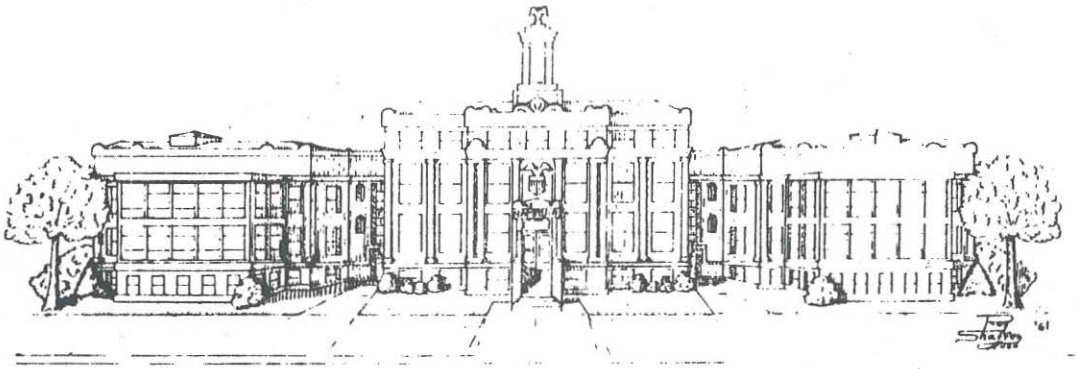
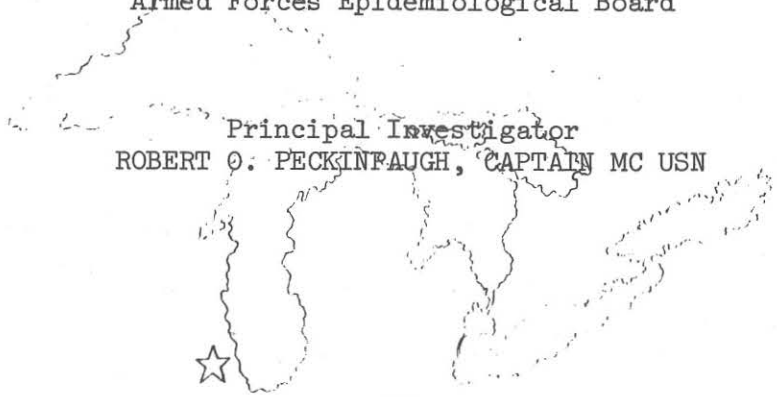
NAVAL MEDICAL RESEARCH

UNIT No. 4



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

Principal Investigator
ROBERT O. PECKINPAUGH, CAPTAIN MC USN



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Epidemiology and Prevention of Acute Respiratory Disease in Navy Recruits

ANNUAL PROGRESS REPORT

by

ROBERT O. PECKINPAUGH, CAPTAIN MC USN

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Annual Report to the Commission on Influenza
of the Armed Forces Epidemiological Board

Navy Research Project Nos: MF 12.524.009-4013B; M4305.12-4001B

Naval Medical Research Unit No. 4
Great Lakes, Illinois 60088

Annual Report to the Commission on Influenza
Armed Forces Epidemiological Board

Epidemiology and Prevention of Acute Respiratory Disease in Navy Recruits

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SUMMARY

Surveillance studies

The incidence of acute respiratory disease (ARD) during 1969-70, as measured by hospital admission rates of recruits at Great Lakes, continued to be low although high rates of adenovirus infection prevailed. In contrast, ARD rates at Orlando were considerably higher during the same reporting period. This discrepancy was due to the different hospital admission policies of the two training centers. Adenovirus type 4 infections appear to have become endemic at Orlando and were associated with the increased illness rates.

A survey of rubella antibody titers of incoming recruits at Great Lakes showed that only 9% had titers less than 1:10. Of these, 70% showed seroconversions. The low incidence of clinical rubella indicated that most of these infections may have been asymptomatic. The increase in rubella seroconversion rates among susceptibles at Orlando indicated that a greater transmissability of these viruses, as well as adenoviruses, had occurred at this new training center during 1969.

An examination of the incidence and distribution of 11 different microbial infections at Great Lakes over the past five years revealed a statistically significant excess number of men either without any infections or with multiple infections. Combinations of Neisseria meningitidis and adenovirus infections occurred in the same men more often than would be expected by chance.

Influenza studies

Serologic evidence indicated that influenza A2 infections occurred during early winter (1969) at Great Lakes (35% seroconversions). No concomitant increase in ARD rates was noted.

A protection study comparing adjuvant with aqueous preparations of ether-extracted hemagglutinin antigens revealed no difference in ARD admission rates between vaccinated subjects and controls.

An acceptability study involving 38 combinations of different preparations of influenza vaccines, additives and routes of inoculation demonstrated that the hemagglutinin vaccine prepared by zonal centrifugation, when administered intramuscularly, in a 0.5 ml dose, produced the least number of local reactions. No difference between vaccine groups was observed when systemic responses were compared.

A study of the effect of influenza vaccination or infection on the detection of rheumatoid factor-like responses showed that individuals who acquired influenza antibodies either artificially or naturally were also more likely to exhibit rheumatoid factor-like substances.

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INCIDENCE OF RESPIRATORY VIRUS INFECTIONS OF NAVY RECRUITS AND RELATION TO ACUTE RESPIRATORY DISEASE AT NTC, GREAT LAKES, ILLINOIS AND ORLANDO, FLORIDA DURING 1969-1970*

Surveillance studies for infectious agents associated with acute respiratory disease (ARD) in Navy recruits at Great Lakes have been described in previous reports to this Commission. These serological and virological studies have been in continuance since 1964. In addition to data from Great Lakes, observations on the new recruit training camp at Orlando, Florida (commissioned 1 October 1968) are included in this report. The results of a special virus isolation program carried out at Great Lakes and Orlando (designated ES and OS, respectively) are also presented. These additional surveys were instituted during the past 3 years to supplement the regular surveillance program.

DISCUSSION OF RESULTS

NTC, Great Lakes, Illinois

Population statistics and incidence of ARD and pneumonia at Great Lakes are shown in Figure 1. There was a considerable decrease in input of recruits during 1969-1970. Hospital admission rates for ARD remained low despite the high percentage of adenovirus infections noted in the surveillance companies (25 - 80%). The paradoxical phenomenon of extensive viral infection without concomitant increase in hospital admissions may be partially explained by the hospital admission policy which has been in effect at Great Lakes. For several years cases of borderline ARD severity instead of being hospitalized have been issued a "rack pass." This permits the patient to return to his barracks for bed rest of limited duration. Thus, this procedure decreases the overall ARD admission incidence. Figure 2 shows the incidence of rack passes and hospitalization for ARD complaints from January 1969 - May 1970. There is general agreement between the trends of the 2 illness categories except for the 10-fold difference in magnitude (1 admission for every 10 rack passes issued) until January of 1970. From January through March, the proportion of men receiving barracks bed rest for ARD increased greatly to 40 - 60-fold the number of men admitted to the hospital. The increase in the excessive number of rack passes issued coincides with the peak rates of adenovirus seroconversions detected during these same months (Fig. 1). Influenza A2 Honk Kong seroconversions increased to 25 - 27% during November-December 1969 without a concomitant increase in hospital admission rates during this time (Fig. 1). Only a few influenza B seroconversions were noted during the present reporting period. Also shown in Figure 1 is the percent

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*From Research Project No. MF 12.524.009-4013B, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

of men with seroconversions to rhinoviruses (types 1a, 1b and 2, combined). During the fall months of this past reporting period, as many as 70% of the surveillance subjects showed rhinovirus seroconversions. A reoccurrence of these infections was observed during the early spring (52%). The high spring and fall incidence of these agents has been fairly consistent over the past 5 years.

Additional information concerning viral infections was obtained from isolation data from a sampling of patients reporting to the dispensary (ES surveillance). Every week, 3 men with a diagnosis of febrile ARD, 3 with afebrile ARD and 3 with nonrespiratory diseases were cultured. Of the 454 men sampled during the past year, 31% yielded adenovirus type 4 and only 9% type 7. Fifty-two percent of the patients with febrile ARD, 24% of the afebrile ARD and 12% of non-ARD cases excreted adenovirus, type 4.

The distribution of these infections by month (1969-1970) and by clinical diagnosis is shown in Figure 3. The month with the lowest association of febrile illness with type 4 isolates was September (25%) and the highest, January (77%). The profile of the incidence of culturally positive infections sampled at the dispensary is similar to the pattern of adenovirus seroconversions in surveillance companies during this period (Fig. 1). This was not the case with the rhinoviruses where relatively few isolates were obtained despite the considerable serological incidence. However, the rhinoviruses which were recovered coincided with the times of greatest incidence of seroconversions.

Only 2 isolates of A2/Hong Kong were recovered -- one in January and the other in February. Both were obtained from patients with febrile ARD.

NTC, Orlando, Florida

The population statistics, ARD and pneumonia incidence, and serological surveillance at NTC, Orlando for 1968-1970 are shown in Fig. 4.

Orlando recruits experienced greater rates of ARD admissions during the 1969-1970 period than in the previous year. During 1968-1969, a rate of 10 cases/1000 men/month was exceeded only in March and April. In 1969-1970, the average monthly rate was over 30 cases/1000 men (with a high of 47/1000 in February 1970). The difference in ARD rates between Orlando and Great Lakes, for the same period, is even more striking. The former experienced almost 5 to 12-fold the amount of ARD hospitalization than was observed at Great Lakes during December 1969 - March 1970. This discrepancy is probably due to the differences in hospital admission policies between the 2 training centers. Unlike Great Lakes, rack passes are not usually given to patients reporting to the Orlando dispensaries for respiratory complaints. All cases of this illness of appropriate severity are admitted to the hospital. There was much greater serological evidence of adenovirus infections

at Orlando, during the fall and winter of 1969-1970 (35 - 50%) than had previously been observed at this center.

A study on the rubella antibody status of incoming Orlando recruits (Serological Survey for Rubella Antibody -- this report) showed that after September 1969 men without pre-existing rubella antibody tititer (<1:10) were more apt to experience rubella infections than before this time. These data indicate that, in general, there was a more efficient transmission of pathogens such as rubella and adenovirus after September than had been previously noted. This is probably due to the establishment of a resevoir of infections similar to that of other recruit training installations. Seroconversions to Hong Kong influenza virus were also evident among Orlando recruits during the Fall of 1969 at approximately the same time they were noted at Great Lakes, but to a lesser extent (18%). Rhinovirus (1a, 1b and 2 combined) infections, as diagnosed serologically, were fairly extensive at Orlando and as was the case at Great Lakes. Like Great Lakes, there appeared to be a seasonal pattern where the peak rates of infection usually were greater in the late fall and spring than during the other seasons.

The Orlando virus isolation data are incomplete. Specimens from dispensary patients (OS surveillance) were obtained in a manner similar to that of the Great Lakes surveillance study. A total of 292 viral cultures from specimen collected between November 1968 and November 1969 have been completed. Of these, 18% were positive for type 4 adenovirus but no type 7 was recovered. Of 95 dispensary patients sampled from the beginning of the study in November 1968 until March 1969 (5 months), only 3 were positive for adenovirus (Fig. 5). Since that time, approximately 25% of the samples have been positive. This change was most noticeable in patients with a diagnosis of febrile ARD where approximately 50% of such patients cultured since March of 1969 have been type 4 positive (Fig. 5). The peaks of adenovirus isolation correlates with the increases noted in ARD admission (Fig. 4). The highest recovery rate of 71% in October of 1969 agrees with the marked increase in adenovirus seroconversions and concomitant elevated illness rates.

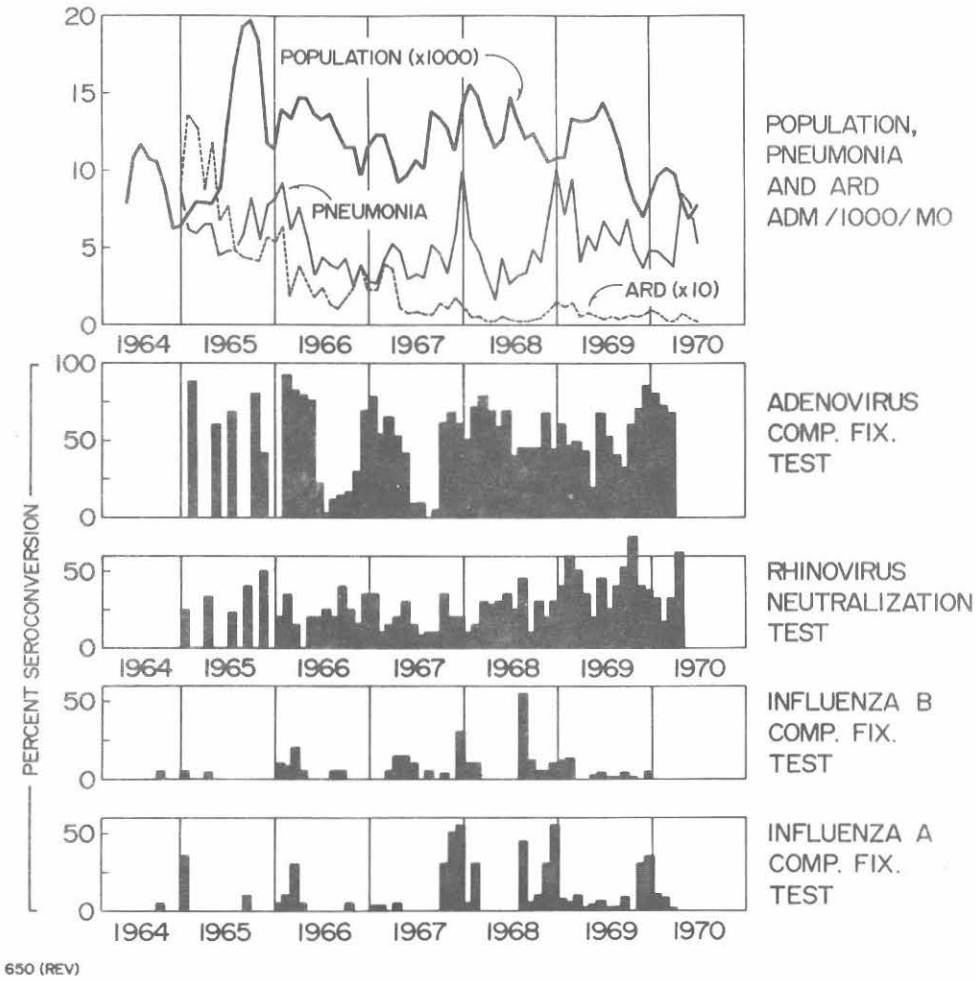

Fig. 1. Population statistics, ARD and pneumonia incidence, and serological responses to ARD viruses, Navy recruits, Great Lakes, Illinois, 1964-1970.

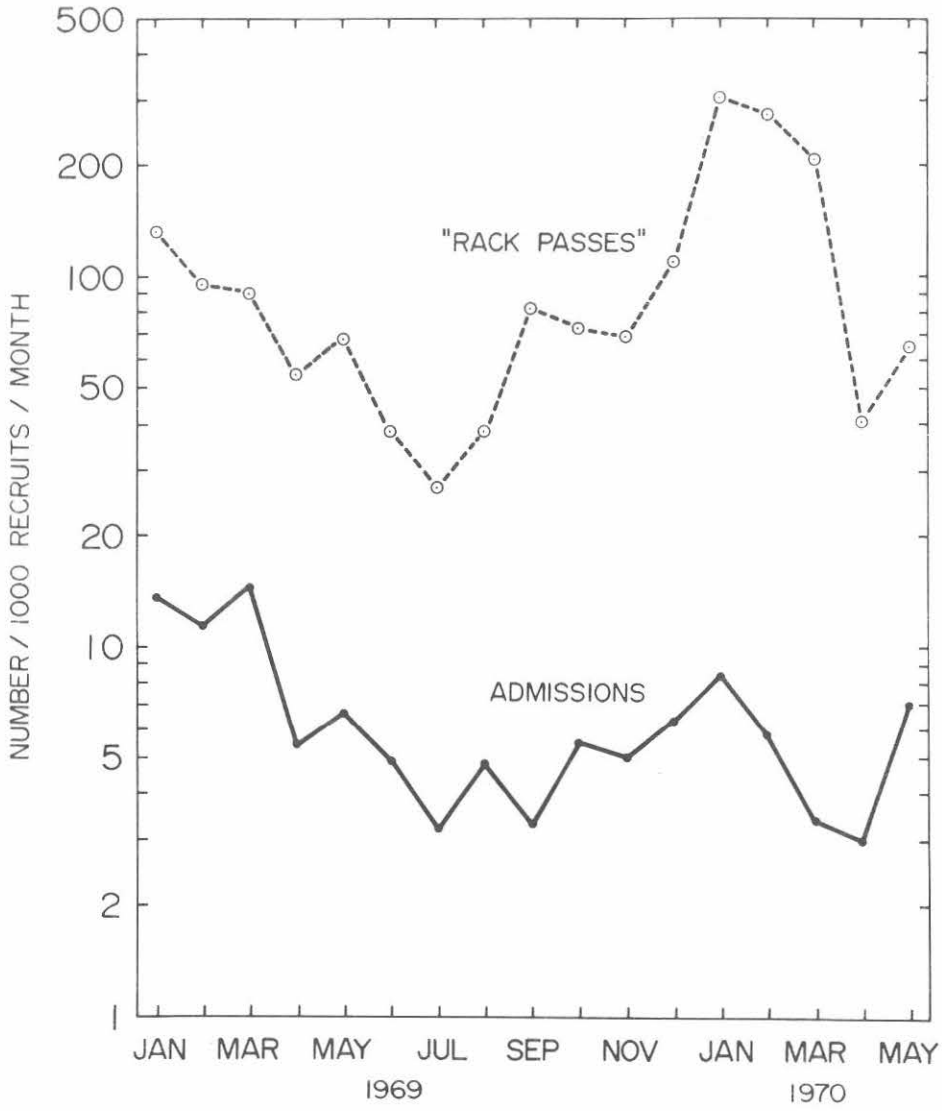

Fig. 2. Incidence of "rack passes"* and hospital admissions for ARD. Navy recruits, Great Lakes, January 1969-May 1970.

*Bed rest in barracks

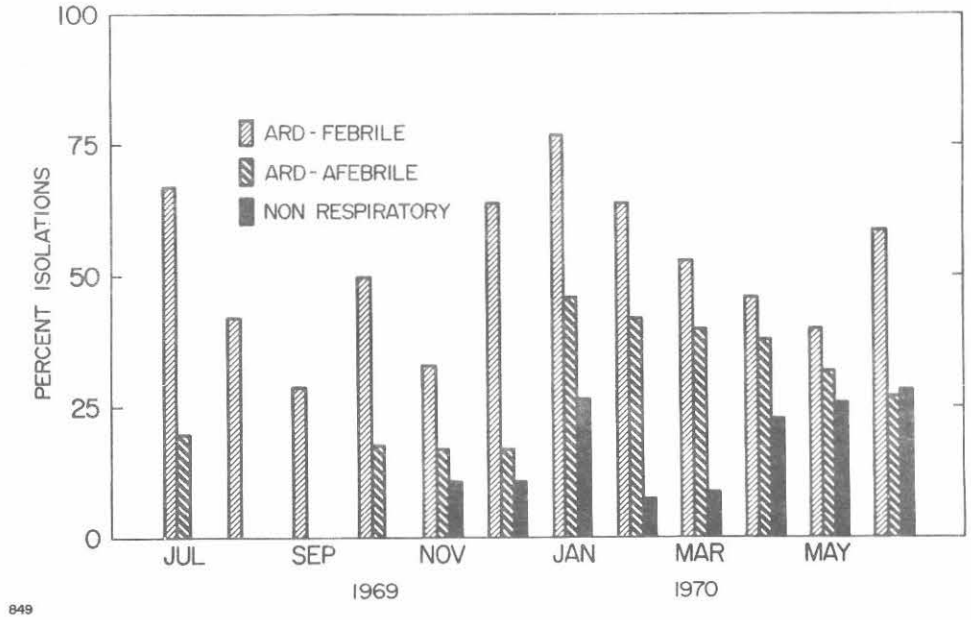

Fig. 3. Monthly distribution of adenovirus type 4 isolations according to clinical diagnosis of recruits sampled, Navy recruits, Great Lakes, Ill., 1 July 1969 - 30 June 1970.

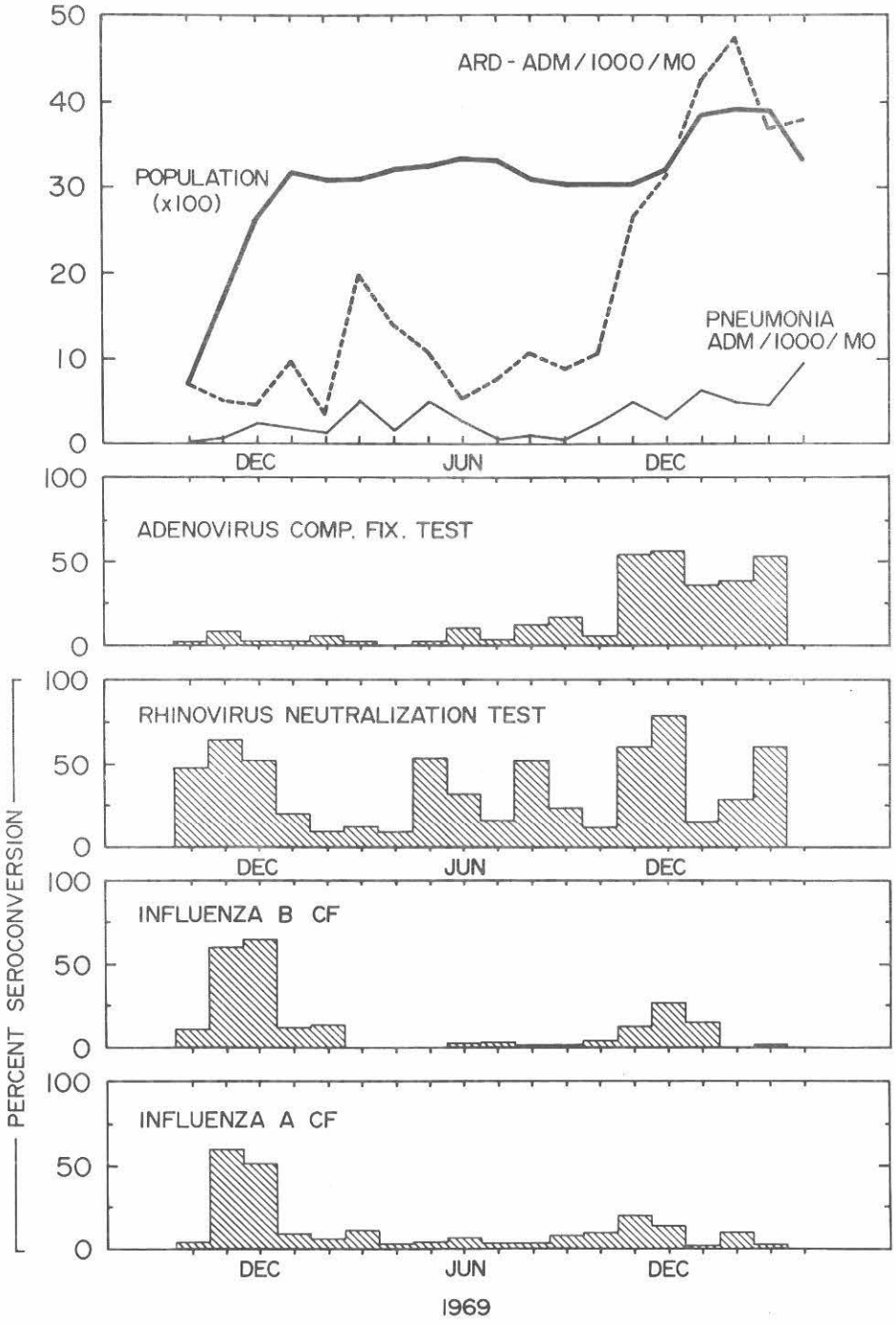

Fig. 4. Population statistics, ARD and pneumonia incidence, and serological responses to ARD viruses. Navy recruits, Orlando, Florida, October 1968 - March 1970.

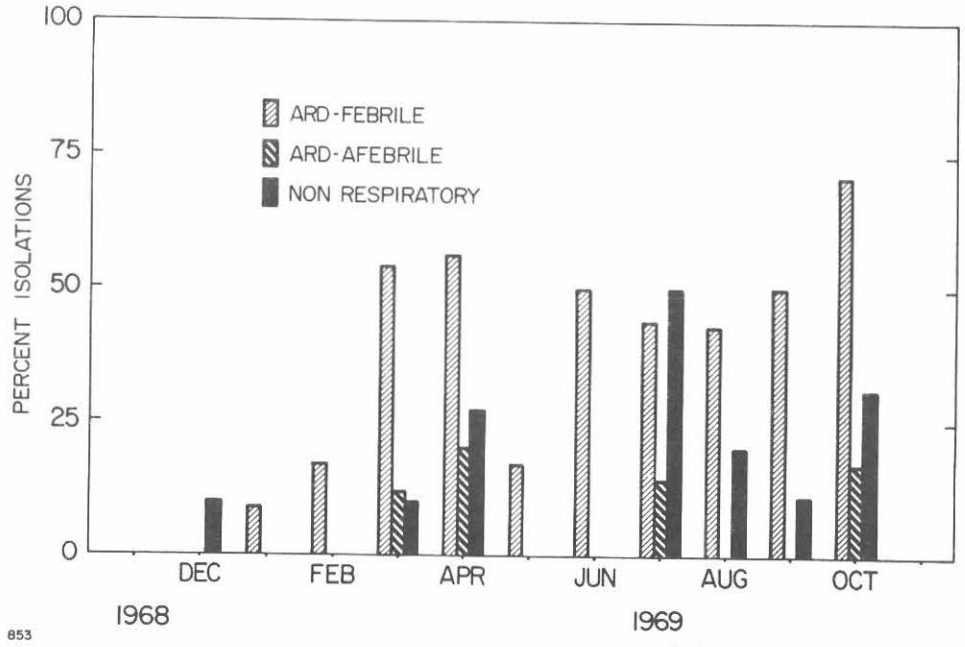

Fig 5. Monthly distribution of adenovirus type 4 isolations according to clinical diagnosis of recruits sampled. Navy recruits, Orlando, Florida, November 1968 - November 1969.

SEROLOGICAL SURVEY OF NEW RECRUITS FOR PRE-EXISTING RUBELLA ANTIBODY TITERS AND RELATION TO SUBSEQUENT INFECTIONS DURING 1969-1970*

Preceding the nationwide rubella epidemic of 1964, this disease was the second most frequent cause for hospitalization of recruits in training at the Naval Training Center, Great Lakes, Illinois. Three to five percent of recruits were admitted for rubella. Half of these cases occurred before the 4th week of training. Since 1964, rubella has ceased to be a major cause of recruit hospital admissions. The number of recruits hospitalized for rubella disease probably did not reflect the total incidence of rubella infections because of the large gradient in the clinical manifestations of the disease.

Little information was available on the ratio of infections to admissions for recruit rubella disease until the hemagglutination-inhibition (HI) test was developed. Using this test, a study was made of initial rubella antibody titers of recruits reporting for training during 1968-1969 and the subsequent seroconversion of these individuals after the 8 - 9 week training period. Information was obtained concerning the susceptibility of these young adults to rubella since the 1964 outbreak, the current extent of these infections in the recruit population and the relationship between the number of infections to clinical disease.

MATERIALS AND METHODS

Sera were obtained from a company of recruits at Great Lakes, Illinois randomly selected for serological surveillance each month for the two-year period of 1968-1969. In addition, sera were collected from recruits at Orlando, Florida during the period October 1968 to December 1969. Specimens were obtained upon company formation and just prior to graduation about nine weeks later. Sera were stored at -20°C until tested. A commercially prepared rubella diagnostic kit (Courtland Scientific Products, Los Angelos, Calif.) was employed in conjunction with the microtiter technique for assay of HI antibodies.

RESULTS

Figure 1 shows the number of monthly admissions for rubella from the recruit population at Great Lakes, Illinois during 1968-69. While the population size was fairly constant, the number of rubella cases was less than 15/month except during the first quarter of 1968.

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*From Research Project No. MF 12.524.009-4013B, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

Table I shows the distribution of initial rubella antibody titers in the approximately 50 men tested each month. Approximately 90% had titers of ten or greater upon arrival at Great Lakes. There was no appreciable shift in the distribution pattern of initial antibody titer levels over the two-year period. Table II shows the number and percent of rubella seroconversions among these recruits according to initial HI antibody titer. Over 70% of the recruits with titer less than 1:10 seroconverted to rubella. There was a significant difference between the percent of infected recruits who had no initial titer (<10) with those with any titer (91%). While the number of recruits reporting with a titer of less than 10 was only 9% of the 1132 recruits studied, 70% of all the subsequent rubella infections occurred in these men.

Results of the serological survey conducted at Orlando are shown in Tables III, IV and V. The distribution of initial rubella antibody titers of incoming recruits was similar to that for Great Lakes (Table III). However, the percent of men without pre-existing antibody titer who seroconverted to rubella was unexpectedly low. Only 25% of Orlando recruits without titers (<1:10) showed seroconversions (Table IV), as compared to 71% at Great Lakes during a similar time period (Table II). Thus it is assumed that considerably less rubella challenge was present at the new training center. However, when the data were reanalyzed using additional serological information obtained from September 1969 through March 1970 (Table V) the percent of susceptibles who became infected (62%) was more like the Great Lakes experience (70%).

DISCUSSION

From this small sampling of recruits, over a two-year period (1968-1969), there has not been any remarkable shift in overall susceptibility to rubella infection at Great Lakes. The data indicate that titers of 40 or greater are indicative of immunity to rubella infection.

It was surprising that while only 10% of the military population was susceptible, the spread of the rubella virus to these susceptibles was unhindered.

The reason for the very low hospital admission rates for rubella during 1968-1969, when 9.4% of the recruits (of 1132) showed serological evidence of rubella infection was probably due to the high proportion of mild or asymptomatic infections. Assuming the sampling was representative, there should have been approximately 12,000 rubella infections during this two-year period. However, only 186 cases were admitted to the hospital for rubella disease. Such high incidence of subclinical infections was previously observed by Buescher et al in Army recruits.

The data from Orlando are of interest not only from the standpoint of rubella but the spread of other infectious agents as well. It should be noted that surveillance data (see Incidence of Respiratory Infections - this report) indicated that few seroconversions to adenovirus were observed at the new training center from the time it was commissioned (October 1968) until November of 1969. After this time the incidence of adenovirus infection rates emulated those at Great Lakes. This increased

adenovirus incidence was associated with increased acute respiratory disease hospital admission rates. It is speculated that these events may signal that Orlando is becoming epidemiologically more similar to Great Lakes and other military training centers that have infectious disease problems. The finding that rubella infections among the Orlando recruits took a dramatic increase during this same period is consistent with these speculations. These data indicate that resevoirs of infection are developed and maintained by continuous input of trainees into recruit training centers. Furthermore, the data also suggest that such resevoirs can be minimized or eliminated by interrupting the continuous input for appropriate intervals. Such administrative hiatus have been previously employed with success in 1963 at San Diego and Fort Ord during outbreaks of meningococcal meningitis and may also be effective in curtailing other infectious disease problems of military recruits.

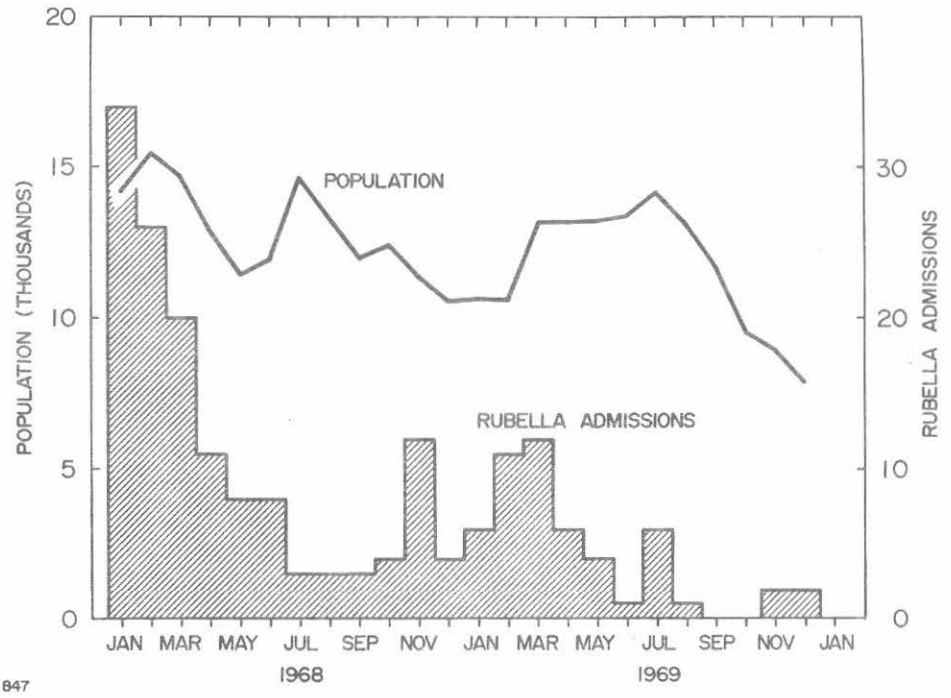

Fig. 1. Average monthly population and number of rubella admissions. Navy recruits, Great Lakes, Ill., 1968 - 1969.

TABLE I

Initial Rubella HI Antibody Titers of Recruits Reporting for Recruit Training, Great Lakes, Illinois During 1968-1969

		Number of recruits with initial titer (I/T)								
Month	Year	<10	10	20	40	80	160	320	640	1280
Jan	1968	5	3	22	18	2	0			
Feb	"	0	2	3	16	15	14			
Mar	"	3	1	6	13	17	10			
Apr	"	2	0	2	15	17	12	2		
May	"	3	4	15	18	7	3			
July	"	7	0	3	16	22	3			
Aug	"	6	1	9	16	11	5	2		
Sept	"	8	0	4	18	13	5	3		
Oct	"	3	0	9	16	13	4	5		
Nov	"	7	1	9	14	14	5	0		
Dec	"	8	4	5	11	9	13	0		
Jan	1969	6	2	4	19	11	8			
Feb	"	8	1	2	14	9	11			
Mar	"	5	0	6	10	15	12			
Apr	"	8	8	13	14	4	0			
May	"	2	0	2	14	15	16			
June	"	1	0	5	14	18	5			
July	"	1	0	1	7	15	26			
Aug	"	6	1	1	5	12	25			
Sept	"	3	0	1	5	7	34			
Oct	"	3	2	4	17	18	2		2	2
Nov	"	6	0	12	17	12	3		0	
Dec	"	3	1	4	13	18	6		2	
Total		104	31	143	320	294	222	12	4	2

TABLE II

Rubella Seroconversions by Navy Recruits in Training at Great Lakes, Illinois during 1968 - 1969

Initial HI Titer (I/T)	Number Men	% of men With Titer	Rises 4-fold or Greater	% Rises
<10	104	9.2	74	71.2
10	31	2.8	5	16.7
20	143	12.6	16	11.2
40	320	28.3	11	3.4
80	535	47.2	0	0
Total	1132		106	

TABLE III

Initial Rubella HI Antibody Titers of Recruits Reporting for Recruit Training, Orlando, Florida, Oct 1968 - Dec 1969

Month	Year	<10	10	20	40	80	160 or greater
Oct	1968	2	0	6	17	16	9
Nov	"	2	3	1	14	20	10
Dec	"	3	0	5	7	15	20
Jan	1969	5	0	11	24	9	1
Feb	"	6	0	4	9	18	13
Mar	"	3	1	5	15	16	10
Apr	"	7	0	6	12	9	10
May	"	5	1	1	3	14	26
Jun	"	6	1	5	6	17	15
July	"	3	2	0	7	16	22
Aug	"	3	0	14	18	11	4
Sept	"	6	1	4	12	20	7
Oct	"	4	1	12	17	11	5
Nov	"	3	1	5	10	19	12
Dec	"	6	1	5	18	13	7

TABLE IV

Rubella Seroconversions by Navy Recruits According to Initial Antibody Titer, Orlando, Florida, Oct 1968-Dec 1969

Initial Titer	Number Men	Percent Total of Men with Titer	Rise 4-fold or >	Percent rise
<10	65	8.65	16	24.6
10	12	1.6	4	33.3
20	84	11.2	13	15.5
40	195	26	15	7.7
80	224	29.8	0	0
160 or >	171	22.8	0	0

TABLE V

Rubella Seroconversion by Navy Recruits in Training at

Orlando, Florida, September 1969 to March 1970

Initial Titer	Number Men	Percent of Men with Titer	Rise 4-fold or >	Percent rise
<10	32	9.14	20	62.5
10	6	1.7	2	33.3
20	42	12.0	10	23.8
40	95	27.1	11	11.6
80	96	27.4	0	0
160 or >	79	22.6	0	0

SEROLOGICAL OBSERVATIONS ON MULTIPLE INFECTIONS IN ACUTE RESPIRATORY DISEASES OVER A FIVE-YEAR PERIOD*

INTRODUCTION AND METHODS

A longitudinal serological surveillance for 11 of the most prevalent microbial infections in Naval recruits has been in effect at Great Lakes since 1964. Those recruits involved were excluded from various prophylaxis programs in order to serve as monitors of natural infections. Serum specimens were collected at the beginning, middle and end of training. The 11 test antigens and method of testing is shown in Table I. The criterion for infection was a rise in homologous antibody titer of 4-fold or greater to a given agent during the training period. The distribution of these serologically diagnosed infections was analyzed for the 5-year period. The diseases resulting from these infections were not considered. Recruit companies (60 - 80 men each) were grouped into 7 clusters according to similar infection rates (Table II). The frequencies of combinations of agents to be expected by chance were compared to the frequencies observed. Because of the large number of possible permutations of agents (2,048) only those combinations actually occurring were considered.

DISCUSSION OF RESULTS

There was a consistent tendency for the number of men with no infection whatever to exceed that expected by chance (Table III). Also, the number of men with multiple infections was greater than chance expectation. An interdependence of agents which appeared in this analysis was the strong positive relationship of seroconversions to both Neisseria meningitidis and adenovirus. These 2 infections tended to appear simultaneously, making it difficult to assign a causal role to either agent. A possible explanation would appear to be that the same type of individual may be susceptible to both agents. In order to determine whether the noninfected population had acquired specific immunity resulting from previous infections the initial antibody titers of the infected and noninfected groups were compared. It was found that the noninfected did not have higher, and in fact to certain agents had slightly lower antibody titer levels (Table IV). Thus, it appears that their immunity was not due to pre-existing antibody titers. These data indicate that the discrepancy between individuals with no infections and those with multiple infections was probably due to innate factors of resistance or susceptibility peculiar to individuals.

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*From Research Project No. MF 12.524.009-4013B, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

TABLE I

Serological Tests Performed on Great Lakes Naval Recruits for 11 Microbial Agents

Agent	Test	Number of men whose sera had test performed
Adenovirus	Complement fixation	2207
Influenza A	Complement fixation	2207
Influenza B	Complement fixation	2207
Rhinovirus 1A	Neutralization	678
Rhinovirus 1B	Neutralization	678
Rhinovirus 2	Neutralization	678
Parainfluenza I	Hemagglutination-inhibition	678
Parainfluenza II	Hemagglutination-inhibition	678
Streptococcus	Antistreptolysin O	2207
Neisseria meningitidis	Complement fixation	2207
Mycoplasma pneumoniae	Complement fixation	2207

TABLE II

Percent Seroconversion for 11 Infectious Agents in Great Lakes Naval Recruit Companies Grouped by Similar Infection Rates

Agent	Company Cluster I	II	III	IV	V	VI	VII
Adenovirus Complement fixation	57.77	79.42	15.46	60.09	3.75	63.33	55.15
Influenza A Complement fixation	1.61	9.68	6.62	4.13	1.50	27.34	13.71
Influenza B Complement fixation	1.61	9.84	1.60	5.33	5.63	14.47	13.27
Rhinovirus 1A Neutralization	17.85	8.58	5.31	12.09	11.45	9.91	8.59
Rhinovirus 2 Neutralization	51.49	11.04	25.00	11.90	12.50	13.33	41.54
Streptococcus	1.34	8.53	5.08	6.60	7.14	6.94	6.61
Parainfluenza I Hemagglutination-inhibition	2.02	0.72	7.64	7.00	15.12	1.65	2.73
Parainfluenza III Hemagglutination-inhibition	8.00	6.96	8.16	14.00	6.00	9.09	6.88
M. pneumoniae Complement fixation	5.08	5.94	5.66	4.61	7.19	13.08	7.06
N. meningitidis Complement fixation	63.55	64.50	20.00	65.42	14.66	64.36	58.80

TABLE III

Expected and Observed Incidence of Noninfected Subjects Among Great Lakes Naval Recruit Companies Grouped by Similar Rates of Infection, and Significances of Differences between Expected and Observed Percent

	Cluster Number	Expected % Noninfected	Observed % Noninfected	P
Population complete for 6 infections	I	14	23	0.04
	II	5	10	0.0001
	VI	6.5	8.6	-
	V	65	70	-
Population complete for 11 infections	II	3.5	8	0.015
	III	30	40	0.01
	V	37	48	-

TABLE IV

Geometric Mean of Initial Antibody Titer Levels of Infected and Noninfected Individuals

	Noninfected Subjects	Infected Subjects
Adenovirus	6.8	4.9
Influenza A	5.8	5.6
Influenza B	6.0	4.9
M. pneumoniae	5.6	9.4
Anti-streptolysin O	160.0 (Todd Units)	182.0 (Todd Units)
N. meningitidis	3.7	<1.0
Rhinovirus 1A	5.5	11.0
Rhinovirus 2	7.1	7.3
Rhinovirus 1B	7.4	9.4
Parainfluenza I	25.0	57.0
Parainfluenza III	154.0	112.0

II. EVALUATION OF VARIOUS INFLUENZA VACCINES

A COMPARISON OF THE PROTECTIVE EFFECT OF AQUEOUS OR ADJUVANT BIVALENT HEMAGGLUTININ VACCINES IN NAVY RECRUITS*

In recent years, vaccines containing a purified hemagglutination fraction (HA) of influenza virus have been tested in Navy recruits. Adjuvant preparations of bivalent and polyvalent HA vaccines have been compared with a standard military polyvalent aqueous whole virus preparation and saline placebo. Adjuvant HA vaccines showed enhanced immunogenicity with low incidence of local or systemic reactions. Since there was no significant natural influenza challenge in this population during 1967-1969, it has not been possible to assess the protective effects of the HA preparations. Hence, another attempt was made during 1969-1970 to obtain protection information.

MATERIALS AND METHODS

The study population consisted of approximately 7500 men. Commencing in December 1969 and continuing through March 1970, all incoming recruits who volunteered to participate were given 1 of 3 treatments randomly. Treatment group I received 0.25 ml, intramuscularly, of the ether-extracted preparation of influenza antigens A2/HK/1968 and B/Mass/1966 (100 CCA units each) emulsified in Arlacel A and mineral oil adjuvant. Group II received 0.5 ml, subcutaneously, of an aqueous preparation of the same antigens, but at a higher CCA concentration (400 each). The third group received 0.5 ml of a sterile saline placebo, subcutaneously.

During each week of the study, blood was obtained from 1 recruit company containing 60 - 80 men (divided equally into the 3 treatment groups) prior to immunizations, and again during the 9th week of training. Serological tests were performed on these sera to determine the antibody responses to the vaccines or natural infection.

At the completion of the study, hospital admissions for acute respiratory disease (ARD) were analyzed for differences in rates which might be attributed to the effect of the vaccines.

DISCUSSION OF RESULTS

The comparative ARD and pneumonia experience of the study population is shown in Table I. The ARD rates are extremely small for the recruit population and indicate a lack of epidemic disease. There

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*From Research Project No. MF 12.524.009-4013B, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

was no significant difference between any of the treatment groups in either ARD or pneumonia incidence. Thus, as in previous attempts to evaluate the protective effect of the HA vaccines, there was no indication of sufficient influenza disease to ascertain this effect.

The serological responses to the vaccine are shown in Table II. The percent of A2/HK seroconversions among placebo subjects indicated that a considerable number of infections had occurred in the study population. Why these were not reflected in the illness data is not clear. It can be seen in Table III that there was a high incidence of these infections among placebo subjects throughout the study period and a sufficient time for a large group of immunized individuals to accumulate to detect a protective effect, had it occurred.

Only 2 influenza isolates were recovered during this study. One was derived from a member of the placebo group, and the other from a recruit who was not in the study and did not receive any influenza vaccine.

TABLE I

Hospital Admission Rates for ARD or Pneumonia of Naval Recruits Treated with Either Influenza Hemagglutinin Vaccines or Placebo (1969-1970)

Treatment	No. Men in group	Illness rate/1000 men/ 9-week period	
		ARD	Pneumonia
HA adjuvant vaccine*	2486	17.3	10.9
HA aqueous vaccine**	2540	12.2	12.2
Placebo†	2522	10.7	14.3

*Bivalent (A2/HK, B/Mass -- 100 CCA each).

**Bivalent (A2/HK, B/Mass -- 400 CCA each).

†Saline

TABLE II

Influenza Serological Responses of Recruits Treated with Either Influenza Hemagglutinin Vaccines or Placebo (1969 - 1970)

Treatment	No. men in group	% HI Antibody Seroconversions A2/HK	B/Mass
HA adjuvant*	176	65	54
HA aqueous**	180	64	50
Placebo†	177	46	7

*Bivalent (A2/HK; B/Mass -- 100 CCA units each)

**Bivalent (A2/HK; B/Mass -- 400 CCA units each).

†Saline

TABLE III

Distribution of Seroconversions to Influenza A2/HK Among Placebo

Subjects Sampled from December 11, 1969 - February 3, 1970

Company Number	Date Entered Study	Number placebo Subjects Tested	% Seroconversion (4-fold> titer rise)
5706	12/11/69	24	21
5712	12/17/69	24	54
5720	12/24/69	16	62
5723	12/31/69	16	50
5007	1/ 8/70	17	82
5019	1/15/70	18	56
5032	1/22/70	20	70
5043	1/28/70	18	33
5058	2/ 4/70	16	50
5076	2/13/70	23	26

REACTOGENICITY OF VARIOUS INFLUENZA VACCINES PREPARED AND ADMINISTERED BY DIFFERENT METHODS*,**

Since numerous new techniques for preparation of influenza vaccines have become available recently, a study of the relative toxicity of these preparations was undertaken. Some of the variables in vaccine preparation and administration examined included: concentration procedures (Sharples or zonal ultracentrifugation); chemical extraction of viral particles or subunits; addition of aluminim phosphate and subcutaneous versus intramuscular routes of administration.

MATERIALS AND METHODS

The study involved 38 different monovalent vaccines and 8 different placebos (Table I). Each vaccine or placebo was administered to approximately 60 men. Each subject had a clinical interview and his temperature recorded before, 24 and 48 hours after injection.

In considering the toxicity of these vaccines, both local and systemic reactions were evaluated. A temperature rise, malaise, headache, chills, and fever, and myalgia were considered to be systemic reactions; while pain at site of injection, erythema, calor, swelling, tenderness, and induration were considered local reactions. All symptoms were given equal weight. A symptom score was then calculated for each man by summing the number of symptoms acquired after the injection. The distributions of symptom scores for those individuals receiving a particular vaccine were compiled (Table II). The distributions for any 2 vaccine production or administration techniques were compared by means of a ranked contingency table. Any symptom present prior to treatment was not included in the total score.

The symptom scores of the recipients of each vaccine prepared by a certain technique were compared to their respective controls. Similarly, all vaccines grouped together were compared to all controls grouped. Furthermore, the scores of recipients of vaccines prepared by one technique were then compared to those who received a vaccine prepared by a different technique; for example, scores from all men who received Sharples centrifuged vaccine were compares to those of subjects vaccinated with the zonal centrifuged preparation.

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*From Research Project No. M4305.12-4001B, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

**A collaborative study with Drs. Nicola M. Tauraso and Martin G. Meyers, Laboratory of Virology and Rickettsiology, DBS, NIH.

DISCUSSION OF RESULTS

The following results were obtained from the initial analysis of the reaction data. There were no differences in systemic reactions to the various vaccines, with the exception of those prepared with formalin and those given in a 1.0 ml dose, which were more toxic systemically than without formalin, and a 0.5 ml dose, respectively.

Considering local reactions, vaccines containing aluminum phosphate were more irritating than those without it. Whole particles or subunit vaccines concentrated by Sharples centrifugation were more irritating locally than were those prepared by zonal centrifugation. All vaccines given subcutaneously, grouped together, were more reactive locally than all vaccines given intramuscularly, and the aggregate of the 1.0 ml dose vaccines was more toxic than the 0.5 ml dose vaccines. However, it should be noted that the 0.5 ml dose was only given for vaccines prepared either by ultracentrifugation or from ether-extracted hemagglutinin.

Locally and systemically, all vaccines produced greater reaction than controls. Grouping the vaccines by their preparation technique and comparing them to their respective controls, showed differences in local but not systemic reactions.

Thus, it appears that the optimum influenza vaccine, for minimum toxicity would be a hemagglutinin antigen, concentrated by zonal ultracentrifugation, without aluminum phosphate, and administered intramuscularly in a 0.5 ml dose.

TABLE I

Schedule of Vaccine

Group no.	Manufacturer	Lot no. Bulk	Lot no. Final	Method of Preparation	$AlPO_4$	Route	Dose (ml)
1a	Lederle #1	2018-15	206-320	S	no	s.c.	1.0
b						i.m.	
2a	Lederle #2	2018-13	200-146	S	no	s.c.	1.0
3a	MSD #1	59308	59308-2	S	no	s.c.	1.0
b						i.m.	
4a	MSD #2	59311	59311-3&4	S	no	s.c.	1.0
5a	Nat Drug #1	7013	6341A9	S	no	s.c.	1.0
b						i.m.	
6a	Nat Drug #2	7022	6359A9	S	no	s.c.	1.0
7a	Nat Drug #3			S	no	s.c.	1.0
8a	Nat Drug #4	7145			no	s.c.	1.0
b						i.m.	
9a	Nat Drug #5	7145		Z	no	s.c.	0.5
10a	Wyeth #1	1089	105901	S	no	s.c.	1.0
b						i.m.	
11a	Wyeth #2	1060	106001	S	no	s.c.	1.0
12a	Lilly #1	2PE20	2NU04A	Z	no	s.c.	0.5
b						i.m.	
13a	Lilly #2	2PE21	2NU05A	Z	no	s.c.	0.5
14a	Lilly #3	2PM84		Z	no	s.c.	0.5
15a	PD #1		988122A	S	yes	s.c.	1.0
b						i.m.	
16a	PD #2		41369	S	no	s.c.	1.0
b						i.m.	
17a	PD #3		41342	S/HA	yes	s.c.	0.5
b						i.m.	
18a	PD #4		41343	S/HA	no	s.c.	0.5
b						i.m.	
19a	Abbott #1		823-8936	Z/HA	no	s.c.	0.5
b						i.m.	
20a	Abbott #2		831-8936	Z/HA	no	s.c.	0.5
21a	Control #1				no	s.c.	0.5
b	(Lilly)					i.m.	
22a	Control #2		42A-78		no	s.c.	1.0
b	(Lederle)					i.m.	
23a	Control #3		59676-1002		no	s.c.	1.0
b	(MSD)					i.m.	

Key to abbreviations: S = Sharples; A = zonal; HA = ether-extracted hemagglutinin

TABLE II

Distribution of Symptom Scores (Local Reactions) of Men Receiving Various Influenza Vaccines

	Symptom Score*					
Vaccine Group	0	1	2	3	4	5
All i.m.	535	80	32	8	7	1
All s.c.	775	175	64	15	2	7
All Sharples	643	176	76	22	6	5
All zonal	284	36	10			
HA added	383	43	10	1	3	3
1.0 dose	643	176	29	9	9	1
0.5 dose	667	79	20	1	3	3
With $AlPO_4$	174	55	19	10	7	1
Without $AlPO_4$	1136	200	77	13	2	7
Placebo	686	16	2	1	0	1

*Symptom score is a composite numerical value of all symptoms giving each one equal weight.

RHEUMATOID FACTOR-LIKE SUBSTANCES IN HUMAN SERA WITH NATURALLY OR ARTIFICIALLY ACQUIRED INFLUENZA ANTIBODY*

The rheumatoid factor, which is found in the sera of the majority of patients suffering from rheumatoid arthritis, has been considered to be antibody to denatured autologous gamma globulin. It has been established that rheumatoid factor-like (RFL) substance can be produced by immunization with denatured autologous IgG globulin, giving support for the above concept. Also, RFL substance can be produced in animals exposed to prolonged immunization with various antigens.

It has been demonstrated that influenza A2 disease and influenza vaccination stimulated the formation of the RFL substance. This suggests the method of vaccine preparations may be an important determinant in the stimulation of this substance. Also, the possibility that other vaccinations given military personnel may stimulate the RFL substance needs investigation.

This report confirms that an RFL substance is associated with antibody stimulated by influenza A2 disease or vaccine. Individuals who fail to produce antibody, even though immunized, fail to produce the substance.

MATERIALS AND METHODS

Sera from Navy recruits who were in an influenza vaccine protection study at Great Lakes were tested for RFL substance. These subjects received one of the following vaccines: (1) standard military influenza vaccine, (2) purified ether-extracted influenza hemagglutinin (HA) vaccine, (3) a whole particle influenza vaccine produced in bovine kidney cells, or (4) placebo.

Sera were collected before and at 6 weeks post-immunization and stored at -20°C until tested. As a source of sera from recruits who were not vaccinated but had sustained influenza infection, samples were obtained from serological surveillance program carried out during an influenza outbreak in 1968. Paired serum specimens were obtained from these subjects at the start and end of training. Those individuals who showed seroconversions (CF) to influenza (1:4 to greater than 1:64) were considered to have been infected.

A source of sera with RFL substance resulting from immunization other than influenza was obtained from samples collected during a period when there was no serological evidence of influenza. Only

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*From Research Project No. M4305.12-4001B, Bureau of Medicine and Surgery, Navy Department, Washington, D. C.

those paired sera which both showed influenza A2 CF titers of 1:4 or less were employed to ascertain the effects on RFL by noninfluenza vaccinations.

The method of Waaler-Rose was used for the detection of the RFL substance. Absorption of the Forssman antibody was accomplished by absorbing 0.9 ml of heat inactivated serum with .05 ml washed packed sheep cells at room temperature for 1 hour and overnight at 6°C. To insure that all Forssman antibody was absorbed, a control was set up for each serum using nonsensitized sheep erythrocytes at the same dilutions that were used in the test. Sera showing a 1+ agglutination was taken as the endpoint in the test.

RESULTS

Table I shows the responses of RFL substance to various types of influenza vaccines or placebo. The greatest number of 4-fold rises in RFL titer occurred in men who received either the aqueous or adjuvant vaccines prepared in bovine kidney (42 and 30%, respectively), and the fewest in recipients of the HA vaccines. Table II shows the distribution of RFL substance reactants among subjects who also showed an 8-fold or greater rise in influenza HI antibody titer. In this comparison, the placebo subjects did not demonstrate such HI responses or titer rises of RFL substance. The vaccinated subjects, however, showed 10 - 55% of the men with RFL titer increases. As in Table I, the bovine kidney groups had the greatest number of RFL rises. Table III compares the effect of natural influenza infection on RFL responses with those stimulated by other routine military inoculations. Over 38% of the infected men had RFL titer rises compared to only 4% in the controls.

DISCUSSION

Rheumatoid factors have been demonstrated, not only in the rheumatic diseases, but also in such diverse conditions as syphilis, sarcoidosis, myocardial infections, liver disease, malignancy, hypertension, dysglobulinemia, diseases of the chest, in psychiatric populations, and even in normal individuals. It is, therefore, difficult to interpret the meaning of the occurrence of the rheumatoid factor. However, the fact that an RFL substance results from either influenza vaccination or from natural infections, but apparently does not result from other inoculations received by military recruits, is of interest. Investigators in Finland (Aho et al, Proc. Soc. Exp. Biol. Med. 124: 229, 1967) have suggested that the rheumatoid factor and immuno-conglutinin responses in military populations result from the net immune response provoked by all vaccinations rather than by any single vaccine. The present study does not support their conclusions. That there is a definite correlation between influenza A2 antibody response and RFL substance is demonstrated in Table II. The exceptions to this were the HA monovalent vaccines, although the number of subjects may be too small to be significant. Why the influenza vaccine produced in bovine

kidney cells showed such a marked ability to induce the RFL substance is not understood. While the number of RFL responses did not differ significantly from responses in the subjects with natural infections, the bovine kidney aqueous vaccine preparation differed significantly from the standard military vaccine.

The question arises: Are rheumatoid factor-like substances important in the pathogenesis of influenza disease or are they induced by the presence of the viral antigens? Local reactions at 24, 48 and 72 hours produced by the HA monovalent vaccine and saline placebo were significantly less than to the other influenza vaccines received (see Commission on Influenza Report, 1969). This suggests that there may be a correlation between the reactogenic quality of the vaccine and RFL substance formation. None of the recruits who met the criteria as having influenza disease were admitted to the dispensary, therefore, if the stimulation of the rheumatoid factor-like substance contributes to the pathogenesis of influenza disease, it was not severe enough to cause the subject to seek treatment.

These questions could not be answered by this study. The fact that there appears to be a relationship between influenza antibody formation and RFL substance suggests the possibility that antigen-antibody complexes provide the stimulus for production of RFL substance. The stimulation of this factor by influenza infection or by influenza vaccinations may be similar to, if not identical with, the rheumatoid factor associated with rheumatic diseases. Also, the appearance of an RFL substance, associated with influenza A2 disease or immunization, raises important questions as to its overall role in immunological homeostasis.

TABLE I

Effect of Different Influenza Vaccine Preparations on Stimulation of Rheumatoid Factor-Like Substance

Vaccine Preparation	Dose	No. Tested	Mean influenza A2 HI titer (log_2) Pre	Post	Rheumatoid factor-like Substance 4-fold or greater rise in titers	Percent
Standard military vaccine	1 cc (jet gun)	20	4.1	5.8	2/20*	10
Placebo	(jet gun)	20	3.85	5.0	2/20	10
HA bivalent	.25 ml IM	20	3.9	4.1	1/20	5
HA monovalent (zonal centrifuge)	.5 ml SQ	20	3.8	5.3	0/20	0
Bovine kidney bivalent aqueous	.5 ml IM	40	3.8	5.8	17/40	42.5
Bovine kidney bivalent adjuvant	.25 ml IM	20	3.35	5.05	6/20	30

*$\frac{\text{Number positive}}{\text{Number tested}}$

TABLE II

The Association of Antibody Response to Rheumatoid Factor-like Substance from Recruits Showing Seroconversions to Different Influenza Vaccines

Vaccine Preparation	Dose	No. Tested	Rheumatoid factor-like substance 4-fold or greater rise in the titer	Percent
Standard military	1 cc jet gun	23	6/23*	26
Placebo**	1 cc jet gun	18	0/18	0
HA bivalent adjuvant	.25 ml IM	6	1/6	17
HA monovalent aqueous	.5 ml SQ	10	1/10	10
Bovine kidney aqueous	.5 ml IM	20	11/20	55
Bovine kidney adjuvant	.25 ml IM	10	3/10	30

*$\frac{\text{Number positive}}{\text{Number tested}}$

**Men who did not show serological evidence of natural infection

TABLE III

Rheumatoid Factor-Like Substance Stimulated by Natural Influenza A2 Infection and by Routine Military Immunization

	No. tested	Rheumatoid factor-like Substance	Percent
Natural influenza A2 infection	26	10	38.5
Noninfluenza A2 infection, but received routine military immunizations (except influenza)	58	2	3.5

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13. ABSTRACT

Surveillance studies

Recruit hospital admission rates for acute respiratory disease (ARD) at NTC, Great Lakes remained low during 1969-70 despite the high incidence of adenovirus type 4 infections. Increased ARD rates at NTC, Orlando were noted during this time and were associated with adenovirus infections. Rubella surveys at Great Lakes indicated that only 9% of incoming recruits were without pre-existing antibody. Seventy percent of these susceptibles became infected, but most were not hospitalized. An increase in rubella infections at Orlando was noted. A 5-year study of various microbial infections at Great Lakes showed that some recruits experienced no infections while others had multiple infections. Combinations of adenovirus and meningococcal infections occurred more frequently than would be expected by chance.

Influenza studies

A2 influenza infections occurred late 1969 and early 1970 (35% seroconversions) without concomitant increase in ARD rates. Protection studies with aqueous and adjuvant hemagglutinin vaccines were inconclusive. Other studies on the reactogenicity of various influenza vaccines or methods of administration showed that the fewest local reactions were produced by a hemagglutinin vaccine, prepared by zonal centrifugation, when given IM in a 0.5 ml dose. An investigation of rheumatoid factor-like substances in recruit sera showed a correlation between titer increases of RFL with increases of naturally or artificially acquired influenza (A2) antibody.

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