose of 10⁴ EID₅₀ of the British vaccine was at least equivalent to 10⁶ EID₅₀ of the Russian vaccine, as judged by serological or virus isolation tests. A finding of practical value was that the administration of vaccine by nasal spray—a very simple procedure—produced similar results to the cumbersome method of instilling nasal drops. The peculiar failure of spray to induce a response in persons already with antibody cannot be explained, but the experiment should be repeated before any conclusion is drawn from the observation. Certainly, inactivated vaccine given by either method was quite ineffective. On the other hand, the antibody responses observed were lower than those which follow intramuscular injection of inactivated virus.

This investigation has also indicated clearly our essential ignorance of what is involved in the preparation and use of live influenza vaccines. Although the Iksha vaccine was prepared in exactly the same way as that used in the 1960 trial (McDonald *et al.*, 1962), the frequency of virus recovery and the antibody responses were much inferior. Perhaps changes with passage of time in some characteristic of the virus strain or the human population, or both, accounted for this. Unfortunately there are no laboratory tests available by which the ability of vaccine strains to multiply in or to damage cells of the human respiratory tract can be detected and compared, and so this kind of question cannot be answered at present. The British strain, picked more or less at random, behaved in a similar manner to the carefully selected Iksha strain, but we cannot conclude that it was sufficiently "attenuated" for safe use on a large scale. The nature and stability of the "attenuation" produced by a small number of egg passages is not known. More reactions might have occurred if the dosage of virus given had been as large as the dose of Iksha; the failure to spread and cause illness may have been partly due to good fortune and partly to the high prevailing level of natural immunity in the population.

The potential importance of a live influenza vaccine and the encouraging results from trials such as ours appear to warrant intensive laboratory study of the characteristics by which stable and avirulent virus strains can be recognized.

Summary

Two live influenza-A vaccines were given to 96 volunteers without neutralizing antibody. The Russian Iksha strain was given in doses of 10⁶ or 10⁷ egg-infectious doses (EID₅₀) to 66

volunteers, and a British strain in doses ranging from 10^3 to 10^6 EID₅₀ to 30 volunteers. One month later 75 of the vaccinated volunteers were given 10^4 EID₅₀ of the British vaccine. In addition, 55 volunteers with neutralizing antibody were given 10^7 EID₅₀ of the Iksha vaccine by drops or spray, and 53 volunteers were given the same dose of vaccine inactivated with formalin.

Of the volunteers without antibody 41% had mild respiratory symptoms after the first dose, and 8% after the challenging dose; 9% of the volunteers with antibody who were given live vaccine had similar symptoms.

Virus was recovered from 25% of the volunteers without antibody after the first dose of vaccine, and from 5% after the challenging dose. Immediately before the challenging dose of vaccine 21% of the volunteers showed fourfold or greater haemagglutination-inhibition antibody response and 21% a complement-fixation response; two weeks after the challenging dose the proportions were 29% and 19% respectively. A much higher proportion of volunteers showed an antibody response after live than after inactivated vaccine.

A dose of 10^4 EID₅₀ of the British vaccine gave similar results to that of 10^6 or 10^7 EID₅₀ of the Iksha vaccine. Except perhaps in volunteers with antibody there was little difference in efficacy of administration by nasal drops or spray.

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Extensive Histological and Cytological Survey of Patients with Acute Leukaemia in "Complete Remission"*

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[WITH SPECIAL PLATE]

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Throughout the world only about 50 patients with acute leukaemia have had a remission lasting longer than five years (Burchenal and Murphy, 1965). In most patients with this disease the remissions that are called "complete" are invariably followed by a recurrence.

It can be questioned whether this recurrence is due to proliferation of the leukaemic cells that have persisted and whose presence has not been detected on routine examination. On the other hand, it is possible that the original leukaemic cells are destroyed and that the recurrence is due to the induction of a new leukaemia by a leukaemogenic factor which remains in the body. It would therefore be logical to obtain very detailed information about the state of the leukaemic cells during a so-called complete remission.

In practice we have made the following observations on patients during a remission:

1. A study of the cells present in the blood by means of a leucoconcentration technique (this had previously been shown to be able to demonstrate circulating cancer cells in patients with haematosarcomata who were not leukaemic (Festing, 1962)).
2. A cytological examination of six bone-marrow biopsies and a histological examination of one. (Bernard and Mathé (1951) have shown that when multiple bone-marrow biopsies are taken simultaneously from patients with acute leukaemia the cellular picture may vary from site to site.) The skeleton is also examined radiologically.

3. The central nervous system is examined by an analysis of the cerebrospinal fluid and by electroencephalography; there is a high frequency of leukaemic infiltration of the meninges and central nervous system during the course of an acute leukaemia (Wells and Silver, 1957).

4. Biopsies are taken from the kidney, liver, and testicles (see Special Plate).

In those patients in whom the investigations revealed the presence of leukaemic cells, the treatment which has led to the remission is continued at twice the dosage for a month. This treatment is generally with corticosteroids. After this period, those tests that revealed the presence of leukaemic cells are repeated.

Methods, and Patients Studied

The methods used for this investigation start when the patient's routine blood count and bone-marrow are found to be in the normal range both quantitatively and qualitatively. Thirty-one patients were studied in the present survey; details of their age, sex, classification according to cell type involved, history of the proliferative phase preceding the remission, and treatment during the proliferative phase are given in Table I.

The technique used in counting the leukaemic blast cells after concentrating the blood is as follows: 30 ml. of blood is taken into A.C.D. (acid-citrate-dextrose, 1 vol. A.C.D. for 9 vol. blood) and allowed to stand at an angle of 45 degrees for three hours. The supernatant plasma containing the leucocytes is then removed and its volume measured. This supernatant is

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