

**Annual Progress Report
to the
Commission on Influenza
Armed Forces Epidemiological Board**

Principal Investigator

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Epidemiology and Prevention of Acute Respiratory Disease in
Navy Recruits

ANNUAL PROGRESS REPORT

by

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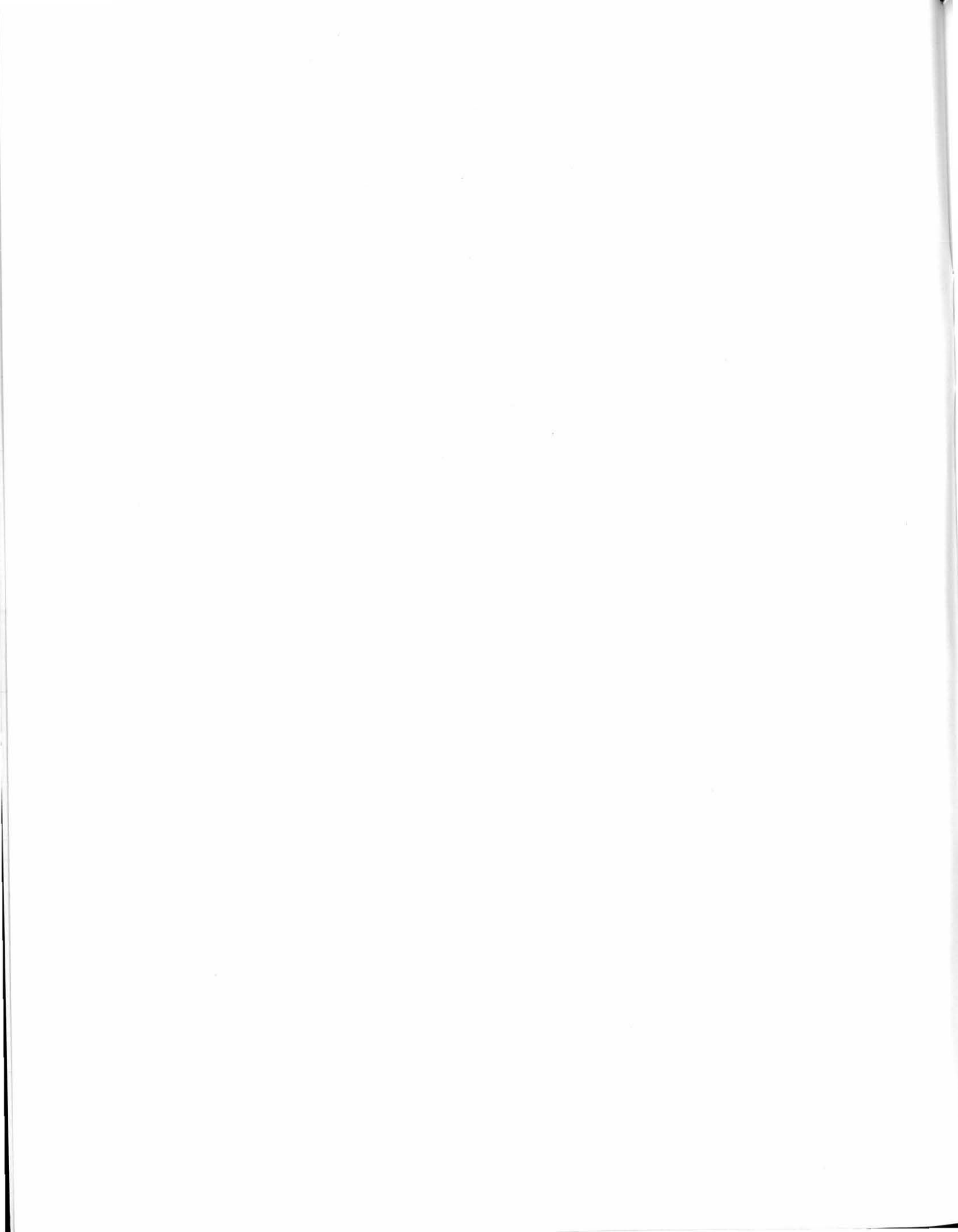
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The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.



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Armed Forces Epidemiological Board

Epidemiology and Prevention of Acute Respiratory Disease in
Navy Recruits

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SUMMARY

Surveillance studies of Great Lakes recruits from June 1, 1970 to May 31, 1971 indicated a high incidence of adenovirus, types 4 and 7, infections. These agents were most prevalent during the fall and early winter of 1970, with increased hospitalization rates for ARD and pneumonia. A limited outbreak of influenza B virus was detected during January and February of 1971. Rhinovirus, type 1A, 1B and 2 infections continue to be endemic in this population. A surveillance study at NTC, Orlando, Florida confirmed the establishment of endemic adenovirus infections with concomitant ARD problems. Adeno-7 infections have not been detected in great extent. Rhinovirus infections continue to be endemic with higher rates in the fall and spring months.

Rubella infections in recruits occurred primarily in individuals with initial titers of 1:10 or less. Although 91% of the recruit population was immune, this did not prevent infection of the susceptibles. The ratio of admitted disease to infection was approximately 1:50.

A comparison was made of various doses of A₀/PR-8 influenza vaccines given by intranasal, subcutaneous and intradermal routes for optimal production of antibody. In general, parenteral vaccines produced higher and more rapid seroresponses than those given by the intranasal route. Maximal antibody titers were observed by the 14th day post-vaccination parenterally, while those provoked by the intranasal route were delayed approximately one week. Some of the seroresponses to parenteral vaccines were not directly dose-dependent and the overall response curves appeared to be biphasic. The magnitude of response to the highest vaccine dose (2139 CCA) given intranasally was less than the next to lowest dose (34 CCA) given intradermally. The responses to either intradermal or subcutaneous routes were similar at comparable amounts of antigenic mass. Nasal secretory antibody responses to intradermal or intranasal vaccination were quite similar. All factors considered, except for ease of administration, the intradermal method of inoculation produced the optimal antibody response with the least amount of antigenic mass.

Live adenovirus vaccines, types 4 and 7, when given in combination, were effective in reducing the incidence of febrile acute respiratory disease by approximately 40%. Similarly, adenovirus type 4 infections, as measured by virus isolations from recruits in the barracks or in the dispensary, were reduced by 40-60% as compared to non-vaccinated controls. Monovalent vaccines (4 or 7), when given separately, were not as effective as the combination of the two. Adeno-7 vaccine showed the least protection against illness or adenovirus infection. This was attributed to the low potency of this vaccine preparation.

TABLE OF CONTENTS

	<u>Page</u>
I. A Continuous Surveillance of Viral Agents Associated with Respiratory Disease in Recruits During Training. MF12.524.009-4013BE6I.	1
A1. Incidence of Respiratory Virus Infections of Navy Recruits and Their Relation to Respiratory Diseases at NTC, Great Lakes, Illinois during 1970-1971.	1
A2. Incidence of Respiratory Virus Infections of Navy Recruits and Their Relation to Respiratory Diseases at NTC, Orlando, Florida, during 1970-1971.	2
B. Viral Etiology of Non-bacterial Pneumonia.	3
C. Seroepidemiology of Rubella in Navy Recruits.	4
II. Effect of Dose and Route of Immunization on the Antibody Responses to A ₀ /PR-8 Influenza Vaccine. M4305.12-5004AGG2.	16
III. Effect of Live Adenovirus Types 4 and 7 Vaccines on Adenovirus Infections and Respiratory Diseases of Naval Recruits. M4305.12-5007BEG6.	24



I. A CONTINUOUS SURVEILLANCE OF VIRAL AGENTS
ASSOCIATED WITH RESPIRATORY DISEASE IN
RECRUITS DURING TRAINING*

A. Incidence of Respiratory Virus Infections of Navy Recruits and
Their Relation to Respiratory Diseases

The purposes and methods of the surveillance programs have been described in previous reports to the Commission on Influenza. These data relate the incidence of respiratory diseases and causative agents found in the Naval recruit populations of Great Lakes, Illinois and Orlando, Florida during the period 1 June 1970 - 31 May 1971. These data are also utilized as background information in other parts of this report dealing with the protective effect of adenovirus vaccines.

DISCUSSION OF RESULTS

1. NTC, Great Lakes, Illinois

The overall incidence of hospital admissions for acute respiratory disease (ARD) and pneumonia from 1964-1971 are shown in Figure 1. Also displayed are the percent of seroconversions to the three main groups of etiological viral agents found among recruits during this period. Like the experience of the past four years, hospital admission rates for ARD continue to remain low (2-11 cases/1000/month) despite the high incidence of adenovirus infections. This paradox was noted in the Commission Report of 1970 and is partially explained by a recent policy which permits the patient to rest in his barracks ("rack pass") rather than be hospitalized in the dispensary. Despite the presence of a considerable challenge by influenza B (35% seroconversion rate and isolation of B/Mass prototypes, Jan - Feb 1971), there was only a slight elevation of ARD hospital admission rates during the past winter. The total rhinovirus seroconversion rate for the three types tested (1A, 1B and 2) during the past year were similar to incidence of previous years. Currently, type 1B emerged as the dominant rhinovirus (20-28%).

An expanded view of the illness rates for the past year, their comparison with "rack pass" incidence and type-specific adenovirus seroconversion rates are shown in Figure 2. The ARD admission rate peaked sharply during January and February. This coincided with a similar peak in pneumonia incidence at this time. Both illness rates declined rapidly in March and remained very low for the remainder of the reporting period. The monthly incidence of "rack passes" for febrile ARD (Fig. 2) generally follow the pattern of hospital admission rates for this illness. This graph represents the number of

*From Research Project No. MF12.524.009-4013BE6I, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

men, beginning training in the specified month, who were issued "rack passes" for febrile ARD at any time during their 11 weeks of training. There was a steady increase in the number of passes issued during the summer (June-Sept) with a slight temporary decrease in October and November. This trend was reversed in December and peaked to its highest rate in January (430 passes/1000 men/11 weeks). The decline in issuance of passes in February appears to precede the decrease in ARD hospital admission rates observed in March. This discrepancy is probably due to the extended period for tabulation of the "rack pass" data. It is assumed that the sudden decrease in illness was due to adenovirus vaccines and this will be discussed elsewhere in this report (Section III -- Live Adenovirus Vaccine Studies).

Much of the illness was due to adenovirus infections. The percent of type 4 and 7 neutralizing antibody seroconversions are shown in Figure 2. These results were obtained from the paired samples of sera which were adenovirus-positive by complement-fixation (Fig. 1). Although adenovirus 4 was the dominant serotype during the summer, type 7 infections were observed during the months of October and January-April. This was the first instance of sustained type 7 superiority which has been observed during the 7-year surveillance for these agents. It is assumed that the continued type 7 dominance and sharp decline of type 4 infections after January was due to the adenovirus vaccine program. These results will also be discussed later in this report (Section III).

The distribution of adenovirus isolates from a sample of recruits who came to the dispensary during June 1970 - February 1971 is shown in Figure 3. Patients with various clinical symptoms were sampled at the dispensary (ESS study). An effort was made to obtain an equal number of specimens from men with febrile ARD, afebrile ARD, as well as from those without respiratory symptoms. The top panel of Figure 3 shows the total adenovirus (4+7) isolation rates from these subjects. Most of these infections were associated with patients reporting ARD. In December the majority of isolates mainly came from patients whose ARD symptoms included fever. The middle and bottom panels of Figure 3 compare the relative proportions of adenovirus types 4 and 7 isolated. Like the serological data (Fig. 2), the dominance of type 7 infections in recruits seeking treatment emerged temporarily during October 1970 and again during January. These data indicated that the setting was ideal to test the efficacy of both the types 4 and 7 live adenovirus vaccines. Such programs were initiated in mid-February 1971.

2. NTC, Orlando, Florida

Figure 4 shows the population statistics, illness data and rate of serologic conversion to various viruses at NTC, Orlando since its commissioning in 1968. As was noted in last year's report, increased rates of ARD admissions became apparent during the winter of 1969-70. These high illness rates coincided with increased prevalence of adenovirus infections. The build-up of adenovirus infections to a peak during December 1969 is shown in Figure 5. These paired serum samples were obtained from a selection of patients who reported to the dispensary (OSS Study). The majority of

these seroconversions were due to adeno-4. Such infections have since become endemic at Orlando following the pattern at other recruit training installations. The ARD illness data for 1970-1971 are somewhat discrepant for January-March of this year since they failed to reach the proportions observed at this time during the previous year. However, in May of 1971, the rates peaked to 61 cases/1000 men, the highest level thus far observed in this population. This is an uncommon finding since this disease, presumably adenovirus-associated, usually is more prevalent during the winter months. Unfortunately, no relation to adenovirus infections can be made since the serological data are incomplete to this time (May). Virus isolation data from specimens obtained at the dispensary in connection with a pneumonia survey (Fig. 6) do indicate, however, that adenovirus infection rates in cases of pneumonia and ARD were higher in April and May than in February and March. The present speculation as to the cause of the low illness (and adenovirus infection) rate during February and March of this year is based on the changes made in the recruit training regimen beginning in November of 1970. These changes resulted from a meningococcal meningitis outbreak at this time. The modifications included decrease in company size from 80 to 60 men and a less strenuous training regimen.

Seroconversions to rhinovirus infections continue at higher rates (15-60%). Of the three rhinovirus test antigens employed (1A, 1B and 2), antibody titer rises to type 1B occurred with the greatest frequency (24-48%). Very few infections to influenza A2 or B were observed during this time:

B. Viral Etiology of Non-bacterial Pneumonia

In November 1970, a survey was instituted at the Orlando dispensary to determine the incidence of agents associated with cases of non-bacterial pneumonia (NPSO Study). Each case of radiologically diagnosed pneumonia was matched with a case of ARD and non-ARD. Nasal wash and throat swab specimens were collected and attempts were made to isolate mycoplasma or viral agents. Acute and convalescent serum samples were also obtained for serological tests. The mycoplasma data are incomplete and will not be presented. To date 200 specimens for virus isolation have been cultured and the results are shown in Table I and Figure 6. Table I summarizes the number of viruses isolated from specimens obtained from pneumonia patients and matched controls. These represent the total results thus far over the 7-month study. Although a greater percent of specimens from pneumonia cases yielded a virus, only Coe virus occurred significantly more often from these patients than from ARD or non-respiratory controls. In Figure 6 the pneumonia study data (NPSO) are presented as an extension of a previous dispensary study which related adenovirus isolation from cases of ARD (febrile and afebrile) with that from non-ARD subjects (OSS Study). The only viral agents isolated in any great numbers from the pneumonia and control groups were adenovirus, type 4. It could not be demonstrated, however, that these agents were more prevalent among cases of pneumonia than in controls. From the data obtained thus far, it would appear that there is a greater tendency for association of adenovirus with pneumonia when the overall incidence of adenovirus is lowest. The serological results shown in Figure 7 indicate a similar interpretation. These studies

will continue and will be correlated later with the mycoplasma data.

C. Seroepidemiology of Rubella in Navy Recruits

Until the epidemic in 1964, rubella was the second highest cause of hospital admissions in recruits at Great Lakes. Since 1963, a serological survey for rubella HI antibodies among recruits has been conducted at Great Lakes. This study determined the initial antibody titers of incoming recruits and the subsequent seroconversions of these individuals after their 9-11-week training period. Such information can be used to determine any changes in the susceptibility of 17-20-year-old males; the extent of recruit rubella infections, and the correlation between the number of infections with cases of clinical disease.

Table II shows the number of men reporting for recruit training with an initial titer of less than 1:10 to a titer of 1:160 or greater. There has not been an appreciable shift in the distribution pattern of initial antibody titer levels over the 3-year period. This pattern of antibody levels shows that only 9% of the 17-20-year-old males have no detectable rubella HI antibody titer and suggests that 91% of the recruit population is partially immune to rubella.

Table III displays the 3-year average number of individuals with initial rubella antibody titers, and shows the low number of susceptibles in this population. The percent of men who had seroconversions to rubella is shown in Table IV. Sixty-three - 81% of the men reporting with a titer of <1:10 showed a seroconversion to rubella during recruit training. The overall seroconversion rate is 9%. The 3-year mean seroconversion rates for those recruits reporting with higher initial antibody titers are shown in Table IV. Men reporting with titers of <1:10, 1:10, 1:20, 1:40 had the following yearly seroconversion rates: 73, 25, 10 and 3%, respectively. There were no seroconversions in those recruits who reported with a titer of 1:80 or greater. These seroconversion rates did not correlate with clinical rubella. Based on these data, 10,000 rubella infections occurred in the recruit population over the 3 years of this study; however, only 190 cases were admitted to the dispensary. This attests to the highly subclinical or mild nature of this disease among recruits.

The infection profile at Great Lakes during the calendar year by initial rubella HI titer is shown in Figure 8. It is apparent that 80-100% of the susceptibles (initial titer <1:10) become infected from November through June of each year. The infection rate during the summer-late summer period ranged from 40-50%. The data in this Figure also show that during the winter-late winter period, recruits who reported with an initial titer of 1:10 also had evidence of having experienced a rubella infection. Those with initial titer of 1:20 or greater showed little evidence of infections. Furthermore, the seasonal pattern of rubella infections is similar to that of ARD, i.e., markedly greater during the winter season.

A similar profile of rubella infections occurred at Orlando, Florida

(Fig. 9). This suggests that there may be a direct relationship between the two illnesses promoted by the greater dissemination of respiratory tract viruses from coughing and sneezing. Recruit training consists of an unusual environment where, even though there are 90% of the population immune to rubella, over 70% of the susceptibles become infected. This attests to the efficiency of dissemination of rubella virus as well as other infectious agents. These data suggest that to insure a "herd effect" against rubella infection in recruits, 97-98% of the men would have to be immunized.

The "herd immune effect" observed in other populations is probably inoperative in unusual environmental and epidemiological situations that exist at recruit training centers. This also emphasized the interrelationship of various disease problems in military populations. It is conceivable that prevention of ARD problems would also reduce the risks of other infections, such as rubella.

TABLE I

Viruses isolated from pneumonia patients and matched
ARD and non-respiratory controls
(Nov 1970-May 1971-Orlando, Fla.)

Viruses Isolated	Patients Sampled					
	Pneumonia (68 men)		ARD (63 men)		Non-respiratory (60 men)	
	No. Pos.	% Pos.	No. Pos.	% Pos.	No. Pos.	% Pos.
ADENO	31	46	25	40	19	32
RHINO	11	16	12	19	9	15
COE	7	10	2	3	2	3
HERPES	3	4	1	2	2	3
TOTAL	52	76	40	64	32	53

TABLE II

Initial rubella HI titers in men reporting for recruit training
Great Lakes, Illinois 1968-1970

Initial Titer	1968		1969		1970	
	Number	% Tested	Number	% Tested	Number	% Tested
<1:10	52	9.21	52	8.98	52	8.84
1:10	30	5.31	15	2.59	16	2.72
1:20	87	15.43	56	9.67	47	8.0
1:40	171	30.31	149	25.73	160	27.21
1:80	140	24.82	154	26.59	185	31.46
1:160	84	14.89	153	26.42	128	21.76
Total	564		579		588	

TABLE III

Rubella infections experienced by navy recruits in training
Great Lakes, Illinois 1968-1970

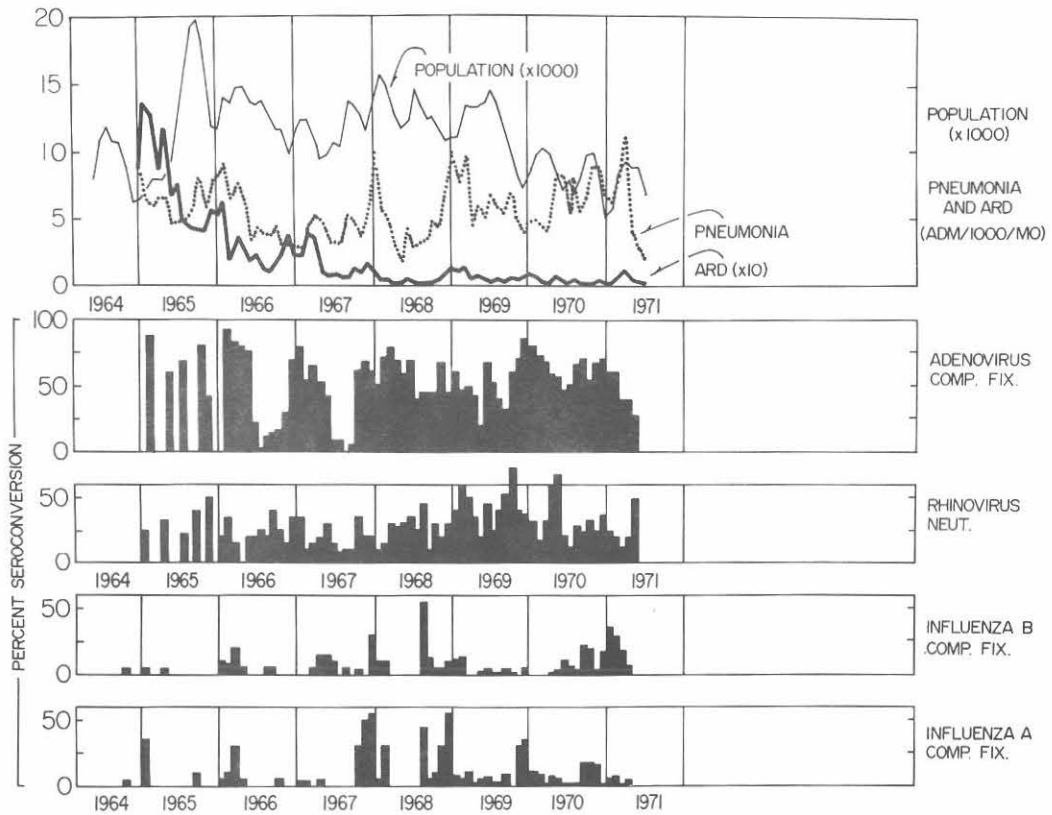
Initial Titer	Number of men	% of men with titer	Rises 4-fold or greater	% of men with 4-fold rise
<10	154	8.96	113	73.37
10	47	2.73	12	25.53
20	190	11.05	20	10.52
40	480	27.93	16	3.33
80	479	27.88	0	0
≥160	368	21.42	0	0
Total	1718		161	

Overall seroconversion rate: $161 / 1718 = 9.37\%$

TABLE IV

Rubella HI seroconversion rate in navy recruits
by initial HI titer and year of training
Great Lakes, Illinois 1968-1970

Initial Titer	1968	1969	1970	3-year average
<1:10	63.46	80.76	75.0	73.37
1:10	20.0	0	43.7	25.53
1:20	13.8	7.14	8.5	10.52
1:40	2.3	4.69	3.1	3.33
1:80	0	0	0	0
<u>≥</u> 1:160	0	0	0	0
Mean sero- conversion rate	9.75	9.15	9.35	



650 (REV 71)

Fig. 1. Population statistics, ARD and pneumonia incidence, and serological responses to respiratory disease viruses. Navy recruits, Great Lakes, Illinois, 1964-1971.

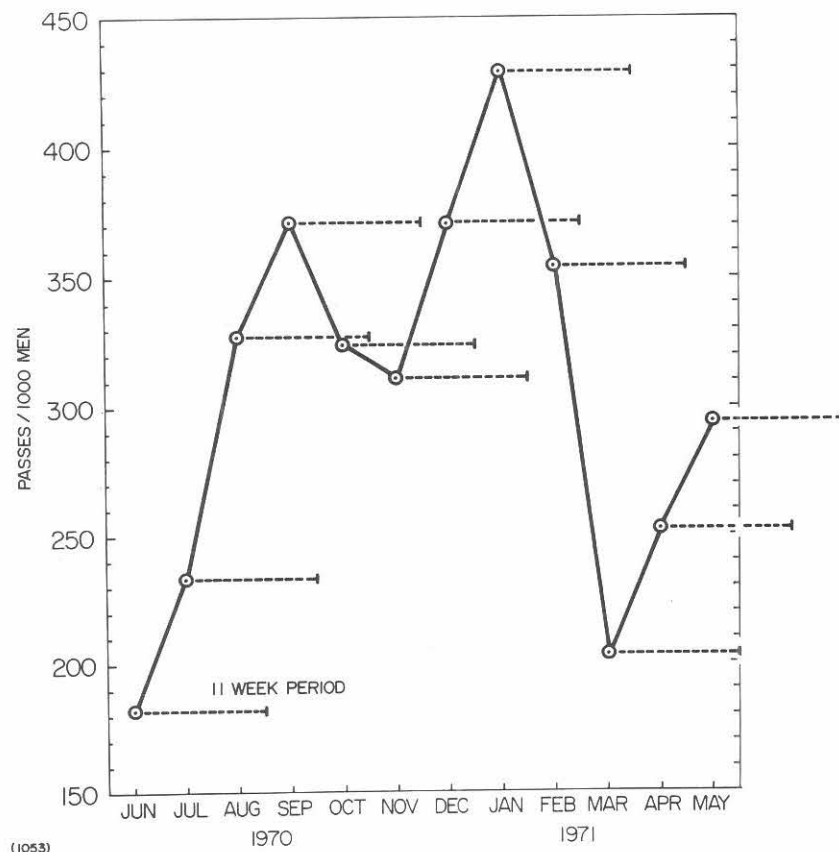
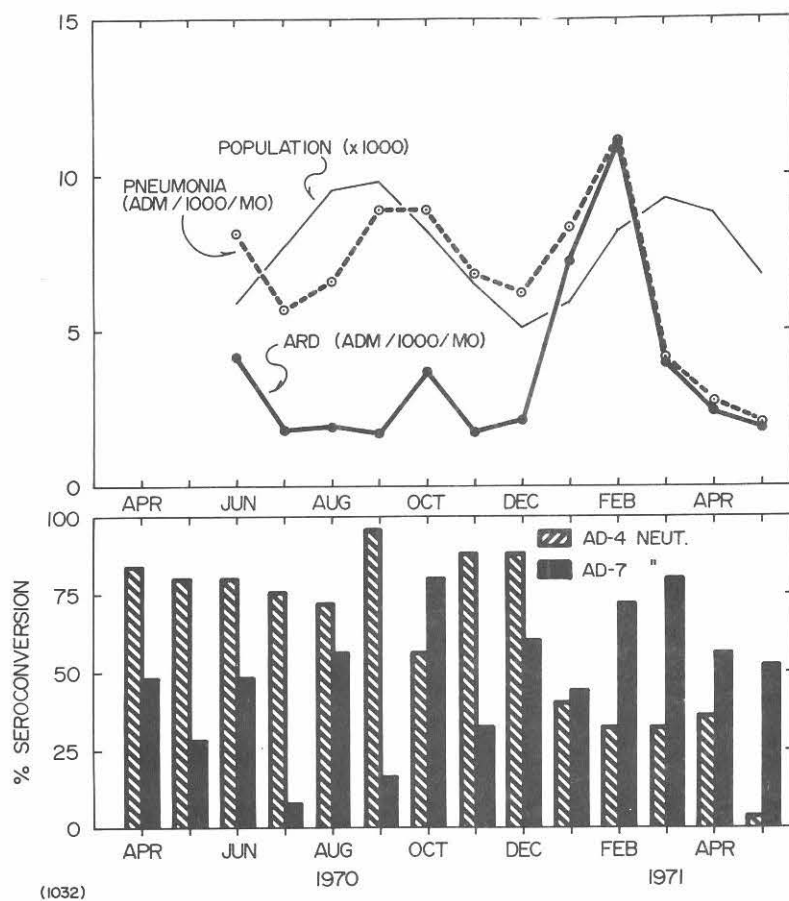


Fig. 2. Incidence of hospital admissions for ARD and pneumonia, and the relative proportions of adenovirus types 4 and 7 infections, and the rate of issue of "rack passes"* for navy recruits, Great Lakes, Illinois, 1970-1971.

*A "rack pass" is permission for bed rest in the barracks.

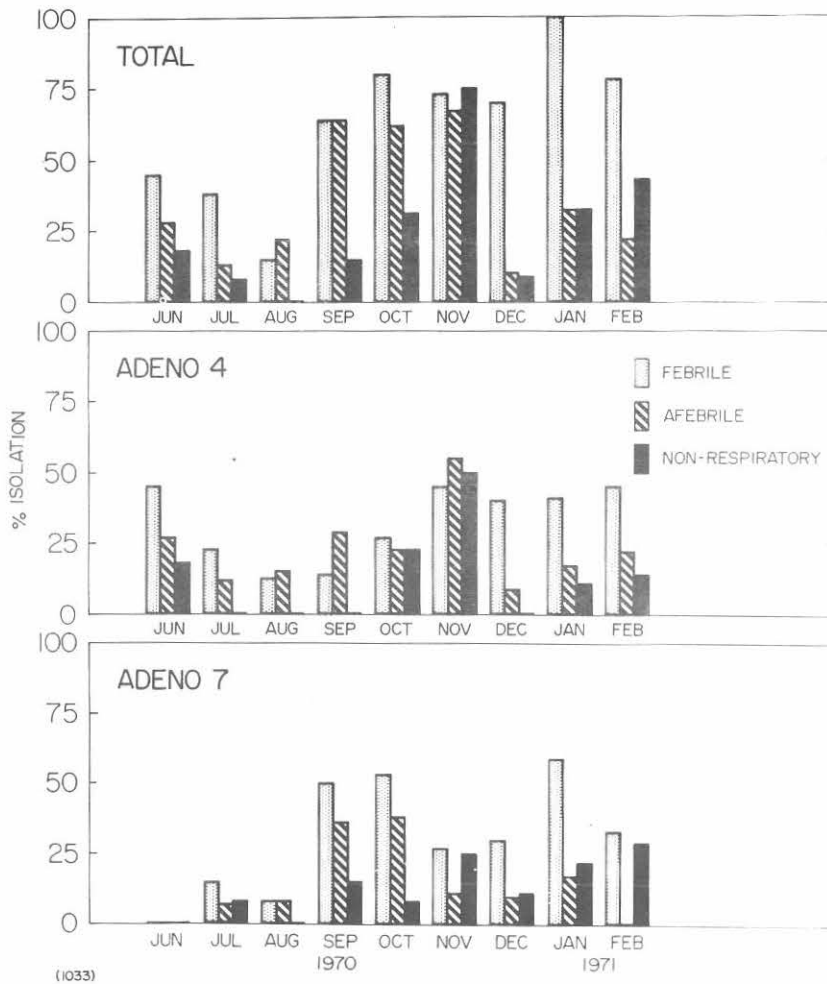


Fig. 3. Adenovirus isolations from recruits sampled at the Dispensary (ESS Study) Great Lakes, Illinois, 1970-1971.

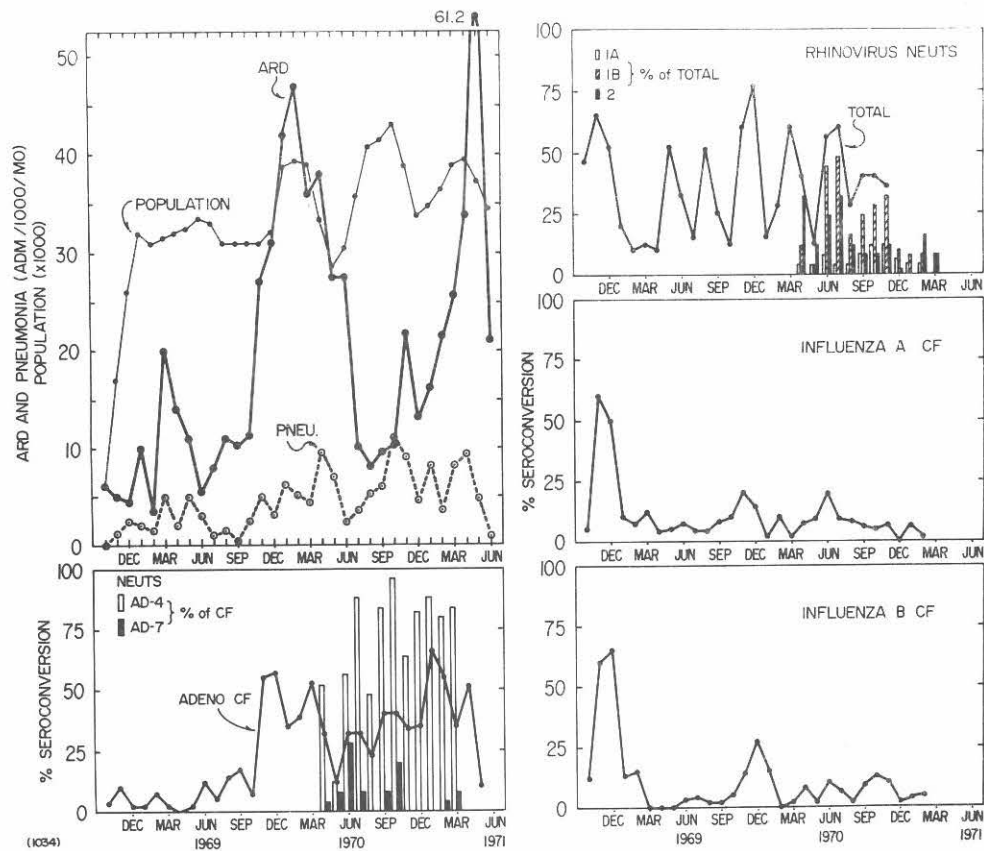


Fig. 4. Population statistics, ARD and pneumonia incidence, and serological responses to respiratory disease viruses. Navy recruits, Orlando, Florida, 1968-1971.

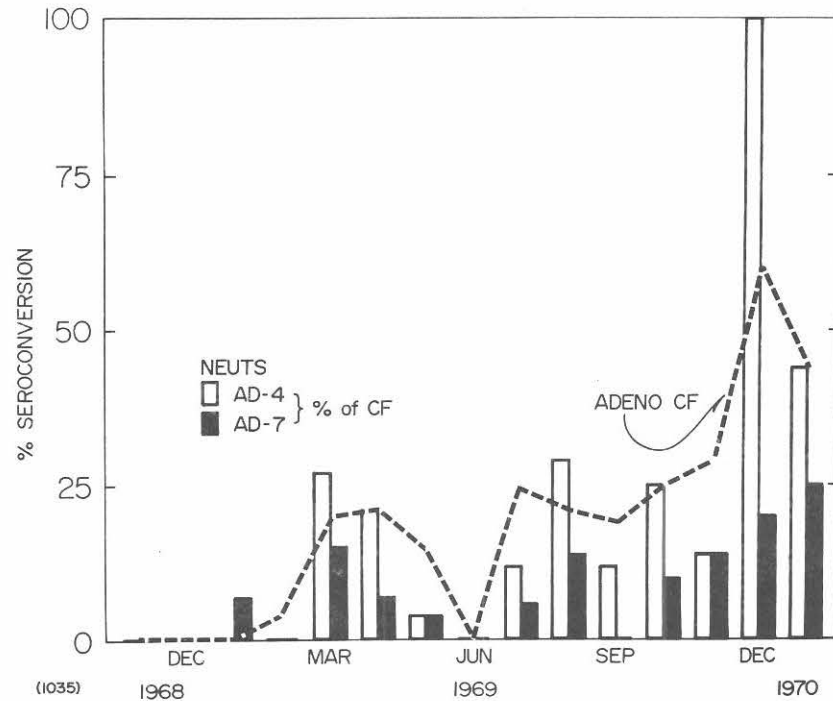


Fig. 5. Adenovirus seroresponses in recruits sampled at the Dispensary (OSS Study), Orlando, Florida, October 1968-January 1970.

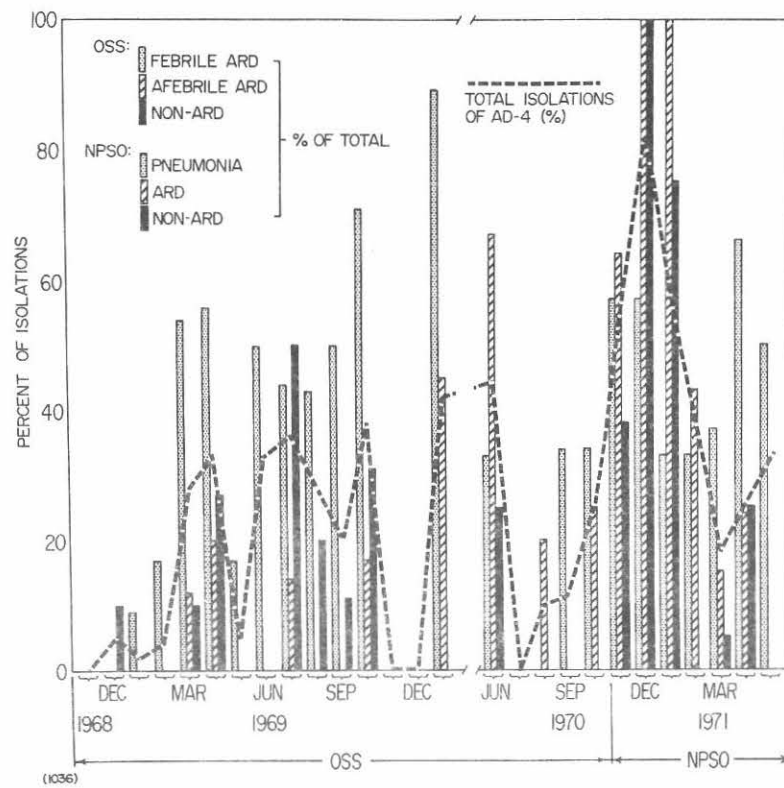


Fig. 6. Adenovirus type 4 isolations from various recruits sampled at the Dispensary (OSS and NPSO studies), Orlando, Florida, 1968-1971.

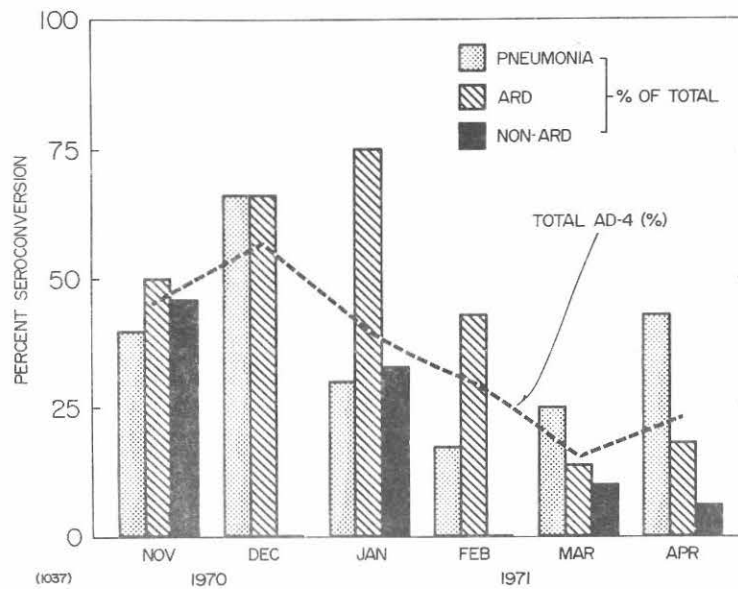


Fig. 7. Adenovirus type 4 seroconversion in recruits sampled at the Dispensary (NPSO Study), Orlando, Florida, November 1970-April 1971.

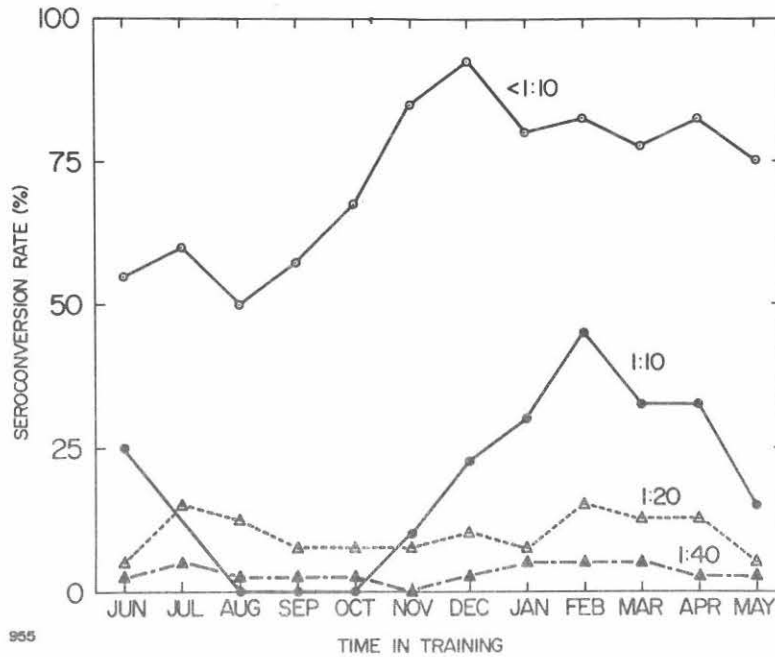


Fig. 8. Three-year monthly averages of rubella seroconversions in recruits entering training with various initial antibody titers. Great Lakes, Illinois, 1968-1970.

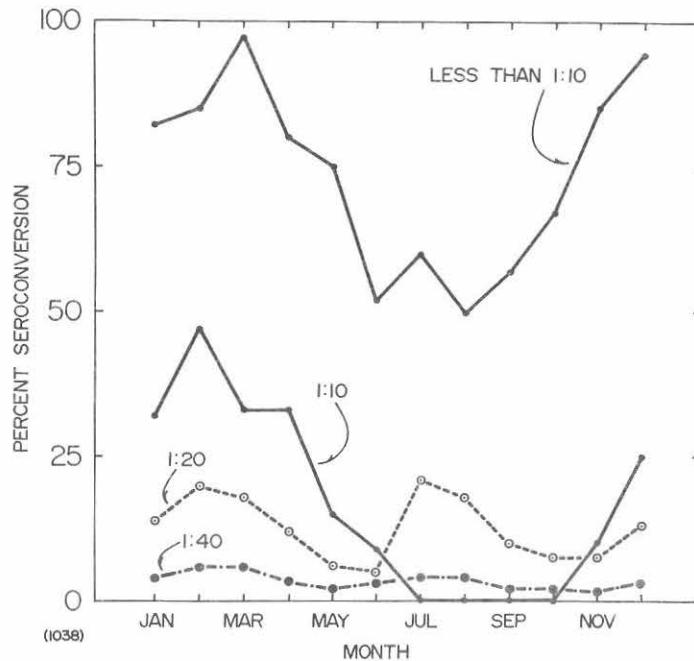


Fig. 9. Three-year monthly averages of rubella seroconversions in recruits entering training with various initial antibody titers. Orlando, Florida, 1968-1970.

II. EFFECT OF DOSE AND ROUTE OF IMMUNIZATION
ON THE ANTIBODY RESPONSES TO A₀/PR-8
INFLUENZA VACCINE*

The objectives of this study were to determine the optimal dose and route of inoculation for influenza vaccination. Three methods of vaccination, at various dosages, were compared for antibody responses in terms of seroconversion, magnitude of resulting antibody titers and rapidity of antibody formation. To make these studies relevant to future antigenic variants, the PR-8 strain of influenza type A₀ was employed. It was reasoned that this antigen was far removed from present strains (A2) of influenza virus and would provoke only primary antibody responses. Such an antigen would emulate, to some extent, the problems which might be encountered with a new variant.

MATERIALS AND METHODS

A zonal centrifuged vaccine purported to contain 6,000 CCA units of A₀/PR-8 was obtained by Dr. N. Tauraso, DBS, NIH. This vaccine was diluted in 3-fold increments to provide a theoretical range of doses from 3,000 to 10 CCA units and packaged in separate vials. Samples from each dilution-lot were assayed and the actual CCA values are shown in Table I.

Three methods of inoculation were employed for the various dose preparations. For intranasal inoculation, a metered nasal atomizer** was calibrated to deliver 0.5 ml of the respective vaccine dilutions to each nostril. Subcutaneous and intradermal vaccination was administered by needle and syringe delivering 1 and 1/10 ml, respectively, of each vaccine dilution. Study subjects were navy recruits from four basic training companies randomly assigned to the various treatment groups shown in Table II.

Doses were given in a step-wise manner to ascertain acceptability and reactogenicity of the four lowest doses in each route of inoculation before administering the highest doses. No adverse reactions, attributable to the vaccine, occurred with any of the doses employed.

*From Research Project No. M4305.12-5004AGG2, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

**DeVilbiss Corp., Toledo, Ohio.

DISCUSSION OF RESULTS

The results of this study are still being analyzed. The data in this report will include only antibody responses determined by hemagglutination-inhibition (HI) tests utilizing the homologous (PR-8) antigen.

Table III and Figure 1 show the serum antibody responses to all doses and routes of inoculation. In Table III the data are presented as geometric mean (\log_2) antibody titers of all men in a given vaccine group before (day 0) and at 7, 14, 28 and 63 days after vaccination. The initial titers of all groups were fairly comparable with the exception of those given 953 CCA units intranasally and 2139 CCA units, subcutaneously. At 7 days post-immunization, only those subjects who received the highest doses intradermally and subcutaneously (515 and 2139 CCA units, respectively), showed significant increases in antibody titers. By the 14th day, subjects in all of the parenteral vaccine groups (SQ and ID) even in the smallest doses (53 and 13 CCA, respectively) showed significant increases ($>2.0 \log_2$) in geometric mean titers. With only a single exception (ID-34CCA) the 14-day titers were the highest observed during the study. By the 63rd day, most of the titers had decreased slightly. The greatest titer increase occurred in those men who had received the largest parenteral doses.

The seroresponses of those subjects who had received the vaccines intranasally were slower in reaching the maximum titer (14-28 days). Moreover, such titers were somewhat lower ($0.8-1.0 \log_2$) than those provoked by the parenteral routes. Even at the greatest doses employed (2139 CCA), the responses of the intranasal group were about $1.0 \log_2$ (2-fold) lower than the comparable dose subcutaneously.

The data in Table III are displayed graphically in Figure 1. The values plotted here are the ratios of the geometric mean titers of the various vaccine groups before and at specified times after immunization.

From these data, it is apparent that vaccination by the parenteral routes produces faster and higher serum antibody responses than by intranasal inoculation. The seroresponses to both parenteral vaccines appeared to be biphasic, characterized by a sharp increase in antibody response to lower doses, followed by a plateau at the respective middle doses with a resurgent response to the highest doses. Unexplained was the considerably lower response to the 214 CCA intradermal vaccine. Whether this impaired response was due to the peculiarity of this group of men or to the vaccine itself is not known. However, a similar, but smaller decline was also observed in the group that received 953 CCA units of vaccine subcutaneously. At comparable amounts of antigenic mass (30-50 and 100-120 CCA units), the responses to either the intradermal or subcutaneous vaccines were quite similar.

The seroresponses to the intranasal vaccines appeared to be dose-dependent over a range of approximately 50 to 500 CCA units. Doses greater than this amount produced no greater antibody titer increases. Whether a second peak similar to that observed with the parenteral vaccines might have been attained with an even more potent vaccine (e.g. 6,000 CCA units) is a matter of conjecture. Nevertheless, the magnitude of responses provoked by even the highest intranasal vaccines at any time was considerably less than that produced by 34 CCA units of intradermal vaccine. Of all three routes of inoculation, the intradermal vaccines, with the exception of the 214 CCA unit-dose noted above, produced the highest responses.

Figure 2 shows the cumulative percent seroconversions ($2.0 \log_2$ or > antibody titer increases) at various times after immunization with the different vaccines. The response curves are grouped roughly according to similar amounts of antigenic mass administered by the various routes of inoculation (panels 1-3). In addition, panel 4 compares the responses to the highest doses of vaccine given by each of the respective routes. Of the three routes, the intradermal method produced the greatest number of seroconversions at any dose 14 days or later after vaccination. The intranasal route produced the fewest and required longest time (28 days or later) to achieve maximum number of seroconversions. The subcutaneous method was almost as good as the intradermal procedure. Both of the parenteral routes were able to seroconvert approximately 50% of the subjects by the 7th day after vaccination, but only at the highest doses employed (Fig. 2, panel 4). Significantly, almost four times the antigenic mass was required by the subcutaneous than by the intradermal method (2193 vs 515 CCA units). Nevertheless, it was remarkable that the intranasal method, albeit slower, was able to produce seroconversions in 80% of the vaccinees given the highest dose (2139 CCA units).

Figure 3 shows the $A_0/PR-8$ HI titers of nasal secretory antibody at various times after immunization by the three routes of inoculation. Considerable difficulty was encountered in interpreting the results of tests on individual specimens. Therefore, all samples from donors who had received the vaccine by a given route were pooled. These pools were concentrated approximately 100-fold and standardized by dilution to an IgA concentration of 10 mg% (± 3 mg). The results shown in Figure 3 are the mean values of the HI titers produced by all the various dosages of each specified route of inoculation. Significant increases in antibody titer were not apparent until 14 days after vaccination by any of the routes. By the 28th day only the responses to the intradermal and intranasal methods of inoculation continued to increase. At the end of the observation period (63rd day) only the antibody titers produced by intranasal vaccination showed continued increase.

From the results of this study, it appears that the intradermal method stimulates the most optimal type of secretory or humoral antibody response in terms of magnitude of titer, rapidity of response, and consistently high numbers of seroconversions with the least amount of antigenic mass.

TABLE I

Theoretical and assayed CCA units of A₀/PR-8 vaccine dilutions

Intranasal and Subcutaneous Vaccine CCA/1 ml		Intradermal Vaccine CCA/0.1 ml	
Theoretical	Actual	Theoretical	Actual
3,000	2,139	600	515
1,000	953	300	214
300	340	100	95
100	127	30	34
30	53	10	13

TABLE II

Numerical distribution of study subjects according to vaccine dose
and route of inoculation

Doses CCA Unit/Inoculum	Intranasal	Subcutaneous	Intradermal	Total
Placebo	15	14	14	
13	ND*	ND	14	
34	ND	ND	14	
53	15	14	ND	
95	ND	ND	14	
127	15	14	ND	
214	ND	ND	14	
340	15	14	ND	
515	ND	ND	14	
953	15	15	ND	
2,139	15	14	ND	
TOTAL	90	85	84	259

*Not done

TABLE III

Geometric mean HI serum antibody titers to A₀/PR-8 before and after vaccination

Inoculation route	CCA/Dose	Reciprocal of Titers at Initial Dilution (log ₂)*					Maximum difference (Post - Pre) at any time
		0	7	14	28	63	
Intranasal	Placebo	2.9	2.9	2.7	2.7	2.5	-
	53	2.5	2.1	3.3	<u>4.1</u>	3.5	1.6
	127	2.5	2.6	3.9	<u>4.5</u>	3.8	2.0
	340	2.5	2.3	4.4	<u>4.9</u>	4.5	2.4
	953	3.7	3.5	5.9	<u>6.3</u>	5.1	2.6
	2,139	3.1	3.3	5.3	<u>5.5</u>	4.5	2.4
Subcutaneous	Placebo	2.8	2.7	2.5	2.3	2.0	-
	53	2.8	3.2	<u>5.5</u>	5.0	5.0	2.7
	127	2.3	2.8	<u>5.5</u>	5.1	4.3	3.2
	340	2.2	2.3	<u>5.5</u>	5.5	4.2	3.3
	953	2.9	3.0	<u>6.1</u>	6.1	5.0	3.2
	2,139	3.6	5.5	<u>7.2</u>	7.2	5.9	3.6
Intradermal	Placebo	3.4	3.4	3.4	3.5	3.4	0.1
	13	2.9	3.4	<u>4.9</u>	4.9	4.0	2.0
	34	2.6	2.7	<u>5.5</u>	<u>5.8</u>	5.0	3.2
	95	2.6	3.1	<u>5.7</u>	<u>5.6</u>	5.7	3.1
	214	3.2	3.4	<u>5.6</u>	4.7	4.7	2.4
	515	3.0	4.2	<u>7.1</u>	7.0	6.9	4.1

*Microtiter tests

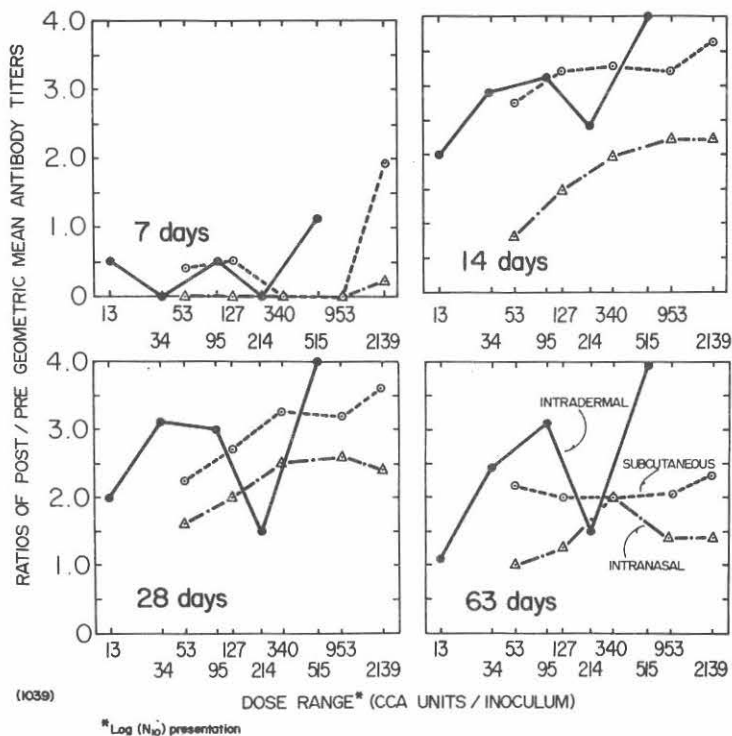


Fig. 1. The ratios of post-immunization versus pre-immunization mean A_0 /PR8 HI antibody titers according to the route of administration and the dosage, at various times (days) following vaccination.

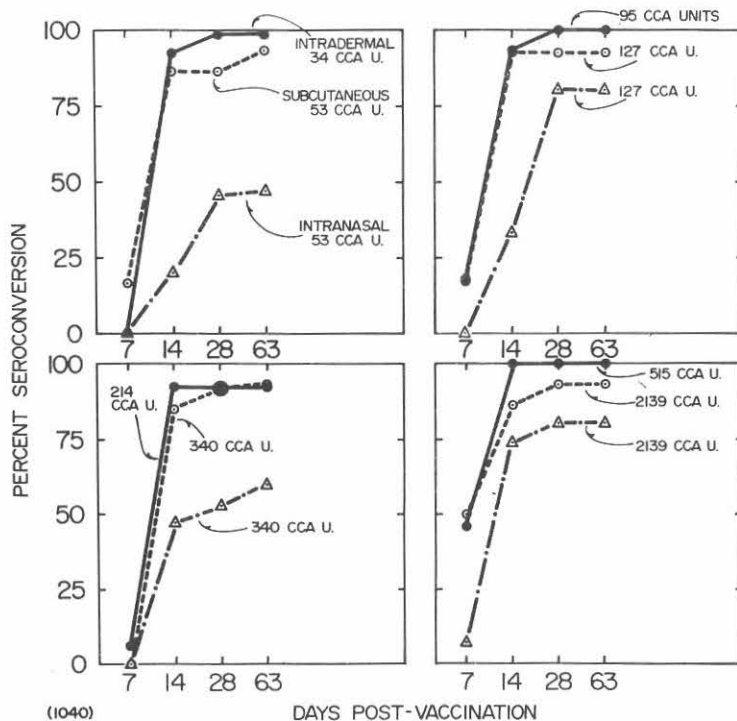


Fig. 2. Cumulative percent seroconversion resulting from various doses (CCA units) of A_0 /PR8 vaccine administered by different routes.

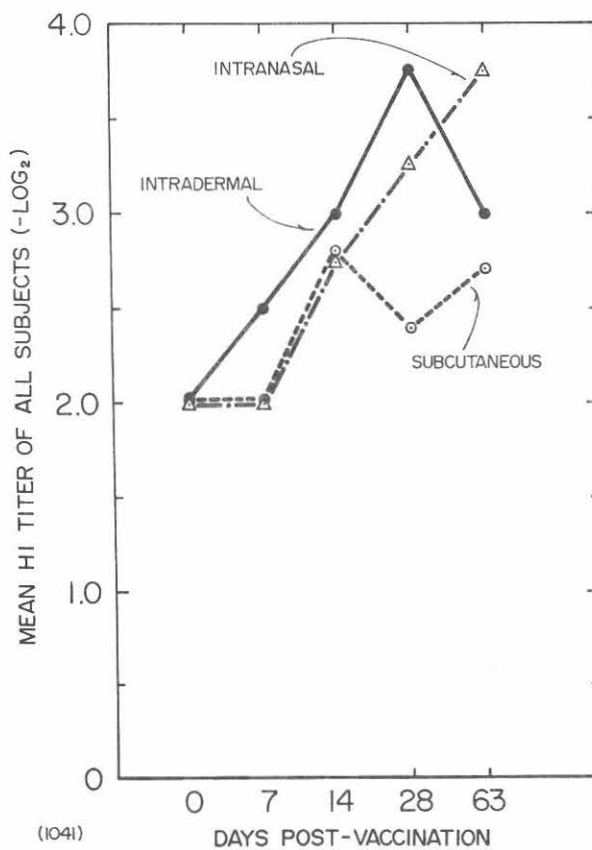


Fig. 3. A₀/PR₀ HI antibody titers of pooled concentrates* of nasal secretions before and after vaccination.**

*Concentrated to 10 mg IgA.

**75 men represented by each point.

III. EFFECT OF LIVE ADENOVIRUS TYPES 4 AND 7 VACCINES
ON ADENOVIRUS INFECTIONS AND RESPIRATORY
DISEASES OF NAVAL RECRUITS*

During the fall and early winter of 1970 surveillance studies indicated a high incidence of adenovirus type 4 and 7 infections among recruits at Great Lakes. To curtail further infections and decrease ARD a study was designed to determine the protective efficacy of live adenovirus vaccines, types 4 and 7, given singly or in combination. Also, the occurrence of the dual adenovirus challenge offered the opportunity to compare the relative efficacies of bivalent (4 & 7) with monovalent (4 or 7) live adenovirus vaccinations.

MATERIALS AND METHODS

Vaccines

Type 4 vaccine (lot #00201) in tablet form had been previously obtained from the Vaccine Development Branch, NIH, in 1967 and had been since stored at 4°C. The titer of this preparation at the time of the study had decreased from the original value of 5.5 TCID₅₀/tablet to 4.1. Because of this low potency, two tablets were administered.

Type 7 vaccine (lot #02301) was obtained from Colonel E. Beuscher, WRAIR in February of 1971. Only one tablet was administered. Unfortunately, the potency of this lot was not assayed until the end of the study (May 1971). At this time, it was found that the titer per tablet was 3.45 TCID₅₀ (average of 9 determinations).

Study Design

The vaccination period extended from 15 Feb to 7 May 1971, but clinical and laboratory observations were carried out until 7 July. Approximately 100 companies of men participated in the study (7,450 volunteers). Each company was divided randomly into 4 groups: (a) Group A - 20% placebo; (b) Group E - 20% type 4 vaccine; (c) Group F - 20% type 7 vaccine, and (d) Group D - 40% both type 4 and 7 vaccines. Groups B and C, respectively, consisted of non-study subjects either within study companies or in other companies composed entirely of non-study men. Treatments were given to each company on the fourth day of training.

*From Research Project No. M4305.12-5007BEG6, Bureau of Medicine and Surgery, Navy Department, Washington, D.C.

Laboratory Tests

Every other week during the study, two companies were bled prior to immunization and again in either the 3rd or 4th week of training. Paired serum specimens were assayed by neutralization test for adenovirus 4 and/or 7 antibody titers.

Also, each week throat swabs were obtained from an entire company in either the 3rd or 4th training week for virus isolation attempts. In addition, 50 men per week who reported to the dispensary for treatment were sampled for virus isolation and evidence of adenovirus seroconversions.

Virus isolation and serological tests were carried out by microplate methods.

Clinical Analyses

Illness incidence data was abstracted from dispensary admission records and analyzed according to the treatment administered to the recipients.

DISCUSSION OF RESULTS

1. Pre-study Adenovirus Infection Rates

The extent of adenovirus infection in the Great Lakes recruit population prior to onset of the live adenovirus vaccine study on 15 Feb is shown in Figure 1. The upper panel shows the percent of CF seroconversions from October through February and the relative proportions of these rises due to adeno-4 and 7 infections.

Both virus types were well represented and alternated in the dominant role. Many individuals exhibited dual seroconversions. At the beginning of the vaccine study (BLAV - 15 Feb), adeno-7 was the dominant serotype encountered in the recruits of the company survey.

The lower panel (Fig. 1) shows the incidence of adeno serotypes isolated from recruits reporting to the dispensary with febrile ARD. Here again a high incidence of adenovirus infection was noted with a representation of adeno-4 and 7 serotypes roughly similar to that shown in the serological survey. These data indicated that adenovirus challenge was ideal to test the efficacy of both adenovirus vaccines.

To properly evaluate the protectiveness of the vaccine(s), it was considered necessary to make an intensive survey of ensuing adenovirus infections (diagnosed by throat virus isolations), not only in the dispensary, but in the barracks as well. Hence, a preliminary survey of the most appropriate time to

obtain specimens from recruits in training was initiated. The results shown in Figure 2 indicate that, while the greatest number of type 7 isolates were recovered during the third week of training, the optimal recovery of type 4 appeared to be during the fourth week. Therefore, weekly collections of virological specimens from companies in the barracks were taken alternately during the 3rd and 4th weeks of training.

2. Immunogenicity of Vaccines

During the 12 weeks of study, each week a company of recruits who had received vaccines as described was bled at the time of vaccination and again 3-4 weeks later. Paired serum specimens were available on approximately 8 to 10 companies and were tested for neutralizing antibody seroconversions to types 4 and 7 adenovirus. The results are shown in Figures 3 and 4. These data were obtained from only those individuals who had received either type 4 or type 7 vaccine, or both. Figure 3 indicates that the type 4 antibody responses varied considerably from 28-71% with a mean rate of approximately 56%. There was no significant overall difference in type 4 responses of men who received adeno-4 vaccine alone from those who had been dually vaccinated.

On the other hand, the responses to the type 7 vaccine were far from optimal (Fig. 4). These seroconversions ranged from 8-50% with a mean of about 22%. This poor response probably reflects the low potency of the vaccine (3.45 TCID₅₀) noted previously. Monovalent 7 vaccine given alone or in combination with type 4 produced seroresponses similar to unvaccinated men.

3. Effect of Vaccines on Adenovirus Infections

Figure 5 shows the complement fixation seroresponses and proportionate involvement of adenovirus types 4 and 7 of men in the surveillance companies. These individuals did not participate in the study and were wholly unvaccinated. The effect of the vaccine program on reducing the overall incidence of adenovirus infection in the entire recruit population is reflected in these surveillance subjects. The total adenovirus CF seroconversion rate declined rapidly during the 3 months of observation after vaccination. Although type 4 infections were virtually eliminated by May, type 7 seroconversions were only moderately affected.

Figures 6, 7 and Table I show the results of adenovirus isolations from men in the barracks or dispensary survey programs. The data are arranged by vaccine and control groups and by adenovirus type isolated. There was an abrupt decline in virus-positive specimens from the barracks survey for either adeno serotype within one month after the vaccine program began (Fig. 6). This was especially noted with respect to type 4 isolations. A similar, but more moderate decrease in adenovirus-positive specimens occurred in the dispensary survey (Fig. 7). While the protective effect of the vaccine is somewhat variable with respect to type 7 infections, consistently less type 4 isolates were recovered from vaccinated men than

from non-vaccinated individuals. When all adeno-4 isolates are grouped together according to the treatment group of the donors (Table I), there were statistically significant less type 4 recovered from type 4 vaccinees than from control subjects sampled at the dispensary or in the barracks (P = .008 and .02, respectively). The reduction of adenovirus isolations in the dispensary was about 50%. No statistically significant protective effect could be demonstrated against type 7 infections.

Figure 8 shows the adenovirus isolation experience from a sampling of recruits reporting to the dispensary from 1966-1971. No differentiation is made as to whether the patient had respiratory or non-respiratory complaints. The data are presented as 4-month averages of the total percent of men adenovirus-positive and proportions due to type 4 or 7. The percent of specimens adenovirus-positive during the 1971 vaccine study (Mar-June) decreased considerably (43% reduction) from the previous 4-month average (Nov-Feb). There was a specific reduction of adeno-4 of about 62%. Little change was noted for the 4 months before and after the vaccine program in the percent of men infected with adeno-7. The only time a similar reduction (44%) of overall adenovirus infection was observed during the height of the adenovirus infection season (Nov-April) occurred during 1966 following a mass live adenovirus type 4 vaccine program (as well as during the 1964-65 LAV studies, not shown).

4. Effect of Vaccines on Respiratory Diseases

Figure 9 compares the previous 5-year mean incidences of ARD and pneumonia with the illness experience before and during the 1971 adenovirus vaccine study. The greatly depressed incidence of hospitalized ARD during the present year is probably due to the "rack pass" policy explained previously (Section I, this report). These data are not shown here. Nevertheless, it is apparent that the illness rates were steadily increasing just prior to the vaccine study and declined sharply afterward. The rates for March and April of 1971 are far less, proportionately, than what had been observed during the previous 5 years. It is believed that this decrease was due to the adenovirus vaccines employed. Moreover, a similar decrease in pneumonia incidence was also noted during this time. Previous studies with monovalent adenovirus vaccines (type 4) have not been associated with this effect.

Figure 10 shows the febrile ARD experience of recruits who received adenovirus vaccines and is compared to that of men who were non-vaccinated or placebo controls. Also indicated are the percents relative reduction between the different experimental groups. The most pronounced difference (45% relative reduction) between the vaccinees and controls occurred with respect to the groups that received both adeno-4 and 7 vaccines (group D) as opposed to those recruit companies wholly composed of individuals that received no vaccine (group C). Those controls [placebo (group A) or non-vaccinees (group B)] who were distributed within companies that received adenovirus vaccines (approximately 60%, either 4 or 7) fared slightly better than their totally unprotected cohorts. This difference, however, did not reach the level of statistical significance (groups A & B vs C; $P \sim .14$).

Those individuals who received only the adeno-4 vaccine (group E) showed a 33% reduction when compared to group C. This difference in protection is probably due to the absence of the lesser, but significant protection provided by the type 7 vaccine (group F). When illness rates of all controls (groups A, B, & C) were compared to that of the group D, who received both vaccines, a reduction of 39% was observed. The illness data of hospitalized patients with ARD or pneumonia (not shown) also indicated the protective effect of the bivalent (4 + 7) vaccines. These vaccines apparently had no effect on ARD that was not associated with fever. Thus, it is assumed that the protective effect was primarily ameliorative.

TABLE I
Adenovirus Isolations From Study Subjects Sampled at Barracks or Dispensary

Study	ADENOVIRUS-4 ISOLATES																	
	BARRACKS SAMPLES									DISPENSARY SAMPLES								
	CONTROLS			VACCINEES			CONTROLS			VACCINEES			CONTROLS			VACCINEES		
	No. Vacc	Pla- cebo	Ad-7 Vacc	Total		Ad-4 Vacc	Ad-4 + 7 Vacc	Total		No. Vacc	Pla- cebo	Ad-7 Vacc	Total		Ad-4 Vacc	Ad-4 + 7 Vacc	Total	
No. Pos.	No. Pos.	No. Pos.	No. Pos.	%	No. Pos.	No. Pos.	No. Pos.	%	No. Pos.	No. Pos.	No. Pos.	No. Pos.	%	No. Pos.	No. Pos.	No. Pos.	%	No. Pos.
Month	No. Test.	No. Test.	No. Test.	No. Test.	Pos.	No. Test.	No. Test.	No. Test.	Pos.	No. Test.	No. Test.	No. Test.	No. Test.	Pos.	No. Test.	No. Test.	No. Test.	Pos.
March	$\frac{0}{10}$	$\frac{2}{38}$	$\frac{0}{38}$	$\frac{2}{86}$	2	$\frac{0}{34}$	$\frac{2}{90}$	$\frac{2}{124}$	2	$\frac{3}{32}$	$\frac{6}{20}$	$\frac{4}{17}$	$\frac{13}{69}$	19	$\frac{2}{13}$	$\frac{4}{34}$	$\frac{6}{47}$	13
April	$\frac{1}{15}$	$\frac{4}{55}$	$\frac{4}{56}$	$\frac{9}{126}$	7	$\frac{0}{58}$	$\frac{2}{118}$	$\frac{2}{176}$	1	$\frac{10}{59}$	$\frac{4}{26}$	$\frac{2}{26}$	$\frac{16}{111}$	14	$\frac{3}{26}$	$\frac{4}{42}$	$\frac{7}{68}$	10
May	$\frac{0}{10}$	$\frac{0}{41}$	$\frac{1}{47}$	$\frac{1}{98}$	1	$\frac{0}{41}$	$\frac{0}{76}$	$\frac{0}{117}$	0	$\frac{5}{61}$	$\frac{6}{24}$	$\frac{1}{22}$	$\frac{12}{107}$	11	$\frac{1}{24}$	$\frac{1}{42}$	$\frac{2}{66}$	3
June	ND†	ND	ND	ND	ND	ND	ND	ND	ND	$\frac{1}{21}$	$\frac{1}{4}$	$\frac{2}{5}$	$\frac{4}{30}$	13	$\frac{0}{12}$	$\frac{0}{8}$	$\frac{0}{20}$	0
TOTAL				$\frac{12}{310}$	4			$\frac{4}{417}$	1*				$\frac{45}{317}$	14			$\frac{15}{201}$	7**

Study	ADENOVIRUS-7 ISOLATES																			
	Ad-4			Ad-7			Ad-4			Ad-7			Ad-4			Ad-7				
Month	No. Pos.	No. Test.	No. Pos.	No. Test.	%	No. Pos.	No. Test.	No. Pos.	No. Test.	%	No. Pos.	No. Test.	No. Pos.	No. Test.	%	No. Pos.	No. Test.	No. Pos.	No. Test.	%
March	$\frac{2}{10}$	$\frac{6}{38}$	$\frac{4}{34}$	$\frac{12}{82}$	15	$\frac{11}{38}$	$\frac{6}{90}$	$\frac{17}{128}$	13	$\frac{7}{32}$	$\frac{7}{20}$	$\frac{4}{13}$	$\frac{18}{65}$	28	$\frac{2}{17}$	$\frac{10}{34}$	$\frac{12}{51}$	24		
April	$\frac{0}{15}$	$\frac{1}{55}$	$\frac{4}{58}$	$\frac{5}{128}$	4	$\frac{0}{56}$	$\frac{3}{118}$	$\frac{3}{174}$	1	$\frac{8}{59}$	$\frac{6}{26}$	$\frac{2}{26}$	$\frac{16}{111}$	14	$\frac{6}{26}$	$\frac{5}{42}$	$\frac{11}{68}$	16		
May	$\frac{0}{10}$	$\frac{0}{41}$	$\frac{0}{41}$	$\frac{0}{92}$	0	$\frac{0}{47}$	$\frac{1}{76}$	$\frac{1}{123}$	1	$\frac{4}{61}$	$\frac{3}{24}$	$\frac{4}{24}$	$\frac{11}{109}$	10	$\frac{2}{22}$	$\frac{2}{42}$	$\frac{4}{64}$	6		
June	ND	ND	ND	ND	ND	ND	ND	ND	ND	$\frac{10}{21}$	$\frac{1}{4}$	$\frac{1}{12}$	$\frac{12}{37}$	32	$\frac{0}{5}$	$\frac{1}{8}$	$\frac{1}{13}$	8		
TOTAL				$\frac{17}{302}$	6			$\frac{21}{425}$	5				$\frac{53}{322}$	18			$\frac{28}{196}$	14		

* P = .008

** P = 0.02

† Not Done

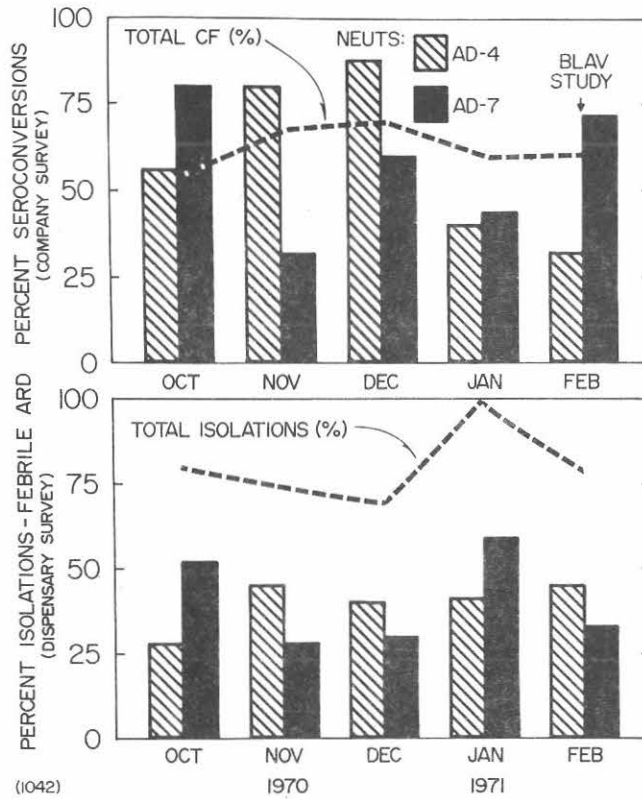


Fig. 1. Incidence of adenovirus infection prior to the Live Adenovirus Vaccine Study.

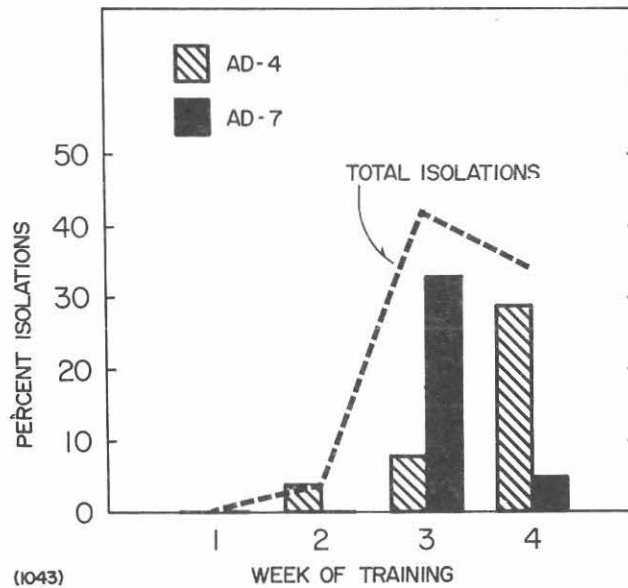


Fig. 2. Adenovirus isolation from randomly selected recruit companies before 14 January, 1971, Live Adenovirus Vaccine Study.

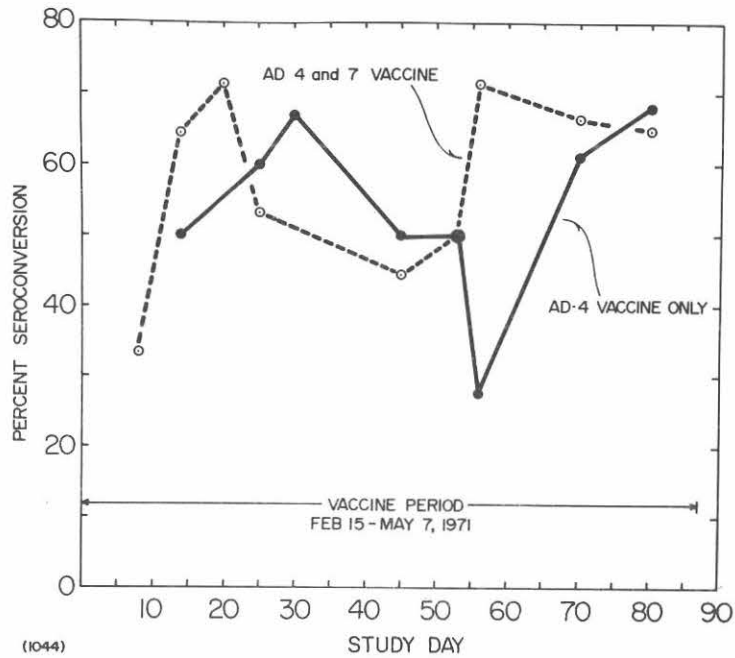


Fig. 3. Adenovirus type 4 seroconversion in navy recruits who received adeno-4 vaccine only, or both adeno-4 and adeno-7 vaccines.*

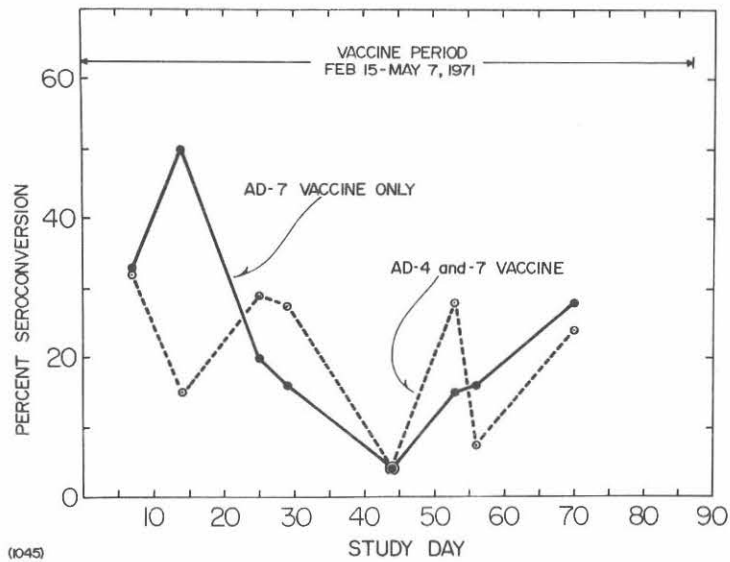


Fig. 4. Adenovirus type 7 seroconversion in navy recruits who received adeno-4 vaccine only, or both adeno-4 and adeno-7 vaccines.*

*In these figures, each point represents seroconversion which occurred at 21 or 28 days after vaccination. The men were vaccinated on the fourth day of training.

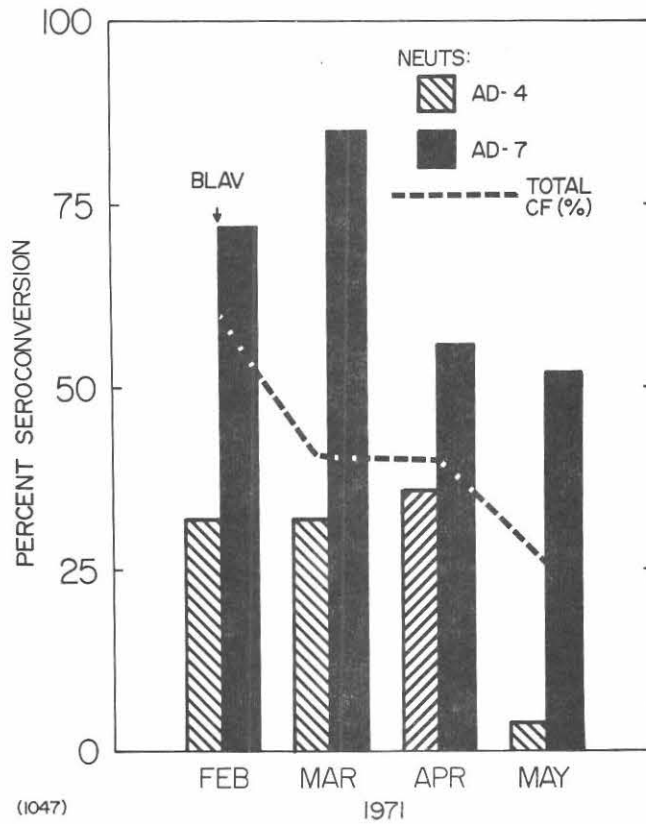


Fig. 5. Adenovirus CF seroresponses, and the proportions contributed by adenovirus type 4 and adenovirus type 7 following the Live Adenovirus Vaccine Study (Company survey, February-May, 1971).

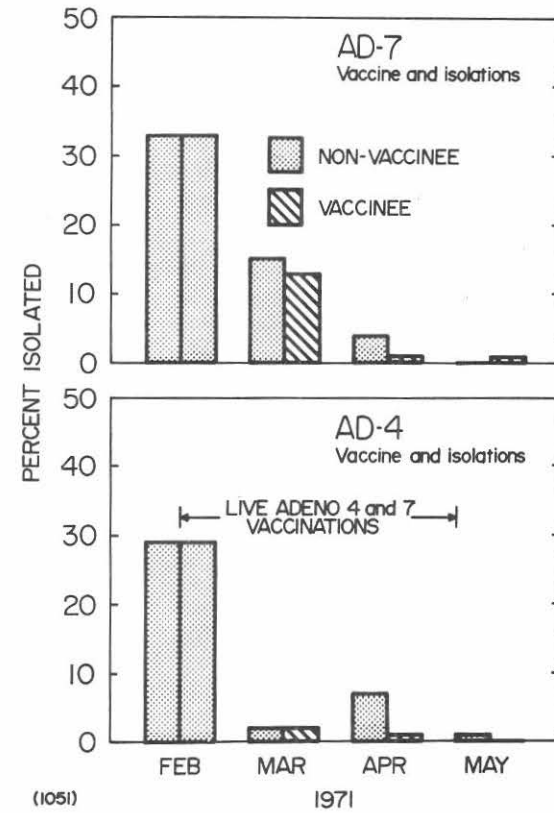


Fig. 6. Adenovirus isolations from recruit companies sampled in the barracks.

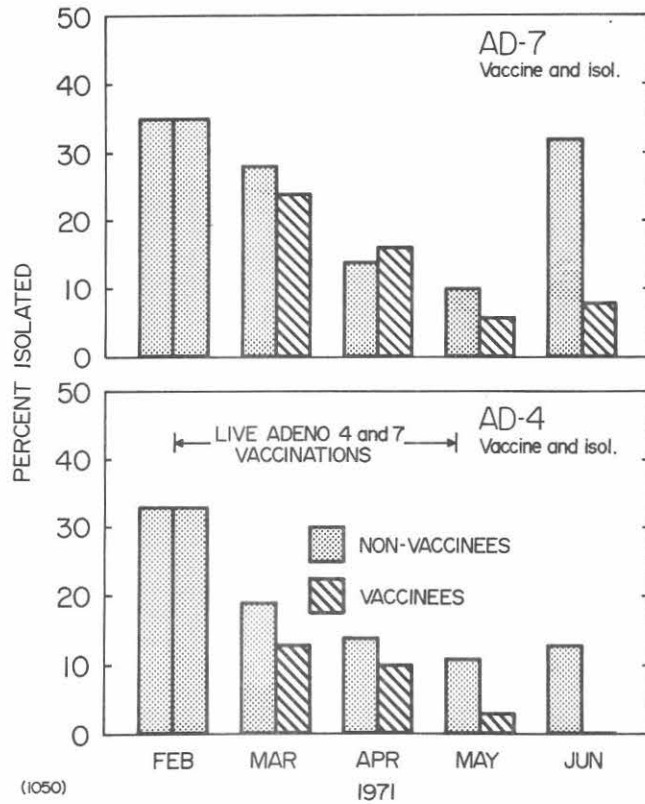


Fig. 7. Adenovirus isolations from recruits sampled at the Dispensary.

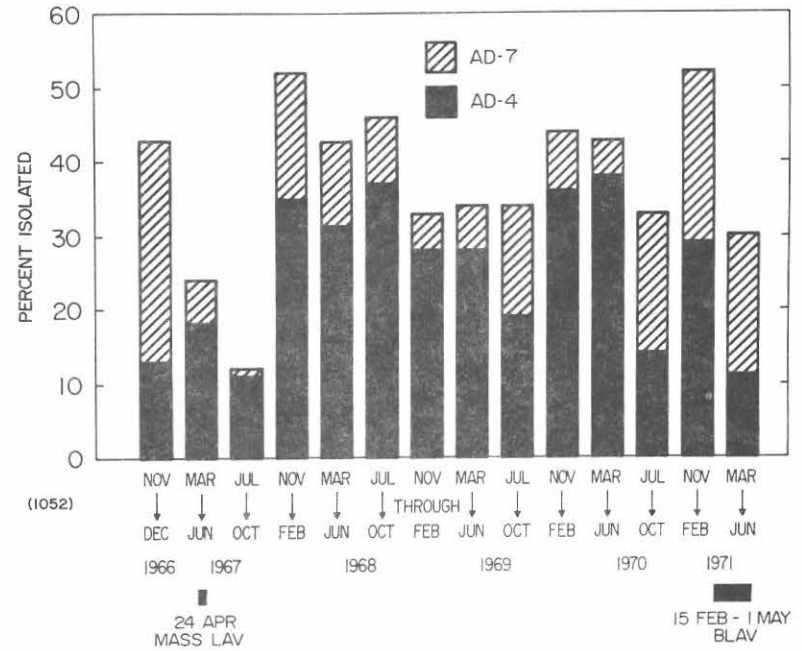


Fig. 8. Adenovirus types 4 and 7 isolation rates from patients sampled at the Dispensary (ESS Study, 1966-1971).

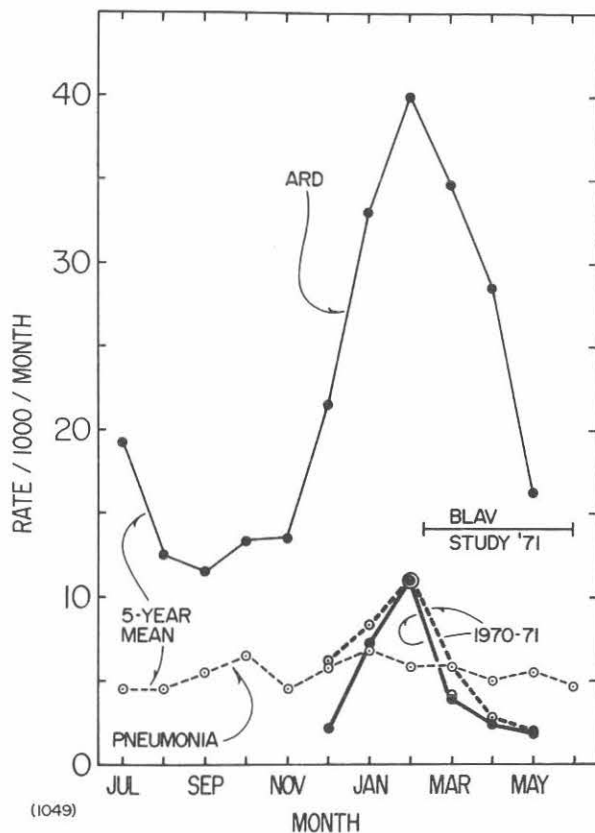


Fig. 9. Five-year (1965-1970) mean ARD and pneumonia incidence in recruits, compared with the 1971 illness experience.

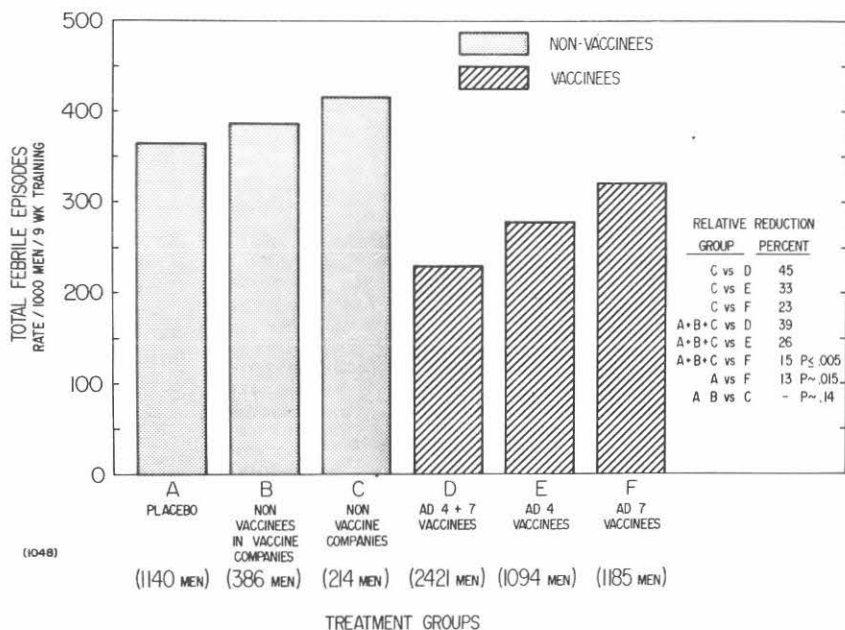


Fig. 10. Febrile ARD experience of non-immunized recruits, and of those who received adenovirus vaccine(s).

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13. ABSTRACT			
<p>Surveillance of naval recruits for viral respiratory diseases at Great Lakes, Illinois and Orlando, Florida showed that the continued illness problems were caused mainly by adenovirus, types 4 or 7. A limited outbreak of B/Mass influenza virus was detected at Great Lakes. Rubella infections continue to occur in the low proportion of susceptible recruits even though they were distributed among an immune population (91%). A study of the best method and dosage for administration of influenza vaccine (A₀/PR-8) showed that the parenteral routes (intradermal and subcutaneous) produced more rapid and greater serum antibody titers than the intranasal route. The intradermal route was as effective as the intranasal method for stimulating nasal secretory antibodies. The intradermal route would appear to be the method of choice, since it produced the most optimal antibody responses with the least amount of antigenic mass. Live adenovirus, types 4 and 7, vaccines of medium potency, when given in combination, were effective in reducing the incidence of febrile respiratory disease (40%), and adenovirus, type 4 infections (40-60%) when compared to non-vaccinated controls.</p>			

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Navy recruits acute respiratory disease virus surveillance viral pneumonia rubella influenza vaccines live adenovirus vaccines adenovirus infection						

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14. **KEY WORDS:** Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical content. The assignment of links, roles, and weights is optional.



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