Prevention of Acute Respiratory Illness in Recruits by Adenovirus* (RI-APC-ARD) Vaccine.† (22484)

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Acute respiratory illness caused by viruses of the RI(1,2) (also called APC(3) or ARD (4)) family is a major medical problem to the Armed Forces. In certain recruit training camps, these viruses have been found responsible for as many as 90% of the hospital admissions for respiratory disease during the winter months(5-8) and for up to 60% of all hospital admissions for these illnesses during the entire year(9). Respiratory illnesses caused by agents of the RI group are included in the general disease category of the febrile catarrhs(10) but belong, more specifically, among the entities of undifferentiated acute respiratory disease (ARD)(11), non-streptococcal exudative pharyngitis(12), primary atypical pneumonia (PAP) unassociated with cold agglutinins(13), bronchitis resembling atypical pneumonia (Br-AP)(14) and pharyngo-conjunctival fever(15). The viruses of the RI family comprise at least 14 distinct serotypes(2,3,8,16,17) but only 3 of these, types 3, 4 and 7, have been found important in the causation of respiratory illness of soldiers, types 4 and 7 being most frequent(2,8, 18). Patients with RI virus infections develop both neutralizing and complement-fixing antibodies against the infecting virus(1). The neutralizing antibody appears to be associated with protection against the disease (4,19), while complement-fixing antibody is directed against a "soluble" group-specific antigen(2,3,20) which is common to the agents of the RI group and appears not to be protective(5,7).

The impact of RI-caused illness on the training of recruits in the Armed Forces makes highly important the development of a vaccine which will protect against the natural disease under field conditions. Huebner, *et al.* (19) have recently reported the effectiveness of a type 3 killed-virus vaccine in the prevention of an experimentally induced disease in volunteers challenged by inoculation of the homologous virus onto the conjunctivae. The present report describes the development, in our laboratory, of a bivalent formalin-killed types 4 and 7 vaccine prepared from infected monkey kidney tissue cultures. This was found highly effective, under field conditions, in reducing the incidence of acute respiratory illness of RI etiology in recruits.

Materials and methods. Vaccine preparation. The RI virus strains used to prepare the vaccine were: RI-67(1), a type 4 virus originally recovered in human tracheal epithelium tissue culture from the throat washing of a case of PAP; and a type 7 virus, RI-4-202(2), recovered in human amniotic epithelium tissue culture from a throat washing of a case of ARD. These strains were adapted from the original human cell isolations to monkey kidney epithelial culture¶ maintained in serum-free synthetic mixture 199¶ containing 200 units of penicillin and 200 μg of streptomycin per ml. The infected whole cultures were harvested 4 to 7 days after viral inoculation, homogenized in a Waring blendor, clarified by spinning at 2500 rpm for 20 minutes in a horizontal centrifuge, and filtered through a sintered glass filter of medium porosity. These filtrates, after neutralization

* Viruses previously called RI, APC or ARD agents have recently been named "Adenoviruses."

† This study was carried out at Fort Dix, N. J. using facilities of U. S. Army Hospital, Col. R. H. Eckhardt, Commanding; Fort Dix Health Center, Lt. Col. J. Cooch, Commanding; and Influenza Vaccine Evaluation Program of Commission on Influenza, Armed Forces Epidemiological Board, Dr. Harry Rose, Director.

¶ Tissue cultures and tissue culture fluids employed were obtained from Microbiological Associates, Bethesda, Md.

with 0.5 M monobasic sodium phosphate solution, had infectivity titers of 10^{-1} or 10^{-2} in HeLa cell cultures, complement-fixation (CF) titers of 1:2 or 1:4 and protein nitrogen values between 0.009 and 0.016 mg/ml. Prior to inactivation, the identity of each filtered virus lot was proved by serum neutralization tests performed with monotypic rabbit antisera. The failure of the mixtures of virus and homotypic serum to produce degeneration in monkey renal epithelium and HeLa† cell cultures identified the virus and showed the absence of other detectable cytopathogenic agents. To prepare vaccine, the filtered virus suspensions were treated with freshly redistilled formalin (assay 35.1%) to a final concentration of 1:4000 and held at 36°C in a water bath with occasional shaking for 6 days. This inactivation period was twice that required to render the virus non-infectious. Twenty ml aliquots of the formalin-treated filtrate were dialyzed at 4°C against Hank-Simms† solution to remove free formaldehyde. This same procedure performed on live virus preparations did not cause measurable reduction in the infectivity titers. To test for completeness of formalin inactivation, 10 ml amounts of the dialyzed vaccine were inoculated in duplicate into 32 oz flask cultures of renal epithelium maintained in mixture 199. These inocula did not induce significant cytopathogenic change during incubation at 36°C for 12 days nor did extracts of the cells from these cultures cause degeneration when passed to tube cultures of monkey kidney and observed for an additional 12-day period. For use in human beings, two bivalent pools (Lots 1 and 2) consisting of equal volumes of type 4 and type 7 formalin-inactivated vaccine were prepared, distributed in 20 ml amounts into rubber-stoppered dispensing bottles and stored at 4°C until used in the field trial.

Safety and sterility tests. The first series of safety tests was carried out on the virus preparations prior to the addition of formalin. Hamsters were inoculated intraperitoneally; suckling mice intracerebrally and intraperitoneally; adult mice intracerebrally; rabbits intraperitoneally, intracerebrally, and intracutaneously; guinea pigs intraperitoneally; and rhesus monkeys intracerebrally and intramuscularly. These animals were observed for periods of 21 to 42 days during which times there were no deaths and no fever or other signs of illness. No gross pathological lesions were noted at autopsy nor did Leptospirae grow from cultures of hamster blood. The monkeys did not develop detectable neutralizing antibody against Types I, II or III poliomyelitis virus, nor did they show evidence of poliomyelitis on histopathological examination‡ of the brain and cord. The virus preparations did not show detectable growth when cultured aerobically and anaerobically at 37°C and at room temperature on a variety of media§ chosen to detect fungi and bacteria, including *Mycobacterium, Brucella, Pasteurella,* pleuropneumonia and other human pathogens. The final safety and sterility tests on the prepared bivalent vaccine pools consisted of inoculating one ml of the vaccine into 400 ml portions of thioglycollate broth in triplicate and intraperitoneal inoculation of guinea pigs and mice. None of these tests showed any detectable microorganisms. *Virus laboratory tests. Virus recovery* attempts from patients' throat washings were carried out in duplicate in HeLa cell cultures and in the Henle strain of human intestine cells† by the same general methods described earlier(1). Passage of the triturated whole cultures was made at 3- to 7-day intervals and from 3 to 5 such serial passages were made before any specimen was discarded as negative. The recovered viruses were identified as agents of the RI family by demonstration of the presence of RI group CF antigen in the triturated whole culture and the strains were typed by methods already described(1) according to the classification system of Rowe, *et al.*(3).

‡ Histopathological examinations were made by Dr. J. E. Smadel.

§ These included Sabouraud's agar, trypticase soy agar, tryptose agar, blood agar, serum ascites agar, Dubos Tween albumin medium, Lowenstein's medium, egg-meat infusion, brain-heart infusion with 0.1% agar, and Brewer's thioglycollate broth. We are indebted to Miss S. Carey of the Dept. of Bacteriology, Walter Reed Army Institute of Research, for performing these tests.

TABLE I. Serological Response in Guinea Pigs to Monovalent Killed RI Virus Vaccines.

Vaccine		Homologous neutralizing antibody titer*		RI group, CF antibody titer*	
Virus type	Pool No.	Aqueous	Adjuvant	Aqueous	Adjuvant
4	1	2	32	0	20
	2	2	32	0	10
7	3	0†	32	0	0

* Titers are expressed as reciprocal of serum dilution.

† 0 titer value in the neutralization test equals less than 1:2 and in the CF test less than 1:5, the lowest serum dilutions tested.

Serology. The methods for the CF and neutralization tests were described previously(1). All serum titers are expressed as the greatest initial dilution of serum which caused complete or nearly complete suppression of virus growth (neutralization) or of fixation of complement.

Results. Neutralizing and CF antibody response to vaccination in guinea pigs and volunteers. Groups of six 800 to 1000 g guinea pigs were given one intramuscular injection of 0.25 ml of inactivated aqueous monovalent vaccine or 0.50 ml of an adjuvant-vaccine emulsion consisting of 0.25 ml of the vaccine and 0.25 ml of Arlacel in mineral oil (10% Arlacel, 90% Drakeol) mixture.‖ The animals were bled 4 weeks post-inoculation and pooled aliquots of the sera tested for content of homologous neutralizing and RI group CF antibodies. As shown in Table I, the neutralizing antibody titer resulting from injection of type 4 aqueous vaccine was 1:2 and from type 7 vaccine was below the detectable level. The neutralizing antibody response to all adjuvant preparations was 1:32. The only vaccine which stimulated CF antibody production was Type 4 in adjuvant.

To test the vaccine in man, each of 12 volunteers, military or civilian personnel of the Walter Reed Army Institute of Research, were given 1 ml of the bivalent preparation into the triceps muscle. Table II, which shows the CF and neutralizing antibody titers of the individual sera from 5 volunteers collected prior to and 3 weeks post-vaccination, illustrates the variety of antibody response in the group as a whole. It is seen in the table that nearly all persons developed an increased amount of neutralizing antibody against not only homotypic 4 and 7 virus but heterotype 3 as well. These increases in titer were as great as ordinarily obtained following the natural disease(1,5,6). Volunteers 1, 2 and 3, who received 2 additional injections of the vaccine at 3- and 5-week intervals, failed to show any further increase in neutralizing antibody. In no instance has repeated injection been found superior to a single injection alone. Measurable CF antibody response was very infrequent and of low titer as illustrated by volunteer 2 in Table II.

Field Evaluation. Study population. The clinical evaluation of the vaccine was made at Fort Dix, N. J., an Army post where recruits are given basic training. At this post, the RI virus infection rates have been consistently high during the winter months in recent years(5,7). *Organization of the study.* At the time this study was undertaken, an influenza vaccine evaluation program was being conducted at Fort Dix by the Commission on Influenza, Armed Forces Epidemiological

‖ Supplied by Dr. I. W. McLean, Parke-Davis and Co., Detroit, Mich.

TABLE II. Serological Response in 5 Volunteers to Bivalent Types 4 and 7 Killed RI Virus Vaccine.

Serum specimen	Neutralizing antibody titer*			RI group CF antibody titer*
	Homotype 4	Homotype 7	Heterotype 3	
Pre-vac.†	0‡	2	0	0‡
3 wk post-vac.	32	32	2	0
Pre-vac.	2	0	0	0
3 wk post-vac.	8	8	8	5
Pre-vac.	2	2	2	0
3 wk post-vac.	2	8	8	0
Pre-vac.	0	0	8	0
3 wk post-vac.	8	128	128	0
Pre-vac.	0	0	0	0
3 wk post-vac.	32	32	2	0

* Titers are expressed as reciprocal of serum dilution.

† Pre-vac. = Pre-vaccination. Post-vac. = post-vaccination.

‡ 0 titer value in the neutralization test equals less than 1:2 and in the CF test less than 1:5, the lowest serum dilutions tested.

Board, under the direction of Dr. Harry Rose. In this program, influenza vaccine was given to the first 100 men assigned to each company with the remaining 100 to 150 men of the unit being retained as controls. By arrangement with Dr. Rose, the control groups of all 6 companies which began basic training between 20 February and 5 March 1956, were made available for the evaluation of the RI virus vaccine. These were divided into two groups by the following procedure. As rosters were received assigning the men to a company, case numbers were given in sequence to the men entering the study. During the precycle week, a team of technicians visited each unit, drew a prevaccination blood sample and gave the initial inoculation into the triceps muscle. In the first company, recruits whose case numbers were odd received 1 ml of the RI vaccine (Lot 1) and those whose case numbers were even were given a formalin-saline placebo and retained as controls. In the second company, the procedure was reversed and the even-numbered men received the vaccine and the odd-numbered men the placebo. This alternation of odd and even numbers by company was carried out until all 6 companies were vaccinated. A 1 ml dose of Lot 2 vaccine or placebo was given approximately 7 days following the first injection. In all, 311 soldiers received vaccine while 313 received placebo. Additional specimens were drawn from all soldiers in the study group at some time during the third and seventh week of training. Complete records were kept of all hospital and dispensary admissions from the 6 companies under study. All recruits admitted to the hospital with a diagnosis of any acute respiratory condition were bled at the time of admission and again approximately 3 weeks later. In addition, a throat washing for virus recovery was collected in nutrient broth from the patients while in the admitting room. These were frozen immediately in dry ice in tightly sealed screw-cap jars and transmitted to the laboratory at the Walter Reed Army Institute of Research where they were maintained in dry ice until tested 3 to 10 weeks later. All blood specimens were collected in vacuum venules and transmitted to the same laboratory where the serum was separated from the clot and stored at 4°C until tested 2 to 3 months later.

TABLE III. Total Hospital Admissions for Respiratory Illness and Admission Rates in Vaccinated and Control Groups.

Inoculum	No. of persons in group	No. of hospitalized cases from group	Admission rate in %
Cases occurring during entire period of observation:			
RI vaccine	311	32	10.3
Formalin-saline control	313	89	28.4
Cases occurring 7 days or more following initial inoculation:			
RI vaccine	293	14	4.8
Formalin-saline control	294	70	23.8

Clinical evaluation of RI vaccine effectiveness. The numbers and percentages of soldiers hospitalized from acute respiratory illness in the 311 vaccinated and 313 control recruits are summarized in Table III. The overall figures for the entire period of observation are not particularly impressive since 10.3% of the soldiers receiving vaccine were hospitalized compared with 28.4% of those given placebo. However, when the cases occurring during the first week post-vaccination are excluded, the findings are dramatic. Only 4.8% of the vaccinated were hospitalized compared with 23.8% of the controls. The importance of considering the interval between vaccination and onset of illness is illustrated in Fig. 1 which shows the admissions for each week from time of first vaccination. Essentially the same number of cases were hospitalized in each group during the prevaccination period and first week following the initial dose. However, during the 2nd, 3rd and 4th weeks, about 11 times as many cases developed in the control group as occurred among an equal number of vaccinated soldiers. During the last 4 weeks of basic training, at a time when the susceptibles were largely depleted, very few cases of respiratory illness occurred and no difference was apparent in the incidence in the two groups. The beneficial effect of vaccination, therefore, did not appear until one week after the initial vaccination and disap-

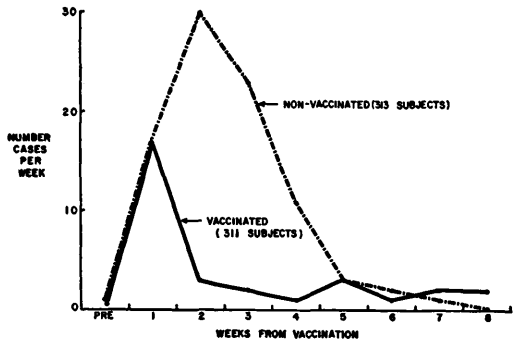

FIG. 1. No. of hospital admissions for acute respiratory illness—vaccinated and control subjects by week from vaccination. Fort Dix, N. J., winter, 1956.

peared during the last 4 weeks.

Laboratory findings. The results of the virus isolation and serological tests showed that approximately 9 of every 10 cases of acute respiratory illness admitted to the hospital during the study period were of RI etiology showing that the vaccine was tested during the course of an epidemic of RI virus infection. Forty-nine RI viruses were recovered from throat washings from 65 patients hospitalized for acute respiratory illness. Virus was recovered from 4 of 12 (33%) vaccinated individuals and 37 of 49 (75%) non-vaccinated persons. Among 41 RI virus isolates typed to date, 24 (59%) were type 7 and 17 (41%) type 4. The percentage distributions of types within the vaccinated and non-vaccinated groups were essentially the same. Acute and convalescent sera from 97 patients, tested for amount of RI CF antibody, revealed a diagnostic (4-fold or greater) increase in antibody titer in 78 which was related temporally to the clinical illness. Eighteen of 28 (64%) vaccinated persons and 60 of 69 (87%) non-vaccinated individuals showed such increase in antibody.

Discussion. The relatively low infectivity titers, 10^{-1} or 10^{-2}, of the virus preparations prior to inactivation would ordinarily suggest insufficient quantity of antigen to permit its use as a vaccine without virus concentration. However, the infectivity titers of such preparations do not reflect the true content of virus. Electron microscopy has shown virus particle counts of 10^9 in similar preparations which titered 10^{-2} or 10^{-3}(20) indicating the presence of amounts of virus material which might reasonably be expected to be immunogenic. The first confirmation of this hypothesis was the demonstration of detectable neutralizing antibody levels in sera of guinea pigs following injection of aqueous or adjuvant preparations. The neutralizing antibody response in guinea pigs to the aqueous vaccine was low, as might be expected in animals with no previous experience with these antigens. The response in man, however, was quite different since relatively high levels of antibody were readily achieved with this vaccine. Further experience(9) has shown that maximal or near maximal levels of neutralizing antibody are achieved within 1 week after a single injection of vaccine. This, probably, is a manifestation of the recall phenomenon in persons with previous experience with RI antigens common to types 4 and 7, if not the result of earlier infection with types 4 and 7 viruses themselves. The fact that additional injections of vaccine after the first failed to increase the antibody level is consistent with the hypothesis that the first dose of vaccine acts to elicit a recall type of response.

The vaccine has now been given to approximately 350 persons, in single or multiple doses, without untoward effect. The low protein content of the vaccine, which is in the range of that of the current poliomyelitis vaccine, suggests that the production of sensitivity to monkey kidney component is not likely to be a problem.

The administration of this RI virus vaccine to newly inducted recruits effected a striking reduction in the incidence of hospitalized cases of acute respiratory illness. Although no beneficial effect was apparent during the first week after vaccination, the reduction of cases beginning in the second week and continuing in the third and fourth weeks post-vaccination was dramatic. This interval corresponds to the time when the vast majority of cases of RI disease have been shown to occur in recruit camps(7). During this period of high incidence, 64 cases occurred among 313 placebo-inoculated troops compared with only 6 among the 311 who received the RI

vaccine. After the fourth week, the incidence fell to low levels in both vaccinated and control groups. This pattern conforms to previous experience in recruit camps(7) and would seem to indicate the emergence of respiratory illnesses of non-RI etiology in troops among whom the RI susceptibles have been reduced to a low level. This hypothesis is strengthened by the fact that most of the late cases tested to date have been shown to be of non-RI etiology. It would appear that one dose of vaccine is sufficient to protect since, as described above, it acts as a recall mechanism. Hence, the second dose, as given in this study, is probably unnecessary. The failure of the vaccine to protect during the first week was expected and can be explained readily when one adds the known 4- to 5-day incubation period of RI virus disease to the 3 or 4 days necessary for recall antibody to develop. No information was obtained with respect to the duration of the immunity gained as a result of RI vaccination. The disappearance of RI illnesses from these companies after the fourth week rendered it impossible to study this question.

It is of interest to note that the pattern of cases of acute respiratory disease among the recruits who received influenza vaccine paralleled that of the control group throughout the training period. These men did not form part of the study group but nonetheless may be used as a secondary control to confirm the efficacy of RI vaccine.

As has been noted, the initial dose of vaccine was given after the men were assigned to their training companies and after cases of RI virus disease had already begun to occur in the men. A more marked overall reduction in the incidence could be expected if the vaccine were administered at the time the recruit is first inducted into the Army or as soon as possible after his arrival at the training base. By doing this, the incidence could be reduced in the first week of the training cycle.

It should also be noted that this trial was conducted during a season when little influenza was present on the base. The total percentage reduction in the incidence of acute respiratory illness brought about by RI vaccine will, of course, vary greatly depending upon the current incidence of influenza and other respiratory conditions of non-RI virus etiology.

Summary. The development, preparation and field evaluation of a highly effective formalin-killed vaccine against acute respiratory illnesses caused by viruses of the RI (Adenovirus) family has been described. The vaccine, prepared from infected tissue cultures of monkey renal epithelium, contained types 4 and 7 virus and was not injurious upon human injection. In human volunteers given a single 1 ml dose of vaccine intramuscularly, it induced high levels of neutralizing antibody. In 311 recruits, vaccinated at Fort Dix, N. J., it produced a marked reduction in the incidence of acute respiratory illness beginning one week after the initial dose of vaccine; only 4.8% of those receiving RI vaccine were hospitalized for respiratory illness compared with 23.8% of the control group of 313 which received placebo.

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Effects of Mescaline in Laboratory Animals and Influence of Ataraxics on Mescaline-Response. (22485)

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Mescaline has been of continuing interest to biologists because of its ability to produce "model psychosis" in man(1) as well as experimental catatonia in laboratory animals (2). Current interest has been stimulated by the findings that α-(4-piperidyl)benzhydrol HCl (azacyclonol, Frenquel) antagonizes mescaline-induced hallucinosis in man(3,4), electroencephalographic changes in man(5) and rabbits(6), and spasm of isolated estrus-rat uteri(7). Because of preliminary clinical reports on the beneficial effects of azacyclonol in mentally-disturbed patients, this drug has been included in a category of drugs known as "ataraxics" or "tranquilizers" together with reserpine and chlorpromazine(4). The present study was undertaken, first, to determine whether or not reasonably objective indications of behavioral response could be obtained following mescaline in laboratory animals and, second, to study the ability of ataractic compounds to antagonize the effects of mescaline on behavior.

Materials and methods. Mescaline sulfate was administered to dogs and cats intravenously, intraperitoneally, and into the lateral ventricle of the brain after the method of Feldberg and Sherwood(8,9). Permanent intraventricular cannulae of stainless steel were implanted in the cranium and covered with a skin-flap, placement being confirmed by x-ray or dissection following death or sacrifice. These cats showed no apparent ill effects from the cannulae. Routinely, antibiotics (500 mg dihydrostreptomycin and 100,000 U penicillin G, intramuscularly) were administered for 3 days after operation, as well as the day of and day after each intraventricular experiment. These experiments were not begun until at least one week after operation and the cats were rested 5-10 days between tests. Drugs were administered intraventricularly in volumes of 0.5 ml or less under sterile conditions, with the cats held under minimal restraint. The volume of the cannulae was approximately 0.1 ml.

Results. Intravenous mescaline in dogs. In 6 experiments on 4 dogs, 20 mg/kg of mescaline caused repeated defecation and urination, tachypnea, salivation, mydriasis, and apparent dizziness. These effects lasted 10-20 minutes and were succeeded by a 4-hour period during which the dogs lay or stood nearly motionless in one place, exhibiting marked disregard for and non-reactivity to visual, auditory, and pain stimuli. This condition resembled that in cats described by de Jong(2), which he termed "catatonia," as well as that recently described in dogs by Hosko and Tislow(10); it also duplicated some of the effects of mescaline in man, such as salivation, emesis, mydriasis, immobility, and decreased reactivity to pain(2,11,12). When the dogs were forced to move, they