AMERICAN JOURNAL OF EPIDEMIOLOGY
Vol. 89, No. 4

Printed in U.S.A.

FIELD EVALUATION OF A RESPIRATORY SYNCYTIAL VIRUS VACCINE AND A TRIVALENT PARAINFLUENZA VIRUS VACCINE IN A PEDIATRIC POPULATION[1]

JAMES CHIN, ROBERT L. MAGOFFIN, LOIS ANN SHEARER, JACK H. SCHIEBLE, AND EDWIN H. LENNETTE

(Received for publication August 8, 1968)

Chin, J. (Calif. State Dep't. Public Health, Berkeley 94704), R. L. Magoffin, L. A. Shearer, J. H. Schieble and E. H. Lennette. Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. *Amer. J. Epid.*, 1969, 89: 449–463.—A field evaluation of two formalin-inactivated respiratory virus vaccines was conducted in a selected pediatric population in California during the 1966–1967 respiratory disease season. A total of 441 children ranging in age from four months to nine years were immunized; 219 with a respiratory syncytial (RS) virus vaccine and 222 with a trivalent parainfluenza virus (types 1, 2 and 3) vaccine. Both vaccines elicited good serum antibody responses. Very high attack rates of parainfluenza virus, types 1 and 3 and RS virus were observed during the study period in infants and children in both vaccine groups. A protective effect was not demonstrable for either vaccine. Infants who received the RS virus vaccine and who subsequently became infected with RS virus tended to have a more severe clinical illness than infants who did not receive this vaccine.

antibody; parainfluenza; pediatric; respiratory; respiratory syncytial; vaccine; virus

INTRODUCTION

The association of the respiratory syncytial (RS) and parainfluenza viruses with acute respiratory illness (ARI) in infants is well documented (1–5). These agents have been shown to account for most of the serious respiratory illnesses in infants. The RS virus has been most frequently associated with bronchiolitis and pneumonia in very young infants while the parainfluenza viruses are associated with croup and severe lower respiratory illness in older infants and in pre-school age children. For these reasons, a high priority has been given to the development of effective immunoprophylaxis against these agents by the Committee for Vaccine Development (CVD) of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. This committee was established in 1961 to promote the development of virus vaccines. In late summer, 1966, the CVD released for field evaluation a trivalent parainfluenza virus

[1] From the Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley, 94704.

This study was supported by a contract (PH-43-63-1141) from the Committee for Vaccine Development, National Institute of Allergy and Infectious Diseases, United States Public Health Service, Department of Health, Education and Welfare.

Reprint requests to Dr. Chin, Calif. State Dept. of Public Health; or to the Vaccine Development Branch, N.I.A.I.D., N.I.H., Bethesda, Md. 20014.

The cooperation of the Office of the Post Surgeon and the medical staff of the U. S. Army Hospital, Fort Ord, in the conduct of this study is gratefully acknowledged. The authors also thank Dr. John C. Bolton and Dr. Peter A. Ahmann for their participation in this study during their active duty as Epidemic Intelligence Service Officers, Communicable Disease Center, U. S. Public Health Service.

vaccine and an RS virus vaccine. This report will describe the results of a field study with these two vaccines in a selected pediatric population at Fort Ord, California, during the 1966–1967 respiratory disease year.

Materials and methods

RS virus vaccine. (Lot No. 100, Pfizer.) The seed virus (Bernett strain) was grown in Vervet monkey kidney cell cultures. After formalinization, the tissue culture fluid harvest was concentrated 25-fold by ultracentrifugation and then further concentrated fourfold by alum precipitation. The final concentration factor was 100.

Trivalent (types 1, 2, and 3) parainfluenza virus vaccine. (Lot 6364, National Drug Company.) This vaccine was derived from amniotic fluid harvested from seven-day-old chick embryos infected with parainfluenza virus, types 1, 2, or 3. The seed virus strains were, type 1 (C39), type 2 (Greer) and type 3 (C-243).

The amniotic fluid for each virus type was concentrated approximately threefold by ultracentrifugation. The three types of parainfluenza virus infected amniotic fluids were inactivated with formalin prior to pooling.

Study area. The field study was located at Fort Ord, California, a permanent Army Post whose primary function is to train recruits from the western part of the United States. Fort Ord is situated on the shores of Monterey Bay, about 100 miles south of San Francisco and Berkeley, and is near the cities of Monterey, Carmel and Salinas, California. The dependents of the permanent party personnel live in about 3,000 single and duplex units on Post and in private residences off Post, mainly in the small continguous communities of Seaside and Marina.

Study population. The population studied consisted of those children of Army personnel living on Post or in the closely adjacent areas. The total pediatric population that received medical care at Fort Ord was estimated to be about 19,000. Of this total, slightly less than half were of preschool age and about one-tenth were infants under one year of age. This population received essentially all of its medical care from the Fort Ord Medical Facilities, thus providing the field staff an opportunity to maintain close surveillance of all medically attended illnesses which occurred in these children.

The following two groups of children at Fort Ord were included in the vaccine field study. Group I was comprised of infants who had been participating in a longitudinal study of ARI since their birth. In September, 1966, infants in this latter study who were four months of age or older, and their siblings under 10 years of age, were asked to participate in the vaccine study. Group II consisted of those children who were attending the pre-school nursery on Post and their siblings under 10 years of age.

Method of vaccine allocation and administration. Parainfluenza or RS virus vaccine was given alternately to infants who were enrolled in the longitudinal study and to children attending the pre-school nursery. The siblings of these children who were under 10 years of age were also given one of the vaccines on an alternate basis. The alternation in Group I was dependent on the vaccine which was assigned to the longitudinal study infant. Thus, all families with more than one child received both vaccines. The only exception to this system of assignment was when a child had a known history of allergy to eggs. These children were automatically assigned to the RS vaccine group and the alternation order of their siblings changed if necessary.

The dosage and schedule of vaccine administration were identical for both vaccine groups. Two 0.5 ml injections were given, intramuscularly, a month apart. The vaccines were given to small infants in their left anterio-lateral thigh. In older children, the left deltoid area was used. A double blind system of vaccine adminis-

tration was used. Neither the parents nor the field staff involved in the follow-up observations of these children were aware which vaccine preparation a child had received. Capillary blood specimens were collected on filter paper discs from all children in Group I at the time of the first vaccine injection and approximately one month after the second vaccine injection.

Surveillance of ARI. In order to establish illness baselines for the pediatric population under study, surveillance of all ARI admitted to the hospital was started in late 1964, and continued through the vaccine study period. Specimens for viral etiologic studies consisting of a throat swab and acute and convalescent phase blood samples were collected. In addition, a throat swab for virus isolation and a medical history were obtained on *all* medically attended ARI occurring in the vaccine study groups. When a child in the vaccine study attended the emergency clinic, a home visit was made to obtain the necessary information and throat swab. The ARI experience of infants in Group I was further followed by routine home visits which were scheduled at four month intervals. During this visit, an interim medical history, a throat swab, and capillary blood absorbed onto filter paper discs were collected.

Laboratory methods

The main laboratory support for this field study was located in the Viral and Rickettsial Disease Laboratory of the California State Department of Public Health, Berkeley. In addition, a small field laboratory was maintained at Fort Ord.

Virus isolation. For virus isolation, two host tissues, human fetal diploid lung (HFDL) and primary rhesus monkey kidney (MK) were routinely employed. The HFDL cell strains used were initiated and maintained in the laboratory at Berkeley according to the method of Hayflick and Moorhead (6). Tube cultures of HFDL cells were seeded with 10^5 cells in Eagle's minimum essential medium with 10 per cent fetal bovine serum and outgrowth was usually complete in three to four days. MK cell cultures were prepared by a modification of the method of Bodian (7). Outgrowth medium for MK cells consisted of 85 ml of Hank's balanced salt solution, 10 ml of 5.0 per cent lactalbumin hydrolysate and 2 ml fetal bovine serum. For maintenance of both cell types, Leibovitz Medium No. 15 with 2 per cent fetal bovine serum was used (8). All media contained 100 u of penicillin, 100 mcg of streptomycin and 10 mcg of amphotericin B per ml.

Throat swabs, collected from children admitted to the hospital for an ARI and from children participating in the vaccine study who were seen in the pediatric clinic for an ARI, were placed in 2-dram screw cap vials containing 3 ml of tryptose phosphate broth and 0.5 per cent gelatin. Within two hours of collection, a 1 ml sample of the specimen was removed, treated with 1000 u penicillin, 5000 u streptomycin and .01 mg of Fungizone for an hour at 4 C and then 0.2 ml of the treated specimen were inoculated into two tubes each of HFDL and MK cell cultures. After inoculation the cell cultures were placed in a roller drum and incubated at 33 C. The inoculated cell cultures were transported to Berkeley weekly and fresh cell cultures sent to the field laboratory at Fort Ord.

The inoculated cell cultures were maintained for approximately two weeks without a change of medium and periodically examined for cytopathic effects and hemadsorption of guinea pig red cells. Negative cultures were routinely passed to new cultures and observed for an additional week. Presumptive identification of viruses was based on the type of cytopathic effect observed in the cultures and on the presence or absence of hemadsorption. Final identification was made by serological procedures. Parainfluenza viruses were identified by serum neutralization tests using

hemadsorption as an index of infection. Identification of RS virus was made by the direct fluorescent antibody technique utilizing anti-RS (Long strain) monkey serum (9).

Serologic methods. The procedures for obtaining and handling specimens of capillary blood collected onto filter paper discs for serologic study have been presented in detail in another paper (10). All tests were conducted in the microtiter system. Antibody titer of blood specimens collected from children hospitalized for an ARI were determined by complement fixation (CF) tests for influenza viruses A and B, adenovirus, RS virus, parainfluenza virus, type 4 and *Mycoplasma pneumoniae,* and by hemagglutination-inhibition (HI) tests for parainfluenza virus types 1, 2, and 3. The antigens employed have been described in detail in a previous paper (10). Antibody response to the RS virus vaccine was measured by the CF test and the response to the parainfluenza virus vaccine by HI tests for virus types 1, 2, and 3. The blood samples collected from infants in the longitudinal study were tested for CF antibody to RS virus, adenovirus and parainfluenza virus, type 4, and for HI antibody to parainfluenza virus types 1, 2, and 3.

Results

Immunizations with the vaccines were started on September 6, 1966. Priority was given to infants in the longitudinal study group as this was considered the group at highest risk to infection with the RS virus and the parainfluenza viruses. Immunization of most of the children was completed by November and no injections with either vaccine were given at Fort Ord after December 15, 1966. A total of 441 children received one of the two vaccines; 56 of these children received only one injection of vaccine. A total of eight children in this study had a history of allergy to eggs. All of these children were given RS vaccine.

Table 1

Age distribution of children in the vaccine field study at Ford Ord, California by type and dose of vaccine received, September through December, 1966

Age at start of immunization	RS vaccine recipients			Parainfluenza vaccine recipients		
	No. of injections			No. of injections		
	Two	One	Total	Two	One	Total
<1 Yr	43	2	45	43	9	52
1–4 Yrs	99	14	113	91	11	102
5–9 Yrs	49	12	61	60	8	68
Totals	191	28	219	194	28	222

The age distribution of all the children who participated in the vaccine field study is presented in table 1, according to the number of injections and type of vaccine received. The distribution is similar for both vaccine groups. For the remainder of this report, only those children who received two injections of their assigned vaccine (191 in the RS group and 194 in the parainfluenza group) will be compared for evaluation of vaccine efficacy. From October, 1966, to July, 1967, a total of 117 children who completed their vaccine injections were lost to the study because of the unexpected transfer of their fathers. The distribution of these children by month and age in the two vaccine goups was essentially similar during the period of vaccine evaluation. Although there was a gradual loss of children from the vaccine study during this period, the results presented in this report will be based on the number of children at the start of the study.

Reaction to the vaccines. No immediate reactions to the vaccines were observed except that one of the vaccines (the parainfluenza vaccine) was noted to elicit smarting at the site of injection, but this usually lasted only a few minutes. The parents of the first 144 children who received the vaccines were questioned about reactions to the vaccines by telephone at 24 or 48 hours after vaccine administration. Of these children, information regarding reactions to the second vaccine dose was obtained on 110. The reactions re-

ported by mothers from this telephone survey are summarized in table 2. No difference can be seen between the two vaccine groups. A placebo group was not available for comparison and thus it cannot be definitely established whether the fevers reported were attributable to the vaccines or to coincidental causes. However, of the 10 children reported to have fever, a rectal temperature was taken by only 3 mothers, and none exceeded 101 F. No reactions to the vaccines were reported by any of the mothers not contacted in the telephone survey.

TABLE 2

Results of telephone survey of reactions to test vaccines. Fort Ord, California, September–October, 1966

	No. of children	Reactions reported		
		Fever	Erythema	Calor
First dose				
RS vaccine	72	3	1	0
Parainfluenza vaccine	72	2	0	0
Totals	144	5	1	0
Second dose				
RS vaccine	52	2	0	1
Parainfluenza vaccine	58	3	0	1
Totals	110	5	0	2

Pre- and post-vaccine blood samples. A total of 113 infants in the longitudinal study group at Fort Ord received the primary series of one of the two vaccines. Pre- and post-vaccine blood specimens were collected from 111 of these infants. Of the 173 siblings of these infants who completed the full primary series, pre- and post-vaccine blood specimens were collected from 168. Of these totals, 243 had their post-vaccine blood specimen collected before December 15, and the antibody responses to the vaccine administered are

PARAINFLUENZA VIRUS VACCINE GROUP

pre-vaccine titer	post-vaccine titer							Total	4x or > rise	
	<1:8	1:8	1:16	1:32	1:64	1:128	1:256		No.	%
<1:8	1	4	18	20	15	8		66	61	92.4
1:8		1	6	9	4	3	1	24	17	70.8
1:16		1	1	2	3	1		8	4	50.0
1:32				1	5	3	2	11	5	45.5
1:64					5	2	2	9	2	22.2
1:128							1	1	-	-
1:256							1	1	-	-
	1	6	25	32	32	17	7	120	89	74.2

RS VIRUS VACCINE

pre-vaccine titer	post-vaccine titer							Total	4x or > rise	
	<1:8	1:8	1:16	1:32	1:64	1:128	1:256		No.	%
<1:8	60	21	3					84	3	3.6
1:8	2	6						8	-	-
1:16		1	6	2				9	-	-
1:32				9	4			13	-	-
1:64				1	5	1		7	-	-
1:128							1	1	-	-
1:256							1	1	-	-
	62	28	9	12	9	1	2	123	3	2.4

FIGURE 1. HI antibody levels to parainfluenza virus, type 1 in children participating in the vaccine field study at Fort Ord, Calif., September to December, 1966.

PARAINFLUENZA VIRUS VACCINE GROUP

pre-vaccine titer	post-vaccine titer								4x or > rise	
	<1:8	1:8	1:16	1:32	1:64	1:128	1:256	Total	No.	%
<1:8	8	13	28	23	12	3	1	88	67	76.1
1:8	2	4	2		1			9	1	11.1
1:16				3	3	1	2	9	6	66.7
1:32				1	2	3	1	7	4	57.1
1:64					1	5		6	-	-
1:128								-	-	-
1:256							1	1	-	-
	10	17	30	27	19	12	5	120	78	65.0

RS VIRUS VACCINE

pre-vaccine titer	post-vaccine titer								4x or > rise	
	<1:8	1:8	1:16	1:32	1:64	1:128	1:256	Total	No.	%
<1:8	90	3	2	1				96	3	-
1:8	2	1	3					6	-	-
1:16		1	5	2				8	-	-
1:32				6	1			7	-	-
1:64				2	2	1		5	-	-
1:128								-	-	-
1:256							1	1	1	-
	92	5	10	11	3	1	1	123	3	2.4

FIGURE 2. HI antibody levels to parainfluenza virus, type 2 in children participating in the vaccine field study at Fort Ord, Calif., September to December, 1966.

presented for these children. Post-vaccine blood specimens collected after December 15 were omitted from the study of vaccine antibody response because of the increased incidence of both RS and parainfluenza virus infections after this date.

HI antibody response to parainfluenza vaccine. Figures 1, 2, and 3 present the pre- and post-vaccine HI antibody titers to parainfluenza virus, types 1, 2, and 3, respectively. In the RS virus vaccine group, which serves as a control group, there were very few (<3 per cent) significant (fourfold or greater) HI antibody rises to parainfluenza virus, types 1 and 2; but 17 children (14 per cent) showed significant HI antibody rises to parainfluenza virus, type 3. These latter antibody rises are in keeping with the surveillance results which indicated that parainfluenza virus, type 3 infections were occurring at Fort Ord during the period when immunizations with these vaccines were being given.

In the parainfluenza virus vaccine group, HI antibody responses to virus types 1 and 2 were very good. Most (92 per cent for type 1 and 76 per cent for type 2) of the children without pre-vaccine antibody (<1:8) to virus types 1 and 2 showed a significant response to the vaccine. Children with pre-vaccine antibody to these virus types developed significant rises in antibody titer less often. Pre-vaccine HI antibody titers to type 3 parainfluenza virus were, as expected, relatively high. The antibody response to the type 3 virus component was not as good as that shown for the other two types. Only 54 per cent of children with no pre-vaccine antibody to parainfluenza 3 developed a significant rise in antibody to the type 3 virus.

CF antibody response to the RS virus

PARAINFLUENZA VIRUS VACCINE GROUP

pre-vaccine titer	post-vaccine titer							Total	4x or >rise	
	<1:8	1:8	1:16	1:32	1:64	1:128	1:256		No.	%
<1:8	8	8	7	4	3	3	2	35	19	54.3
1:8	3	2	1	2	2			10	4	40.0
1:16	1	3	2	5	3	3	2	19	8	42.1
1:32				5	13	4	1	23	5	21.7
1:64					4	6	6	16	6	37.5
1:128					2	5	3	10	-	-
1:256						1	5	6	-	-
	12	13	10	16	27	22	19	119	42	35.3

RS VIRUS VACCINE

pre-vaccine titer	post-vaccine titer							Total	4x or >rise	
	<1:8	1:8	1:16	1:32	1:64	1:128	1:256		No.	%
<1:8	22	1		3	2		1	29	6	20.6
1:8	2	9	3	3	1	1	1	20	6	30.0
1:16	2	2	9	5	2		1	21	3	14.3
1:32			4	16	4		1	25	1	4.0
1:64				1	4	3	1	9	1	11.1
1:128					4	4	4	12	-	-
1:256						2	4	6	-	-
	26	12	16	28	17	10	13	122	17	13.9

FIGURE 3. HI antibody levels to parainfluenza virus, type 3 in children participating in the vaccine field study at Fort Ord, Calif., September to December, 1966.

vaccine. Figure 4 presents the pre- and post-vaccine CF antibody titers to the RS virus for the two vaccine groups. In the parainfluenza vaccine group which serves as a control group for the RS vaccine, only one fourfold rise occurred. The frequency of CF antibody response in the RS vaccine recipients was very high. Of the 120 children tested in this group, 82 (68 per cent) had fourfold or greater rises. Of interest is the finding that the significant antibody responses to the RS vaccine occurred as frequently, or more so, in children with detectable pre-vaccine antibody levels, as in those with no pre-vaccine antibody. Of the 75 children initially seronegative (CF antibody <1:8), 47 (63 per cent) had a fourfold or greater antibody response while 35 of the 45 (78 per cent) children initially seropositive (CF antibody $\geq$1:8) had a significant antibody rise to the vaccine. The antibody response to the RS vaccine by age at the start of immunizations is shown in table 3. Response to this vaccine was markedly less satisfactory in infants than in older children.

Surveillance of ARI. Virus isolation and serologic test results confirmed that the RS virus and the parainfluenza viruses are the most frequent agents associated with severe respiratory illnesses in a pediatric population. With the exception of the adenoviruses, no other virus or group of viruses was detected in any significant number. The pattern of pediatric hospital admissions at Fort Ord for ARI by months, with reference to admissions associated with an RS or a parainfluenza virus infection is shown in figure 5. High rates of parainfluenza (mainly type 1) and RS virus infections were found in the portion of the 1964–1965 respiratory disease sea-

RS VIRUS VACCINE GROUP

pre-vaccine titer	post-vaccine titer							4x or >rise	
	<1:8	1:8	1:16	1:32	1:64	≥1:128	Total	No.	%
<1:8	12	16	18	8	10	11	75	47	62.7
1:8	1	1	4	4	13	5	28	22	78.6
1:16		1		2	4	5	12	9	75.0
1:32					1	4	5	4	80.0
	13	18	22	14	28	25	120	82	68.3

PARAINFLUENZA VIRUS VACCINE GROUP

pre-vaccine titer	post-vaccine titer							4x or >rise	
	<1:8	1:8	1:16	1:32	1:64	≥1:128	Total	No.	%
<1:8	67	6	1				74	1	1.4
1:8	10	22					32	-	-
1:16		2	4				6	-	-
1:32									
	77	30	5				112	1	0.9

FIGURE 4. CF antibody levels to RS virus before and after test vaccine administration in two groups of children at Fort Ord, Calif., September to December, 1966.

TABLE 3

Complement fixing antibody response to the respiratory syncytial (RS) virus in RS virus vaccine recipients, Fort Ord, California, September–December, 1966

Age at start of immunization	No.	4X or greater antibody rise	
		No.	Per cent
<6 Months	14	5	35.7
6-18 Months	42	23	54.8
2-9 Years (Mean ~4 yrs)	64	54	84.4
Totals	120	82	68.3

son which was studied. During the 1965–1966 respiratory disease season, relatively low rates of parainfluenza viruses (mainly type 3) and RS virus infections were detected. The 1966–1967 season, which spans the whole vaccine field study was marked by an intense outbreak of RS virus infections which peaked in January. Parainfluenza virus infections were encountered frequently early in the season (September through November, mainly type 3) and also late in the season (March through May, mainly type 1).

Respiratory illnesses in the two vaccine groups. Table 4 shows the cases of medically attended ARI observed in the two vaccine groups from October through July. No significant difference (at the 5 per cent level of significance) is seen between the two groups for the entire study period or for any portion of the study period. As expected, the ARI rates decreased sharply with increasing age. The attack rate of those children who were <1 year of age at the start of their test immunizations was about eight times higher than the rate in children who were of school age at the start of the immunizations. In the one to four year age group, there was a consistently lower ARI rate for children who received the RS vaccine compared to the children who received parainfluenza vaccine, but this difference was not significant at the 5 per cent level.

Parainfluenza virus infections in the vaccine study groups. Parainfluenza virus infections (mainly type 3) were detected in the hospital surveillance at the end of

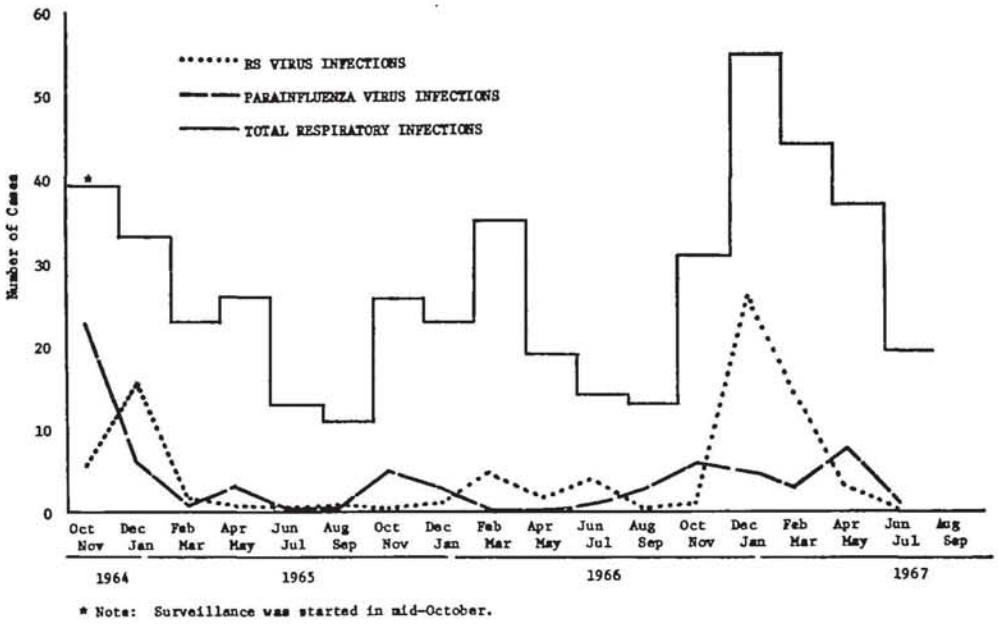

FIGURE 5. The distribution by months of admission of RS and parainfluenza virus infections, as diagnosed by virus isolation and/or a fourfold or greater antibody rise among children admitted to the Fort Ord Hospital because of an acute respiratory illness.

TABLE 4

Distribution by age and month of medically attended acute respiratory illness occurring in children in the viral respiratory vaccine field study at Fort Ord, California, 1966–1967

		No. of respiratory illnesses							
Age* (years)	Vaccine group	Oct., Nov.	Dec., Jan.	Feb., March	April, May	June, July	Total	No. at risk	AR/100
<1	RS	13	25	31	22	14	105	43	244.2
	Para	22	32	22	13	17	106	43	246.5
1–4	RS	16	26	21	16	17	96	99	97.0
	Para	17	33	24	20	15	109	91	120.0
5–9	RS	2	3	3	2	3	13	49	26.5
	Para	1	4	4	2	6	17	60	28.3
Total	RS	31	54	55	40	34	214	191	112.0
	Para	40	69	50	35	38	232	194	119.6

* Age at the start of immunization.

September, 1966, when immunization with the two vaccines was in progress. Table 5 shows the parainfluenza viruses isolated from children after completion of two injections of their assigned vaccine. No significant difference between the two vaccine groups with regard to the parainfluenza types and to the infection rate with these parainfluenza virus types was found. Clinically, the parainfluenza virus infections detected in both vaccine groups were similar and tended to be mild. Only one

TABLE 5

Distribution by age and month of isolation of parainfluenza virus infections in children in the viral respiratory vaccine field study at Fort Ord, California, 1966–1967

Age* (years)	Vaccine group	Parainfluenza virus isolations: Month of isolation: Oct., Nov.	Dec., Jan.	Feb., March	April, May	June, July	Total	No. at risk	AR/100
<1	Para†	2	4		1		7	43	16.3
	RS	2	2		3	1	8	43	18.6
1–4	Para		1	2	5		8	91	8.8
	RS		2		6	1	9	99	9.1
5–9	Para					1	1	60	1.7
	RS						0	49	0.0
Total	Para	2	5	2	6	1	16	194	8.2
	RS	2	4		9	2	17	191	8.9

* Age at the start of immunizations.
† Parainfluenza vaccine.

TABLE 6

Summary of parainfluenza viruses isolated from parainfluenza vaccine recipients, Fort Ord, California, September, 1966 to July, 1967

Child No.	Age*	Virus type isolated	Post vaccine HIA titer†	Virus isolation-days post immun.	Clinical diagnosis
1	4	1	1:64	188	Otitis media
2	14	1	1:128	167	Mild ARI
3	16	1	1:128	150	Croup
4	23	1	1:32	177	ARI
5	26	1	1:8	209	ARI with wheezing
6	32	1	1:8	161	Sore throat, ARI
7	64	1	1:16	196	Sore throat, ARI
8	6	2	1:32‡	24	Bronchiolitis
9	11	2	1:16	57	Otitis media
10	4	3	1:8	115	Croup§
11	5	3	1:8	55	ARI
12	5	3	1:64‡	11	ARI
13	8	3	1:512‡	7	ARI
14	12	3	1:32	63	Bronchiolitis

* Age in months at the start of immunizations.
† All of the pre-vaccine titers were <1:8.
‡ The post-vaccine blood specimen was obtained after parainfluenza virus isolation.
§ Hospitalized.

child required hospitalization for a parainfluenza virus infection and that was a child from the parainfluenza vaccine group. Additional data on the parainfluenza virus infections observed in the parainfluenza vaccine group are given in table 6. Of the 14 children in this group who developed parainfluenza virus infections after completion of their primary immunizations (two doses) 11 had a post-vaccine blood specimen collected before onset of the parainfluenza virus infection. From these, pre-infection homotypic antibody titers could be determined. All of these children had demonstrable pre-infection HI antibody (1:8 or greater) but the numbers of children with a parainfluenza virus type 2 or 3 infection are too small to draw any meaningful conclusions regarding protective antibody levels. However, of the seven children with a type 1 infection, post-vaccine homotypic HI antibody titers of 1:64 to 1:128 in three of these children were not protective against subsequent infection with this virus type.

RS virus infections in the vaccine study groups. RS virus infections were first detected in the general pediatric population in mid-December, 1966, and hence no vaccines were administered after that time. Table 7 shows the RS virus infections in the two vaccine groups by age and month of onset. A significantly higher incidence of RS virus infections is seen in

TABLE 7

Distribution by age and month of onset of respiratory syncytial virus infections in children in the viral respiratory vaccine field study at Fort Ord, California, 1966–1967

		RS virus infections*							
Age† (years)	Vaccine group	Oct., Nov.	Dec., Jan.	Feb., March	April, May	June July	Total	Number at risk	AR/100
<1	RS		8	5			13	43	30.2
	Para		3	1	1		5	43	11.6
1–4	RS		1				1	99	1.0
	Para		2	1			3	91	3.3
5–9	RS		1				1	49	2.0
	Para		1				1	60	1.7
Total	RS		10	5			15	191	7.9
	Para		6	2	1		9	194	4.7

* Diagnosed by virus isolation and/or a 4× or greater antibody rise between acute and convalescent blood specimens.

† Age at the start of immunizations.

the RS vaccine recipients compared to the parainfluenza vaccine recipients in the infant age group, but the difference between the two vaccine groups in total is not statistically significant.

There was a significantly greater number of RS virus infections admitted to the hospital among RS vaccine recipients compared to the parainfluenza vaccine recipients (9 vs 2, $\chi^2 = 4.8$ c̄ 1 df). Clinically the illnesses of hospitalized children in the RS vaccine group with RS virus infections appeared more severe than the hospitalized cases in non-vaccinated children. To quantitate this clinical impression, the RS vaccine recipients hospitalized with a laboratory confirmed RS virus infection were matched by age and sex with non-immunized children who where also hospitalized with a confirmed RS virus infection during the same time period. Table 8 compares these infections in RS vaccinees and matched controls by several laboratory and clinical factors. Although the numbers are small, clear differences between the two groups can be seen for most of the factors compared. A higher proportion of the cases was confirmed by virus isolation (versus antibody titer rises) in the RS vaccine group, and all of these isolations were made in the first tissue culture passage (average 8.1 days) whereas only about half of the isolations in non-vaccinees were obtained in first passage (average 15.2 days). An increased eosinophilia in the RS vaccine group compared to the controls was noted.

TABLE 8

Selected criteria of response to infection in vaccine recipients and matched controls hospitalized with confirmed RS virus infection, Fort Ord, California, December, 1966 to March, 1967*

Criteria of response to infection	RS virus vaccine recipients			Matched controls		
	Total examined	No. positive	Per cent positive	Total examined	No. positive	Per cent positive
Clinical severity†	9	4	44.4	18	1	5.6
X-ray results‡	9	8	88.9	18	14	77.8
Eosinophilia§	9	5	55.6	18	2	11.1
RS virus isolated	9	7	77.8	18	8	44.4

* Confirmed by virus isolation and/or antibody titer rise.

† Illness was graded clinically as mild, moderate, severe or very severe. A classification of severe or very severe was considered positive.

‡ X-ray evidence of an infiltrate was considered positive.

§ An eosinophilic index was calculated by multiplying the percentage of eosinophiles found in the routine white blood count by the total white blood count. An index of greater than 250 was considered positive.

CF antibody to RS virus in the two vaccine groups. Most of the infants in the

TABLE 9

Complement fixing (CF) antibody titers to respiratory syncytial (RS) virus in children who developed an RS virus infection in the vaccine field study at Fort Ord, California, 1966–1967

RS vaccine recipients			Parainfluenza vaccine recipients		
No.	Pre infection titer	Post infection titer	No.	Pre infection titer	Post infection titer
1	8	256	1	<8	64
2	16	256	2	<8	<8
3	8	128	3	<8	8
4	8	128	4	<8	8
5	8	512	5	<8	16
6	<8	128	6	<8	8
7	16	256			
8	8	8			
9	16	512			

vaccine study were also participating in a longitudinal study of acute respiratory illness. Periodic blood specimens (approximately four months apart) have been collected from these infants since their birth. Most of the infants in both groups had elevated CF antibody levels to the RS virus at birth. The geometric mean CF antibody titers of the cord blood specimens in both vaccine groups were similar. These levels of maternal CF antibody dropped off sharply so that within two months, the serum CF antibody was uniformly not demonstrable (i.e., <1:8). Table 9 shows the RS virus CF antibody levels before and after natural RS virus infection for those infants in the study from whom an RS virus was isolated. Most of the infants in the parainfluenza vaccine group had only a minimal or no demonstrable rise in CF antibody after isolation of the RS virus. Infants in the RS vaccine group generally showed low to moderate CF antibody titer rises to the vaccine. However, the CF antibody responses of these infants to natural RS virus infection which occurred after receiving RS vaccine were very pronounced, with titers rising almost uniformly to very high levels (1:128 or greater).

DISCUSSION

Although a multiplicity of agents is involved, clinical and epidemiologic studies have clearly established that the RS virus and the parainfluenza viruses are the most frequent causal agents of severe respiratory illness in infants and children.

The first field study in pediatric population of a vaccine containing the parainfluenza viruses was reported by Laxdal et al. in 1964 (11). They tested an inactivated polyvalent vaccine containing influenza types A, A1, A2 and B, adenovirus types 3, 4, and 7 and parainfluenza virus types 1 and 3. The antibody responses elicited by this polyvalent vaccine were in the word of the authors "disappointing". No details of the antibody response to the parainfluenza virus components of the vaccine were given. Viral etiologic studies were not performed, and the evaluation of protective efficacy was dependent on observations of the overall incidence of ARI in immunized children compared to controls. No significant reduction of ARI in the vaccine recipients was observed.

A more detailed field study of an inactivated polyvalent respiratory virus vaccine conducted in a nursery school and kindergarten population was reported by Weibel et al. in 1966 (12). This vaccine contained RS virus, parainfluenza virus types 1, 2, and 3, influenza virus types A2 and B and *Mycoplasma pneumoniae*. Antibody response to this vaccine was good except to the RS virus component. In this study, a significant reduction in the occurrence of severe respiratory illness in the vaccine recipients, as compared to the controls, was observed during the period of high ARI incidence. The rates of isolation of viruses and mycoplasma from the study population were too low to determine agent-specific attack rates, but the results suggested that the protective efficacy of the vaccine was due mainly to the parainfluenza and mycoplasma components.

After the encouraging results of the heptavalent vaccine evaluation, Weibel et

al. in 1967 (13) reported on tests of monovalent RS, monovalent *Mycoplasma pneumoniae* and trivalent parainfluenza virus types 1, 2, and 3 vaccines in three to five year old children. To improve the antibody response to the RS vaccine, the amount of antigen in the monovalent preparation was increased over that used in the previous heptavalent vaccine. During the course of this field evaluation, ARI rates were lower than those experienced in the earlier heptavalent vaccine study and no statistically significant reduction of ARI was observed in any of the vaccine groups compared to the control group. This study, however, showed that antigenically potent vaccines to RS and parainfluenza viruses could be produced and indicated that the demonstration of a significant reduction in specific etiologically defined illnesses would probably require field evaluations to be conducted in infants and very young children in whom attack rates of RS and parainfluenza virus infections are highest.

In the present study the rates of respiratory illnesses and of specific virus infections, especially in infants, were sufficient to show clearly that neither of the vaccines were protective. Both vaccines elicited substantial serum antibody titers in a high proportion of the subjects, but these antibody levels failed to protect against subsequent natural challenge with these respiratory viruses.

Recent studies by Smith et al. (14) concerning immune response to inactivated parainfluenza, type 1 vaccine indicate that although the vaccine can evoke serum antibody responses similar to those which follow upper respiratory tract infection with the type 1 virus, the vaccine is not as effective as natural infection in stimulating the development of neutralizing antibodies in nasal secretions. These same authors, in volunteer studies, also showed that the level of virus neutralizing antibody, in nasal secretions was a better index of host resistance to parainfluenza virus, type 1 infection than was the level of serum antibody. These findings may well explain the lack of protection to parainfluenza virus infections conferred by the inactivated trivalent vaccine even though it elicited good serum antibody responses to the parainfluenza viruses.

The findings related to the RS virus vaccine indicated that aside from failing to protect, this vaccine in some manner altered the host response to natural RS virus infection. The attack rate and the clinical severity of RS virus infections in infants up to 18 months of age who received the RS vaccine seemed greater than those experienced by infants who did not receive this vaccine. However, in older children the response to natural infections was apparently unaltered by the vaccine. These observations are consistent with the epidemiology of RS virus infections in that the highest risk of severe clinical illness due to this virus is among infants young enough to possess maternal antibody. This is in contrast to the pattern of infections with other common viruses where young infants are relatively protected from severe infection presumably because of maternal antibody. To account for this unique age distribution of severe RS virus infections, Chanock (15) has recently postulated that maternal antibody to this virus may contribute to the pathogenesis of illness rather than protect against infection.

Perhaps, as has been demonstrated for parainfluenza virus, type 1, local antibodies to RS virus in respiratory secretions may be more significant for protection than serum antibody and these local antibodies are only effectively produced in response to natural infection. It is thus conceivable that infants unprotected by local neutralizing antibodies, can undergo severe infection of the lower respiratory tract with RS virus because of their maternally acquired serum antibody. Similarly, serum antibody induced by inactivated vaccine might result in a more

severe clinical response to natural infection in RS vaccinees who lack natural local immunity from a prior RS virus infection.

The mechanism of this altered host response to natural infection is not clear, but one may speculate that the observed exaggeration in clinical response reflects an enhancement of viral growth. Immunologic enhancement of viral infectivity has been demonstrated experimentally *in vitro* with an arbovirus (16) and a poxvirus (17) and it seems theoretically possible that such enhancement might occur in certain *in vivo* situations. Such an enhancement would fit with most of the observations in this study pertaining to RS virus infections in infants who received the RS vaccine. Specific enhancement of RS virus growth in vaccine recipients would not increase the rate of RS virus infections in this group *per se*, but could increase the observed attack rate by increasing the clinical severity of these infections and thereby bring more of the infections to medical attention. The relative facility with which the virus was recovered from immunized infants who subsequently developed RS virus infection suggests that these children may have been shedding more RS virus than other children with a similar RS virus infection. Laboratory studies are being conducted to investigate this hypothesis.

Regardless of the mechanism of the altered host response to RS virus infection following inactivated RS virus vaccine, the results obtained with these vaccines require a reappraisal of the conventional approach, attempted here, to control these common pediatric respiratory viruses.

References

1. Chanock, R. M., Parrott, R. H., Cook, M. K., Andrews, B. E., Bell, J. A., Reichelderfer, T., Kapikian, A. Z., Mastrota, F. M. and Huebner, R. J. Newly recognized myxoviruses from children with respiratory disease. New Engl. J. Med., 1958, *258:* 207–213.
2. Beem, M., Wright, F. H., Hamre, D., Egerer, R. and Oehme, M. Association of the chimpanzee coryza agent with acute respiratory disease in children. New Engl. J. Med., 1960, *263:* 523–530.
3. McClelland, L., Hilleman, M. R., Hamparian, V. V., Ketler, A., Reilly, C. M, Cornfeld, D. and Stokes, J., Jr. Studies of acute respiratory illnesses caused by respiratory syncytial virus. 2. Epidemiology and assessment of importance. New Engl. J. Med., 1961, *264:* 1169–1175.
4. Kapikian, A. Z., Bell, J. A., Mastrota, F. M., Johnson, K. M., Huebner, R. J. and Chanock, R. M. An outbreak of febrile illness and pneumonia associated with respiratory syncytial virus infection. Amer. J. Hyg., 1961, *74:* 234–248.
5. Hilleman, M. R., Hamparian, V. V., Ketler, A., Reilly, C. M., McClelland, L., Cornfeld, D. and Stokes, J., Jr. Acute respiratory illnesses among children and adults. J.AM.A., 1962, *180:* 445–453.
6. Hayflick, L. and Moorhead, P. S. The serial cultivation of human diploid cell strains. J. Exp. Cell. Res., 1961, *25:* 585–621.
7. Bodian, D. Simplified method of dispersion of monkey kidney cells with trypsin. Virology, 1956, *4:* 575–577.
8. Leibovitz, A. The growth and maintenance of tissue-cell cultures in free gas exchange with the atmosphere. Amer. J. Hyg., 1963, *78:* 173–180.
9. Schieble, J. H., Lennette, E. H. and Kase, A. An immunofluorescent staining method for rapid identification of respiratory syncytial virus. Proc. Soc. Exper. Biol. Med., 1965, *120:* 203–208.
10. Chin, J., Schmidt, N. J., Lennette, E. H. and Hanahoe, M. Filter paper disc method of collecting whole blood for serologic studies in children. Amer. J. Epid., 1966, *84:* 74–80.
11. Laxdal, O. E., Evans, G. E., Braaten, V. and Robertson, H. E. Acute respiratory infections in children. II. A trial of polyvalent virus vaccine. Canad. Med. Assn. J., 1964, *90:* 15–19.
12. Weibel, R. E., Stokes, J., Jr., Leagus, M. B., Mascoli, C. C. and Hilleman, M. R. Respiratory virus vaccines. V. Field evaluation for efficacy of heptavalent vaccine. Amer. Rev. Resp. Dis., 1966, *94:* 362–379.
13. Weibel, R. E., Stokes, J., Jr., Mascoli, C. C., Leagus, M. B., Woodhour, A. F., Tytell, A. A., Vella, P. P. and Hilleman, M. R. Respiratory virus vaccines. VII. Field evaluation of respiratory syncytial, parainfluenza 1, 2, 3 and *Mycoplasma pneumoniae* vaccines, 1965 to 1966. Amer. Rev. Resp. Dis., 1967, *96:* 724–739.
14. Smith, C. B., Purcell, R. H., Bellanti, J. A.

and Chanock, R. M. The protective effect of antibody to parainfluenza type-1 virus. New Engl. J. Med., 1966, *275:* 1145–1152.

15. Chanock, R. M., Smith, C. B., Friedewald, W. T., Parrott, R. H., Forsyth, B. R., Coates, H. V., Kapikian, A. Z. and Gharpure, M. A. Resistance to parainfluenza and respiratory syncytial virus infection—implications for effective immunization and preliminary study of an attenuated strain of respiratory syncytial virus. First International Conference on Vaccines Against Viral and Rickettsial Diseases of Man, Wash., D.C., Pan American Health Organization Scientific Publication No. 147, May, 1967, pp. 53–61.
16. Hawkes, R. A. Enhancement of the infectivity of arbovirus by specific antisera produced in domestic fowls. Australian J. Exptl. Biol. Med. Sci., 1964, *12:* 465–482.
17. Hawkes, R. A. and Lafferty, K. J. The enhancement of virus infectivity by antibody. Virology, 1967, *33:* 250–261.