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TRIAL OF ADENOVIRUS VACCINE IN ROYAL AIR FORCE RECRUITS

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Several large field trials of adenovirus vaccines in the United States have shown them to be effective against infection with viruses of the same type. Hilleman and his co-workers, using a vaccine prepared from adenovirus types 4 and 7 during an epidemic of infection with these two types, reported that the acute respiratory illness rate was reduced from 23.8% in the control group to 4.8% in the vaccinated group (Hilleman *et al.*, 1956; Stallones *et al.*, 1957). Bell and his co-workers, using vaccine prepared from types 3, 4, and 7, obtained a reduction of 55% in the incidence of febrile respiratory disease when adenovirus type 4 was prevalent (Bell *et al.*, 1956; Gundelfinger *et al.*, 1958). In another trial of a trivalent vaccine, Culver *et al.* (1959) obtained a 43% reduction in the total sickness rate; during this trial also adenovirus type 4 infection was predominant.

In a study of acute respiratory disease in the Royal Air Force, McDonald *et al.* (1958) confirmed that new recruits to the Service had a far higher illness rate than other personnel. This led to a considerable disruption of training programmes. In addition, they found that a substantial number of recruits were discharged from the

R.A.F. with serious lung damage before any useful service had been performed. Indeed, the major cause of discharge due to respiratory disease in the R.A.F. was illness acquired at recruit camps. Moreover, they observed the great importance of adenovirus infection as a cause of acute respiratory disease among recruits. This corresponded to findings in the United States (Hilleman, 1958), in Holland (van der Veen and Kok, 1957), in France (Sohier *et al.*, 1957), and in Switzerland (Löffler *et al.*, 1956). Apart from some radical change in the method of accommodating recruits on reception and during training, vaccines appeared to offer the best hope of reducing the problem. A trial of adenovirus type 7 vaccine conducted in the R.A.F. early in 1958 was inconclusive, though the results suggested that it afforded some protection against type 7 infection and possibly protection of a lesser degree against type 4 infection, which was predominant at the time (unpublished results of the Medical Research Council Adenovirus Vaccine Working Party).

Early in 1959, 400 doses of a commercial vaccine containing adenovirus types 3, 4, and 7 (prepared by Parke, Davis & Co.) became available to the R.A.F. at short notice. Clinical trials in the United States had left little doubt about the efficacy of the vaccine, but it was decided to use these doses in a way which might demonstrate their value in this country. They were therefore administered to R.A.F. recruits at Bridgnorth, where a comprehensive survey of acute respiratory disease was already in progress.

Material and Methods

On arrival at Bridgnorth each week recruits are divided into flights of about 70 to 80 men each. The flights are made up of an amalgamation of smaller groups formed at the recruit reception unit as the men arrive; there is some tendency for men who live furthest away to arrive later and so to be grouped together, but no other apparent selection in the composition of groups. Men in each flight sleep, work, and live together during the whole of their two months' training, but they also have contact with members of other flights when off duty. It was decided to offer protection to two incoming flights each week for three weeks during February, 1959, and to compare respiratory infection rates in them with those in three uninoculated flights arriving each week during the same period. In this way it was hoped to demonstrate the value of having a high proportion of protected persons in a flight. The nature of the vaccine and what it was hoped to achieve were explained to the recruits. A total of 402, 91% of the selected flights, volunteered. Of the remainder, some were rejected because they were already ill or appeared to be developing a respiratory illness, and it was thought undesirable to inoculate them.

Inoculation Procedure.—About 70% of the volunteers were inoculated within 24 hours of their arrival at Bridgnorth, and the remainder within 48 hours. A single dose of 1 ml. was injected into the deep subcutaneous tissues of the upper arm, using a separate syringe and needle for each volunteer. All volunteers were inspected 48 hours later for reactions to the inoculation. Local reactions were recorded in 25 men (6.2%); all were mild except for one with a moderate degree of local erythema and induration. A further 10 (2.5%) had mild general reactions, and two had both mild general and mild local reactions.

Recording and Laboratory Procedure

No special records of illnesses occurring in the trial groups were necessary, because in the survey already in progress an illness-record card was completed for every recruit admitted with acute respiratory disease to the station sick-quarters or to hospital until the end of March. Medical officers were asked to take throat swabs for virus-isolation tests, and acute and convalescent samples of blood from as many cases of respiratory illness as possible. During the early part of the trial the proportion from whom specimens were collected was low because of the intensity of the epidemic at the time.

Throat swabs were broken off into 5-ml. bottles containing Hanks's balanced salt solution with 0.5% lactalbumin and sodium bicarbonate; these were transported on solid carbon dioxide to the laboratory at Colindale, where they were stored at -70°C . until tested two to three months later. About three-quarters of them were kept at -10°C . for up to two weeks before being taken to Colindale. Strains of adenovirus were isolated from throat swabs by inoculating each of two tubes of HeLa cell cultures with 0.1 ml. of the transport medium. The cells were maintained in tubes, using Earle's balanced salt solution with 0.5% lactalbumin, 2% horse serum, sodium bicarbonate, and 200 units each of penicillin, streptomycin, and nystatin. Cultures were maintained for 28 days, the medium being changed and passage carried out as necessary. The tissue-culture fluid of cultures showing a cytopathic effect was tested by means of a complement-fixation test against a positive serum. Those which were positive were sent to the Virus Reference Laboratory for typing by means of a neutralization test.

Blood samples were sent to the laboratory by post, where, after separation, the serum was stored at -20°C . until tested. Complement-fixation tests were carried out in cups on plastic plates, using volumes of 0.1 ml. The haemolytic system consisted of a 2% suspension of optimally sensitized sheep red cells together with 2.5 HC_{50} units of complement. The source of complement was guinea-pig serum preserved by the method of Richardson (1941). Antigens were used at their optimal concentration. Sera and antigens were allowed to react in the presence of complement at 4°C . overnight before the haemolytic system was added. Then, after a period of incubation at 37°C ., the plates were left at 4°C . to allow remaining red cells to settle before reading. Convalescent-stage sera showing more than 50% fixation in the screening test were titrated in parallel with acute-stage specimens. A fourfold, or greater, rise in titre between paired specimens was regarded as diagnostic of recent infection.

Results

Epidemiological Background.—The incidence of respiratory illness at Bridgnorth was low during the early

part of the winter but rose sharply in the week ending January 23, 1959. In early February there was an explosive outbreak with exceptionally high sickness rates; this coincided with the time when the first adenovirus vaccine was being given to new arrivals. The rate of admission to the sick-quarters continued at a high level for four weeks before beginning to fall one week after the last of the inoculations had been given. The number of admissions returned to a low level by the end of March. Evidence obtained from tests on throat swabs and paired specimens of serum from sample illnesses suggested that infection with adenovirus type 3 was occurring throughout this period, but that the main epidemic in early February was probably due mostly to influenza A virus with some influenza B virus infection as well.

Clinical Results

The total number of admissions due to respiratory illness in the protected and in the unprotected flights is shown in Table I. For both groups the illnesses are divided into those in which the onset was on or before the seventh day from the date of inoculation, and those with onset after that time. The seventh day from inoculation was chosen as a time after which the vaccine could be expected to have a protective effect. The admission rate was 36% in the uninoculated flights, compared with 26% for all persons in inoculated flights, whether or not they had been given the vaccine. The difference between these two rates was statistically significant (difference $\div$ standard error = $10 \div 2.8 = 3.6$). The reduction was relatively greater when only illnesses starting more than seven days from injection were considered. The comparable rates of admission were then 20% and 12%, representing a reduction of 40% in the protected flights. The persons who received no

TABLE I.—Admissions to Sick-quarters with Acute Respiratory Illness (Rate per cent. in Parentheses)

	No. of Men	All Illnesses	Illnesses within 7 Days of Inoculation	Illnesses More than 7 Days from Inoculation
Unprotected flights ..	661	236 (36)	106 (16)	130 (20)
Protected ..	442	113 (26)	58 (13)	55 (12)
Inoculated persons ..	402	95 (24)	48 (12)	47 (12)
Uninoculated ..	40	18 (45)	10 (25)	8 (20)
All flights ..	1,103	349	164	185

TABLE II.—Adenovirus Isolation Results

	Illnesses within 7 Days of Inoculation		Illnesses More than 7 Days from Inoculation		Adenovirus Types		
	Tested	Adenovirus Isolated	Tested	Adenovirus Isolated	3	4	7
Unprotected flights ..	10	0	43	20	18	1	1
Protected ..	8	0	14	3	2	1	0
Inoculated persons ..	6	0	12	1	1	0	0
Uninoculated ..	2	0	2	2	1	1	0
All flights ..	18	0	57	23	20	2	1

TABLE III.—Serological Results

	Illnesses within 7 Days of Inoculation				Illnesses More than 7 Days from Inoculation			
	Tested	Adenovirus	Influenza A	Influenza B	Tested	Adenovirus	Influenza A	Influenza B
Unprotected flights ..	12	0	7	2	46	16 (2)	1 (1)	4 (1)
Protected ..	11	3 (2)	5 (3)	2 (1)	17	1	3	1
Inoculated persons ..	8	3 (2)	4 (3)	1 (1)	14	0	3	1
Uninoculated ..	3	0	1	1	3	1	0	0
All flights ..	23	3 (2)	12 (3)	4 (1)	63	17 (2)	4 (1)	5 (1)

Figures in parentheses denote numbers showing a rise in antibody content to more than one antigen.

vaccine in inoculated flights apparently fared worse than those in the totally unprotected flights, but this was probably because some of them were not inoculated as they were already ill. When only those illnesses which occurred more than seven days from the inoculation date are considered, the difference disappears, but the fact that most of their colleagues were inoculated did not appear to protect them from infection.

Laboratory Results

Adenovirus strains were isolated from 23 (31%) of the 75 throat swabs tested (Table II). The isolations were all from men taken ill more than seven days after the date of inoculation. All but three were from persons in unprotected flights, and, of these three, only one came from a man who had been given the vaccine. Of the 23 adenovirus strains isolated, 20 were of type 3, two were type 4, and one was type 7. The strain isolated from the inoculated man belonged to type 3.

The serological findings (Table III) were similar to the isolation results. Of the 86 pairs of serum tested, 20 (23%) showed a fourfold or greater rise in complement-fixing antibody to adenovirus. Only three of these pairs were from inoculated persons, all of whom were ill within the first week after inoculation. Two of the three showed a rise in titre to influenza A as well as to adenovirus, so that the rise in titre to adenovirus may have been in response to the vaccine rather than to natural infection.

The distribution of positive serological tests for influenza A and influenza B is also shown in Table III. Most of the influenza virus infections occurred at, or soon after, arrival at the recruit camp, whereas nearly all the adenovirus infections occurred after the first week. The influenza virus infections were distributed more evenly among the inoculated and uninoculated flights than were the adenovirus infections.

Discussion

The reduction in illness was less impressive than results reported from American trials. This may have been partly due to the fact that many of the respiratory illnesses leading to admission to sick-quarters in both the inoculated and uninoculated were not caused by adenovirus infection. Some of these illnesses were due to influenza virus infection, and in nearly half of those tested no cause was found. A better assessment of the vaccine can probably be obtained by using the virus isolation results from the patients tested to estimate the proportion of illnesses in the two groups due to adenovirus infection.

In the inoculated flights specimens from 14 of the 55 illnesses that occurred more than seven days from injection were tested and adenovirus was isolated from three of them. We may therefore estimate that adenovirus might have been isolated from about 12 patients—that is, $3 \times 55/14$ —had specimens from all 55 illnesses been tested. Similarly, in the unprotected flights the number of illnesses from which adenovirus might have been isolated was probably about 60—that is, $20 \times 130/43$. Admission rates due to adenovirus infection, based on these estimates, are 2.7% ($12/442 \times 100$) for the inoculated flights, and 9.1% ($60/661 \times 100$) for the uninoculated flights. These figures represent a reduction in the admission rate of 70%. This is probably a conservative estimate of the protective effect of the vaccine, as only one of the three patients in protected

flights from whom virus was isolated had actually received the vaccine.

The value of adenovirus vaccines, previously demonstrated among military recruits in the United States, has thus been confirmed in recruits to the Royal Air Force. Huebner *et al.* (1955) gave a type 3 vaccine to volunteers, and showed that it induced neutralizing antibodies and protected against challenge with virus of the same type. There has been no previous report, however, of the efficacy of a vaccine against type 3 infection under natural conditions.

Summary

A limited supply of commercially prepared trivalent adenovirus vaccine containing strains of types 3, 4, and 7 was administered to 402 R.A.F. recruits to test its value. Two out of five flights of recruits arriving at Bridgnorth each week for three weeks during February, 1959, were inoculated.

Influenza A, influenza B, and adenovirus infections were all highly prevalent at the time the injections were given and during the weeks that followed. The adenovirus strains isolated were predominantly type 3.

There was a total reduction of 40% in the admission rate for acute respiratory illness occurring in the inoculated flights more than one week from inoculation. The protective effect of the vaccine against adenovirus infection, estimated from virus isolation results, was 70%.

We are indebted to the staff of the Station Sick Quarters at R.A.F., Bridgnorth, for their help with records and collection of specimens; to Dr. M. S. Pereira for typing the adenovirus strains; and to Mr. G. V. Heimer and Sergeant C. Woodward for their technical assistance. Acknowledgment is also made to the Director-General of Medical Services, Royal Air Force, for permission to publish results.

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The level of natural radiation from the rocks varies in different parts of the world. The collection of information about its effects in man from those areas where its level is exceptionally high was recommended by the W.H.O. Committee on Radiation in 1958. The committee's report was previously issued in mimeographed form (W.H.O./Rad/5) and is now published as No. 166 in the W.H.O.'s Technical Report Series. Briefly, it recommends that a study be carried out in Kerala, South India. There a relatively stable population of about 80,000 is exposed to natural radiation that is "10–15 times that normally encountered." The report is available from H.M.S.O. for 1s. 9d.