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EFFICACY OF A "MIXED BACTERIAL VACCINE" IN PROPHYLAXIS OF ACUTE RESPIRATORY INFECTIONS: POSSIBLE ROLE OF INTERFERON[1]

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Rytel, M. W. (Milwaukee County General Hospital, Milwaukee, Wisconsin 53226), J. E. Ferstenfeld, H. D. Rose, J. Balay, W. E. Pierce and K. L. Lynch. Efficacy of a "mixed bacterial vaccine" in prophylaxis of acute respiratory infections: possible role of interferon. *Am J Epidemiol* 99:347–359, 1974.—The efficacy of "mixed bacterial vaccines without U.S. standard of potency" (MBV's) in prophylaxis of respiratory infections remains controversial. Recent studies have shown that MBV's are capable of interferon induction and afford protection against influenza and parainfluenza viruses in mice. With this in mind, a study was undertaken to assess the ability of MBV to induce interferon in man, and to determine its prophylactic efficacy against naturally acquired respiratory infections. Commercial MBV was given according to the manufacturer's recommendations to 51 volunteers and a placebo to 44 matched controls at a Veterans Administration Domiciliary. Subjects were followed in a double-blind manner, over a 10-month period. Throat swabs and sera for viral diagnostic studies were obtained at each illness episode. In addition, throat cultures for predominant bacteria and sera for viral complement-fixing antibody determinations were obtained before and twice after administration of the vaccine. There was no difference between the two groups in cumulative respiratory illness rates, clinic visits, or hospital admissions. Viral infections, including influenza A/England/42/72, were identified with equal frequency. No alterations in normal throat flora were observed as a result of immunization. There was no interferon response following MBV administration. It is concluded that the effects of MBV in man differ from those reported in mice.

antigen-antibody reactions; bacterial throat flora; bacterial vaccines; interferon; virus diseases

The prospect of employing interferon inducers in control of viral infections remains an attractive though elusive possibility. The presently available inducers such as poly I:C, tilorone or attenuated viral vaccines are of limited usefulness in man because of toxicity or low potential for interferon induction (1). A number of recent studies have shown that bacterial vaccines induce interferon in mice (2, 3)

Abbreviations: CF, complement fixation; HI, hemagglutination inhibition; MBV, mixed bacterial vaccine; PHA, phytohemagglutinin.

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and afford protection against several types of viruses including influenza A_2 and parainfluenza (Sendai) (4, 5). Of special interest is the observation by Degré and Dahl (6) that injections of animals previously immunized with the same vaccine afforded a higher degree of protection against influenza A_2 than that obtained in animals not so immunized. These authors were unable to conclude, however, whether this enhanced protective effect was mediated by interferon or other nonspecific mechanisms of resistance, although interferon response was enhanced to a proportional degree in animals previously immunized (6).

The efficacy of the so-called "licensed bacterial vaccines without U.S. standard of potency" in prophylaxis of respiratory infections in man remains a controversial subject, with at least two double-blind studies showing beneficial effect (7, 8), while other reports cast doubt on their activity (9, 10). On the basis of the favorable results obtained in the animal studies alluded to above, we became interested in the possibility that some of the currently available bacterial vaccines may indeed possess a degree of efficacy against acute respiratory (primarily viral) infections in man, and that this effect may be mediated through interferon induction. It was obvious that only a very thorough longitudinal study which would assess a number of clinical and laboratory parameters of infection could settle the question of efficacy. We have, therefore, initiated such a study in a population of military veterans, residents of the Veterans Administration Domiciliary at Wood, Wisconsin.

Materials and methods

Study population. Initially, 100 residents of the V.A. Domiciliary volunteered to participate in this study. Most were elderly, all were males, and many had underlying medical conditions. Signed informed consent was obtained from each subject. The clinical records of the volunteers were reviewed to obtain background demographic information. The study population was then divided into the vaccine and the placebo groups with a further subdivision into Tuesday and Thursday clinical survey groups. Assignments into the treatment groups were not randomly made, but rather were stratified in an attempt to insure equal representation according to certain characteristics. Thus, the groups were comparable in the mean age of the subjects (vaccine, 58.2 years; placebo, 58.0 years), their length of stay in the Domiciliary (vaccine, 4.95 years; placebo, 5.02 years), the number of patients with history of chronic obstructive pulmonary disease (vaccine, 12; placebo, 13), of cigarette smoking (vaccine, 31; placebo, 27) and of allergies (vaccine, 1; placebo, 1).

Vaccine. It was decided to use the "Mixed Vaccine #4 with *Hemophilus influenzae*" (Eli Lilly and Co., Indianapolis, Ind., Control No 6MH52A) because it had been previously demonstrated that *H. influenzae* antigens are capable of producing a more prolonged interferon response with diphasic peaks a week apart (2, 11). The vaccine contains whole heat-killed bacteria in isotonic saline in the following concentrations per ml: *Hemophilus influenzae*, 500 million; *Neisseria catarrhalis*, 100 million; Streptococci, 500 million; *Klebsiella pneumoniae*, 250 million; Staphylococci, 1000 million; and *Diplococcus pneumoniae*, 1500 million. The finished vaccine, standardized to the desired bacterial count, is preserved with Merthiolate (Eli Lilly and Co.), 1:10,000. A sample of the lot to be employed in the immunizations was submitted to Dr. M. Carolyn Hardegree (Bureau of Biologics, Food and Drug Administration, Bethesda, Md.) for assessment of its interferon inducing ability in vivo in mice. The preparation was found to be active, giving serum interferon titers in mice of at least 500 units at two hours following intravenous injection of 0.5 ml.

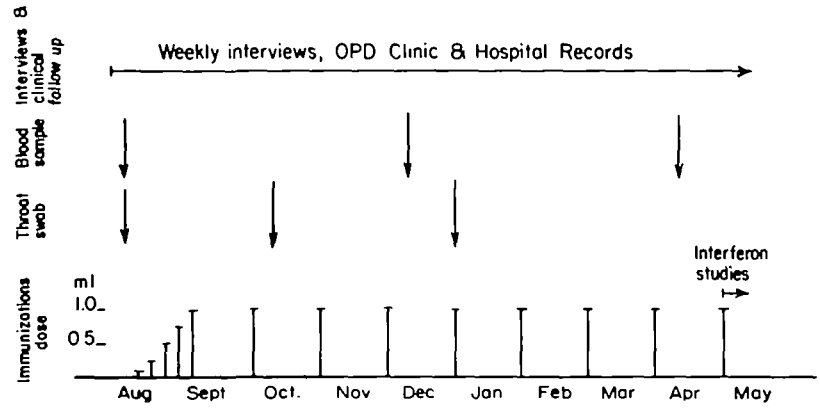

FIGURE 1. Design of the mixed bacterial vaccine study, Veterans Administration Domiciliary, Wood, Wisconsin, August 1972 through May 1973. (For detailed explanation, see text.)

Study design. The schematic outline of the study is depicted in figure 1. The series of immunizations was commenced on August 14, 1972 with an initial dose of 0.1 ml given subcutaneously. The injections were continued with gradually increasing increments of 0.25 ml per week until September 11, when a dose of 1 ml was given. Fifty-one individuals completed the initial five-injection immunizing course of the vaccine, and 44 received corresponding doses of isotonic saline (Eli Lilly and Co.) as placebo. The subjects were not aware of the type of injection they received. Booster injections were then continued at a dose of 1 ml given at monthly intervals. The subjects were seen once a week (either on Tuesday or Thursday) by the same examiners (H.D.R. and J.E.F.), who were not aware of the immunization status of the participants, thus insuring the double-blind character of the study.

At each visit, the subjects were queried as to the presence of respiratory and other symptoms, and if indicated, a brief clinical examination was performed and the findings listed on an IBM card. If a subject had symptoms of an upper respiratory tract infection, a throat swab was obtained for viral studies and an acute and, subsequently, a three-week convalescent serum specimen was collected for serodiagnostic tests. This gave 10 months of close observation, and included the "respiratory disease season" of Fall 1972 to Spring 1973. At the end of the study, patients' clinic and hospital records were reviewed and the incidence, nature and characteristics of their respiratory illnesses were analyzed.

In addition, throat cultures for bacterial flora were performed (on August 9, 1972) just before the initiation of immunizations and at two months (October 18, 1972) and five months (January 8, 1973) afterwards. Serum samples for viral antibody determinations were also obtained on August 9, 1972 and then at four and one-half months (December 18, 1972) and nine months (April 16, 1973) post-immunization.

Interferon studies. 1) *Interferon induction in human lymphocytes in vitro.* Interferon response was determined in lymphocyte cultures from eight vaccinees and three control subjects. These studies were conducted beginning in February 1973. Blood was drawn into a syringe containing phenol-free sodium heparin (U-1394, Upjohn Co., Kalamazoo, Mich.). Lymphocytes were separated according to a technique reported in detail previously (12). They were then adjusted to a concentration of 8×10^6 cells per 3 ml of Eagle's minimum essential medium with 10 per cent fetal calf serum (Microbiological As-

sociates, Bethesda, Md.), for each culture tube. A 10^{-1} dilution of the vaccine was added to each tube. In preliminary studies, this concentration was found to be the highest amount still relatively nontoxic to the lymphocytes (i.e. lymphocyte viability by the erythrosin-B dye exclusion test ≥ 50 per cent after overnight incubation). Phytohemagglutinin-M (PHA) (Difco Laboratories, Detroit, Mich.) was employed as a positive control in a volume of 0.7 ml per culture in cohort lymphocytes incubated with PHA (13). The tubes were incubated overnight in a roller drum rotating at 12 revolutions per hour. They were then centrifuged at 900 revolutions per minute for 10 minutes (Universal Centrifuge, model UV, with head No. 279). The supernatant was collected and stored at −20 C until ready for interferon titration.

2) *In vivo studies.* At the completion of the routine immunizations, in May 1973, subjects from the immunized and placebo groups were compared for their ability to produce humoral interferon following administration of 1 ml and 0.25 ml of the vaccine, respectively. The latter amount was felt to be the highest amount that could be safely given to individuals who had not received the initial 0.1 ml test dose. Five subjects from each group were studied. Serum samples were obtained at two, six and 96 hours following injection, and stored as above until ready for interferon titration.

3) *Interferon titration.* The technique employed was reported by us previously in detail (12). It is based on the 50 per cent plaque reduction of vesicular stomatitis virus (Indiana strain) in human foreskin fibroblast cultures. On repeat titrations of British Human Interferon Research Standard A69/19, the following titers were obtained employing this assay system: 5120, 2560 and 6758 units per 1 ml.

Viral studies. Throat swabs were immersed in 2.5 ml of Earle's balanced salt solution containing 0.5 per cent lactalbumin hydrolysate, 0.5 per cent bovine albumin, with 200 units of penicillin, 20 μg of gentamicin, and 100 units of nystatin per 1 ml. Specimens were inoculated immediately (or quick frozen and stored at −90 C and then inoculated) in 0.2 ml volumes into each of the following cell culture systems: 1) WI-38, two tubes, which were then incubated at 33 C on a roller drum at 12 revolutions per hour; 2) primary rhesus monkey kidney, three tubes incubated on a roller drum as above; and 3) HEp-2, two tubes incubated at 35 C in stationary culture. All cell cultures were obtained from Microbiological Associates, Bethesda, Md. Observations for cytopathogenic effect were carried out three times weekly for 14 days, and then one blind passage of pooled culture cell scraping was made into fresh tubes of corresponding culture. Additional passages were made, if necessary, to follow suspicious cytopathogenic effect. Hemadsorption on primary rhesus monkey kidney was done at seven and 14 days, and at the end of the blind passage using 0.4 per cent guinea pig erythrocytes. Identification of isolates was as follows: suspected rhinovirus isolates were confirmed by checking for acid lability at pH 3.0. Herpes simplex isolates were neutralized with specific antiserum (Microbiological Associates, Bethesda, Md.). Positive hemadsorbing isolates were identified as influenza A/England/42/72, by hemagglutination inhibition (HI) employing specific guinea pig antiserum (Center for Disease Control (CDC), Atlanta, Ga.).

Serodiagnostic tests. Sera from the survey, as well as acute and convalescent specimens, were tested at the same time (at the completion of the study) for the presence of complement-fixing (CF) antibodies against a panel of respiratory viral antigens. These included influenza A and B, parainfluenza 1, 2, and 3, respiratory syncytial virus, adenovirus, and *Mycoplasma pneumoniae* (Microbiological Associates, Bethesda, Md.). The microplate CF

test developed originally by Sever (14) with modification by Edwards (15) was used, utilizing four units of antigen. Sera found positive for CF antibodies to influenza A group antigen were tested by HI (15) for the presence of type specific antibodies to influenza A_2. All sera were treated with receptor destroying enzyme of *Vibrio cholerae* (Microbiological Associates, Bethesda, Md.). Four hemagglutinating units of influenza A/England/42/72 (CDC, Atlanta, Ga.) were used in the test.

Bacterial cultures. Quantitative throat cultures were done according to the following technique: an area of 1 cm diameter was sampled on the posterior wall of the oral pharynx immediately behind the uvula using a sterile nonabsorbent dacron swab. The swab was immediately placed in a tube of 10 ml phosphate buffered saline at pH 7.0, and shaken to suspend the microorganisms present. From this tube, dilutions were plated onto Columbia blood agar (5 per cent sheep blood), EMB agar, hemophilus agar (containing X and V factors), and Sabouraud's dextrose agar (all from Difco Laboratories, Detroit, Mich.). Colony counts and identifications were made after growth for at least 24 hours at 37 C.

Antibody assay. Counterimmunoelectrophoresis was employed for detection of antibodies to antigens contained in the vaccine. The details of the technique were reported by us previously (16). The reaction was conducted on 1 per cent agarose slides, in pH 8.2 veronal buffer, at a constant voltage of 400 V, for 60 minutes at room temperature.

Statistical analyses. Unless otherwise indicated, all statistical analyses were by the chi square (χ^2) test with one degree of freedom, and employing a correction factor for small numbers.

Results

Safety of the vaccine. There were no systemic reactions and only five vaccinees had mild local reactions consisting of erythema, induration and pain at the site of injection. These did not occur until a dose of at least 0.75 ml was administered, and were not severe enough to necessitate decreasing the dose or discontinuing the immunizations for those particular individuals. Two of the five subjects had reactions after two consecutive injections; others had only one documented reaction. There were no reactions of any kind in the control subjects.

One subject, B.J., No. 009, in the vaccine group, developed transient cold agglutinin titers as follows: October 25, 1:256; November 2, 1:128; November 15, 1:64; January 11, 1:16. He remained asymptomatic and there was no evidence of hemolysis. It is unlikely that this was related to the immunizations because he received his usual dose of the vaccine on November 8, and yet, the cold agglutinin titer on November 15 continued its downward trend. There was no serologic evidence of *M. pneumoniae* (or any viral) infection in this individual, however, to account for the cold agglutinin rise.

The lack of side effects is further attested to by the low attrition rate in the vaccine group which was not different from that in the placebo group (table 1). A total of 83 subjects (or 87.5 per cent) completed the study out of the original group of 95.

Interferon studies. Interferon determinations, as indicated in the outline, were done towards the end of the clinical part of the study to maximize the previously alluded

TABLE 1

Makeup of the experimental groups and attrition rates of the subjects during the course of the study

Time in study	Vaccine group		Placebo group		Total	
	No.	%	No.	%	No.	%
Inception	51	100	44	100	95	100
5 months*	49	96.0	40	91.0	89	94.0
10 months*,†	44	86.5	39	88.7	83	87.5

* Differences not significant by χ^2 test.
† Completion of study.

to interferon enhancing effect obtained with repeated immunizations of mice (6). As seen in table 2, no interferon could be detected in supernates of lymphocyte cultures obtained from the vaccinees and control subjects, either at 24 or 96 hours following incubation with the maximum nontoxic amount of the vaccine (10^{-1}). The subjects had received a minimum of 10 injections at the time of these studies. PHA employed as a positive control in cohort cultures stimulated high interferon titers, which were generally higher at 96 than at 24 hours. Green et al. (17) reported similar kinetics for PHA induced interferon response.

Negative results were likewise obtained in our attempts to induce interferon in vaccinees given their final (13th) 1.0 ml of the vaccine and in control subjects given the highest permissible initial dose of 0.25 ml (table 3). No interferon was detected at two, six and 96 hours, which corresponded to the periods of peak interferonemia in the mouse studies reported previously (2, 3).

Effect of the vaccine on respiratory infections. There was no significant difference between the immunized and control groups in respiratory infection episodes in terms of monthly incidence rates (lower curves) or cumulative illness rates (upper curves) (figure 2). The denominator was the "running total" of the patients participating in the study (i.e., who received immunizations) in a given month. The difference in the patterns of cumulative percentages is not significant using the Kolmogorov-Smirnov test (18). At the end

TABLE 2

Stimulation of interferon in lymphocytes in vitro with mixed bacterial vaccine or PHA†*

Subjects		Immunization group‡	Inducers	Interferon titer (units/2 ml) time after incubation	
No.	Initials		Vaccine (10^{-1}) PHA (0.7 ml)	24 hr	96 hr
040	HV	Vaccine	Vaccine	<8	<8
			PHA	*358*	*512*
031	FW	Vaccine	Vaccine	<8	<8
			PHA	*100*	*512*
005	BR	Vaccine	Vaccine	<8	<8
			PHA	*<8*	*128*
075	MV	Vaccine	Vaccine	<8	<8
			PHA	*128*	*256*
077	MJ	Vaccine	Vaccine	<8	<8
			PHA	*320*	*358*
107	WW	Vaccine	Vaccine	ND§	<8
			PHA		*200*
014	CC	Vaccine	Vaccine	ND	<8
			PHA		*256*
106	WF	Vaccine	Vaccine	ND	<8
			PHA		*512*
041	HE	Placebo	Vaccine	ND	<8
			PHA		*512*
038	GM	Placebo	Vaccine	ND	<8
			PHA		*64*
025	DT	Placebo	Vaccine	ND	<8
			PHA		*128*

* 8×10^6 lymphocytes per culture.

† PHA, phytohemagglutinin. PHA induced interferon titers are italicized.

‡ Lymphocytes were obtained from subjects who received either the vaccine or isotonic saline as a placebo.

§ ND: not done.

TABLE 3

Stimulation of interferon induction in vivo with mixed bacterial vaccine

Subjects		Immunization group*	Vaccine dose (ml/S. Q.)	Interferon titer (units/2 ml) time after injection		
No.	Initials			2 hr	6 hr	96 hr
012	BM	Vaccine	1.0	<8	<8	<8
080	OA	Vaccine	1.0	<8	<8	<8
089	SL	Vaccine	1.0	*10*	<8	*8*
108	WR	Vaccine	1.0	<8	<8	<8
059	LW	Vaccine	1.0	<8	<8	<8
090	SH	Placebo	0.25	<8	<8	<8
020	LJ	Placebo	0.25	<8	<8	<8
010	BK	Placebo	0.25	<8	<8	<8
072	McJ	Placebo	0.25	<8	<8	<8
045	HA	Placebo	0.25	<8	<8	<8

* Status of subjects before interferon induction was attempted with the vaccine.

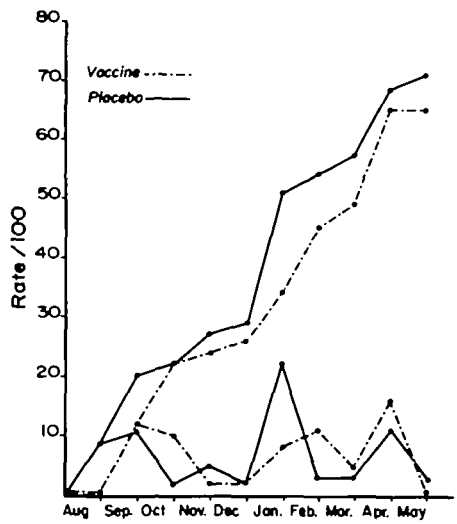

FIGURE 2. Infection rates in the vaccine and the placebo group as derived from data from the weekly surveys. The upper curves depict cumulative respiratory infection rates, and the lower illustrate the incidence of infections per month of the study (corrected for population size).

of the study, the total number of infections in the vaccine group was 30, and in the placebo group, 29. The apparent difference in the January infection rates is not significant. Similarity in the illness experience of the two groups is further brought out by the identical number of infectious episodes (i.e., 12) during the four months classically constituting the peak "respiratory disease season" (December–March).

Cumulative "illness score" is a useful parameter to measure severity of illness in individuals or groups under study. Empirically determined score code is applied for different symptoms and signs, or their combinations (19, 20). The more severe the episode of illness, the higher the score for the individual and, consequently, for his group. Employing this technique, no statistically significant differences (by the *t*-test) were found in the cumulative illness scores between the subjects in the vaccine and the placebo groups (figure 3). The denominator employed in the calculations of the results depicted in figure 3 was the number of subjects present at a given survey session. Since the vaccine is purported to reduce bacterial superinfections accompanying viral respiratory infections, it would be expected to decrease the severity (if not the number) of such infections. This effect, if present, should be evaluable by the parameter of cumulative illness score. However, it was obviously not obtained.

Likewise, as shown in table 4, no difference could be discerned between the two groups in respiratory illness rates based on the data derived from outpatient clinic visits ($p < .5$). The same was true for hospital admissions for respiratory infections (table 5). Death rates and the causes

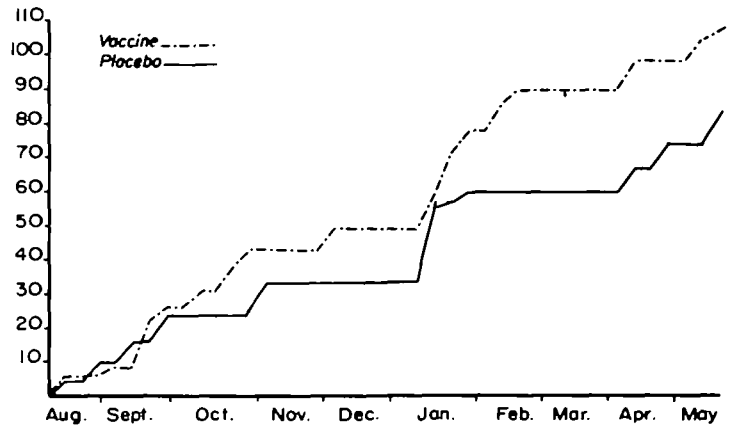

FIGURE 3. Cumulative weighed illness scores (corrected for population size) for the vaccine and the placebo group. The intervals on the abscissa vary depending on the number of weekly interviews conducted in a given month.

TABLE 4

Rates of respiratory infections as determined by clinic visits

Immunization group	No. of subjects in group	Infection episodes		Patients with infection*	
		No.	%	No.	%
Vaccine	51	18	35.3	13	25.5
Placebo	44	14	31.8	12	27.3
Total	95	32	33.6	25	26.4

* Differences not significant by χ^2 test.

of death are also listed in table 5. One patient from each group died with bronchopneumonia. Bacterial studies were noncontributory.

Etiologic agents associated with respiratory infections. The etiologic agents of infections diagnosed during the weekly surveys are depicted in table 6. The results are based both on isolation data as well as serodiagnostic tests. Influenza A_2 was responsible for 13.4 per cent of the 30 respiratory infections in the vaccine group and for 17.2 per cent of the 29 episodes in the placebo group ($p < .9$). There were two influenza virus isolates in the vaccine and one in the placebo groups, all identified as influenza A/England/42/72. Other agents accounted for fewer infections scattered throughout both groups. Herpes simplex virus was isolated from two patients with acute respiratory infections, but the data were not included in the table in view of the uncertain etiologic role of this virus in respiratory infections (21). Moreover, there was no CF antibody rise to herpes simplex virus in either case. Several double infections were also seen. The difference between the two groups in the number of single infections associated with a specific etiologic agent approached, but did not reach, statistical significance ($.05 < p < .1$). An etiologic agent could be identified in approximately half of the infection episodes: 33.3 per cent in the vaccine, 51.7 per cent in the placebo groups (and 42.4 per cent overall). This difference is also not statistically significant ($p < .5$). The percentage of etiologically identified infections in this study compares favorably with resports in the literature (22).

Results of the serologic survey are summarized in table 7 in terms of geometric mean CF antibody titers against various antigens for each group. The higher initial titers for influenza A are most likely due to the fact that the subjects in the study receive annual booster injections of influenza vaccine. With the possible exception of influenza (April specimens), there were

TABLE 5

Hospitalization and death rates*

Immunization group	No. of subjects in group	Hospitalization				Deaths†	
		No. of episodes	%	No. of patients‡	%	No. of patients	%
Vaccine	51	6	11.8	2	3.9	3	5.9
Placebo	44	1	2.3	1	2.3	2	4.6
Total	95	7	7.4	3	3.2	5	5.3

* For respiratory infections only.

† Causes of death (and group): 1) Suicide (placebo), 2) Dead on arrival (vaccine), 3) Arteriosclerotic cardiovascular disease with congestive heart failure (vaccine), 4) Bronchopneumonia with multiple pulmonary abscesses (placebo), 5) Bronchopneumonia in chronic obstructive pulmonary disease (vaccine).

‡ Differences not significant by χ^2 test.

TABLE 6

Association of viral agents with respiratory infection episodes diagnosed during weekly interviews

Infections*	Vaccine group		Placebo group		Total	
	No.	%	No.	%	No.	%
Viral						
Influenza A_2†	4	13.4	5	17.2	9	15.3
Influenza B	0		0		0	
Parainfluenza 1	0	0	2	6.9	2	3.4
Parainfluenza 2	0		0		0	
Parainfluenza 3	3	10.0	1	3.4	4	6.8
Respiratory syncytial	2	6.6	2	6.9	4	6.8
Adenovirus	1	3.3	3	10.3	4	6.8
Rhinovirus	1	3.3	4	13.8	5	8.5
Mycoplasma pneumoniae	1	3.3	1	3.4	2	3.4
Single infections identified†	8	26.6	12	41.4	20	33.9
Double infections identified†	2	6.7	3	10.3	5	8.5
Total single and double infections identified	10	33.3	15	51.7	25	42.4
Etiology unknown†	20	66.7	14	48.3	34	57.6
Total infection episodes	30	100	29	100	59	100

* Identified by isolation and/or fourfold or greater changes in CF or HI antibody titer.

† Differences not significant by χ^2 test.

no discernible rises in geometric mean antibody titers to any of the antigens tested in the post-immunization specimens. There were no significant differences in any of the results between the vaccine and the placebo groups (paired *t*-test).

Immunogenicity of the vaccine. Serial throat cultures persistently revealed normal upper respiratory tract bacterial flora. The only organism represented in the antigenic make-up of the vaccine which was isolated with any frequency was *N. catarrhalis*. As seen in table 8, there was no significant difference in isolation rates between the two groups or in the pre- and post-immunization specimens (pre-immunization, $p < .2$; two months, $p < .2$; and at five months, $p < .6$). Isolates were expressed in actual colony counts, but this information is not presented because it would not have altered the results or the conclusions. We cannot account for the

TABLE 7

Geometric mean viral CF antibody titers

	Vaccine group			Placebo group		
	Aug. '72	Dec. '72	April '73	Aug. '72	Dec. '72	April '73
Viral antigen*						
Influenza A	12.7	12.7	15.3	9.1	9.7	11.9
Influenza B	5.1	5.3	4.9	3.8	3.8	3.8
Parainfluenza 1	3.3	3.3	3.0	3.1	3.1	2.9
Parainfluenza 2	2.1	2.1	2.1	2.1	2.1	2.2
Parainfluenza 3	3.5	3.6	4.0	3.8	3.8	4.2
Respiratory syncytial	2.9	3.4	3.4	2.5	2.7	2.9
Adenovirus	3.6	3.6	3.6	3.6	4.0	4.0
Mycoplasma pneumoniae	5.5	5.8	5.4	5.3	5.6	5.2
No. of sera analyzed	49	49	43	42	40	36

* None of these differences are significant by paired *t*-test.

TABLE 8

The effect of immunizations on rates of Neisseria catharralis isolation from throat cultures

Time of sampling	Vaccine group			Placebo group		
	No. of samples	No. of positives	%	No. of samples	No. of positives	%
Pre-immunization* (Aug. '72)	53	29	54.7	44	18	40.9
2 months post-immunization* (Oct. '72)	50	12	24.0	42	6	14.3
5 months post-immunization* (Jan. '73)	45	22	48.8	40	17	42.5

* Differences not significant by χ^2 test.

drop in *N. catarrhalis* isolation rates observed in the October sampling in both groups.

Detection of circulating antibodies to the vaccine components was attempted by counterimmunoelectrophoresis. A soluble preparation of a bacterial vaccine was used ("Respiratory UBA," Eli Lilly and Co.) to insure diffusion and migration of antigens on the agarose slides in electrical field. The make-up of this vaccine is identical to that of the vaccine actually employed in immunizations, except that it does not contain the antigens of *K. pneumoniae*. Eight of 20 subjects in the vaccine, and seven of 20 individuals in the placebo group whose sera were studied had antibodies to the vaccine components in both the pre- and the post-immunization (April) specimens. Three subjects in the placebo group developed *de novo* antibodies during the period of the study. In addition, the same sera were tested for antibodies to pneumococcal types 1, 2, 5 and 7 (purported to be present in the vaccine) employing 1.0 μg/ml of purified polysaccharide antigens, previously found to give optimal reactions in this system. No pneumococcal precipitins were detected in any of the sera.

DISCUSSION

Experiments conducted in mice by two independent groups of investigators have clearly established that bacterial vaccines are capable of interferon induction and afford protection against challenge with certain viruses, including influenza A_2 and parainfluenza (Sendai) (2–6). These observations are in agreement with our findings reported previously which indicated that interferon induced by Newcastle disease virus protected mice against aerosol transmitted influenza A_2 (23). Because one cannot safely employ Newcastle disease

virus as an interferon inducer in man, and other currently available inducers are equally unsatisfactory, we decided to test the interferon stimulating ability of a commercially available bacterial vaccine, and also to measure its efficacy against the challenge of naturally acquired respiratory infections.

We recognized the fact that after the initial weekly course of five injections, the vaccine could only be given once every four to five weeks; otherwise, most of the subjects would have withdrawn from the study. This, however, has been the correct manner of administration of the vaccine according to the manufacturer's instructions, and the usual practice of physicians employing this type of vaccine for various indications (7, 24). Moreover, Kleinschmidt and Murphy (25), employing another interferon inducer of microbial origin, statolon, showed that protection lasted for two to four weeks following interferon induction in mice, even at the time when circulating interferon could no longer be detected. Finally, it has been shown that antigens of *H. influenzae* type b (which were purportedly included in the vaccine employed in the present study) give a prolonged or diphasic period of interferonemia lasting for up to one week. With these considerations in mind, it was our hope that if the bacterial vaccine proved to be an effective interferon inducer in man, subjects receiving it could be protected for at least one to two weeks out of a month. If, indeed, such were the case, we would have expected a detectable decrease in the overall respiratory infection rates in the group which had received the vaccine. The vaccine proved to be inactive as a human interferon inducer, however, and was totally ineffective in reducing respiratory infection rates as determined by a number of parameters.

The original rationale for the mixed bacterial vaccines (reiterated in product information which accompanied the particular preparation employed in this study) was that they would "decrease the incidence or severity of respiratory infections" providing "immunity against organisms represented in the formula." This was predicated on the hypothesis that acute viral respiratory diseases are often complicated by superinfections with respiratory tract bacteria (24). This mechanism also did not appear to be operative in our study since the vaccine did not decrease the incidence of respiratory infections, and more importantly did not ameliorate the severity of illness as was brought out by the comparable cumulative illness scores in the vaccinated and placebo groups.

Moreover, these vaccines are touted as being particularly effective in patients with "frequently recurring infections" or with chronic lung conditions. However, even in this group of patients who comprised 25.5 per cent of the vaccine group, we found that they were of no benefit. There was no difference in respiratory illness rates as determined by the survey ($p > .5$) or by clinic visits ($.05 < p < .1$) between the patients with these conditions in the vaccine and in the placebo groups. The clinical experience of one patient (K.J., No. 057) is typical: He had five hospitalizations (depicted in table 5) for exacerbations of his chronic bronchitis. This patient received 10 of his scheduled immunizations, missing only three during his hospitalizations. In addition, one of the deaths in a vaccinee was due to bronchopneumonia.

Finally, these findings tend to raise questions regarding the immunogenicity of the vaccine. The vaccine did not effect *N. catarrhalis* throat carriage rates even though it putatively contains antigens of that organism. However, the highly potent meningococcal group C vaccines similarly have no influence on the carriage of *Neisseria meningitidis* group C in subjects who were carriers prior to the immunization (26, 27). Attempts in the present study to identify antibodies to pneumococci and

other vaccine components by the counterimmunoelectrophoresis technique (16) have been unsuccessful with the former, or inconclusive with the latter. The vaccinees' sera were either negative for antibodies or gave evidence of their presence in both the pre- and post-immunization samples. There was also no difference in the total number of subjects with antibody-positive sera between the two groups. The relative insensitivity of the counterimmunoelectrophoresis method for detection of pneumococcal antibodies, however, has been previously alluded to by Kenny and associates (28).

We realize that our study subjects were, for the most part, elderly, institutionalized males. It cannot, therefore, be construed with certainty whether our findings and conclusions would equally apply to the general population. We have chosen to study the efficacy of a mixed bacterial vaccine in these particular subjects because they were felt to be at greater risk from respiratory infections due to their age, underlying diseases, and institutionalized domicile. In this study population, the vaccine was found to be ineffective as an interferon inducer and as a prophylactic agent against naturally acquired respiratory infections.

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