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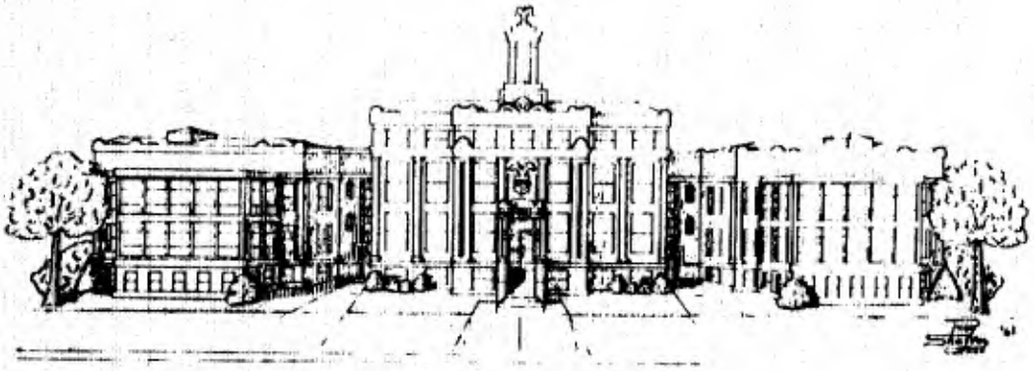
NAVAL MEDICAL RESEARCH

UNIT No. 4



CONFERENCE ON LIVE ORAL ADENOVIRUS VACCINE IN MARINE AND NAVY RECRUITS

In

Celebration of the Twentieth Anniversary of NAMRU-4

RU 66.37

December 1966

UNITED STATES NAVAL MEDICAL RESEARCH UNIT 4

UNITED STATES NAVAL HOSPITAL GREAT LAKES, ILL.

2

Proceedings of the

CONFERENCE ON LIVE ORAL ADENOVIRUS VACCINE IN MARINE AND NAVY RECRUITS

In

Celebration of the Twentieth Anniversary of NAMRU-4

Naval Medical Research Unit No. 4

Great Lakes, Illinois
60088

June 10-11, 1966

The opinions expressed herein are those of the authors and cannot be construed as reflecting the views of the Navy Department or the Naval Service at large. The use of commercially available products does not imply endorsement of these products or preference to other similar products on the market.

The experiments reported herein were conducted according to the principles enunicated 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.

FOREWORD

Upon the occasion of the 20th anniversary celebration of NAMRU-4, noteworthy achievements throughout the years were scrutinized to select a presentation worthy of the event. It was with this thought in mind that the Conference on the Live Adenovirus Vaccine in Navy and Marine recruits was conceived. Some of the most recent work was presented as preliminary reports at the conference, but the reader will note that the data have been brought up to date for publication.

It should be noted by the reader that the differences in the efficacy of the vaccine in the different recruit camps are based on variations of experimental designs and not on variable efficacy of the vaccine. Further studies are anticipated with the live adenovirus vaccines, however, the results to date are most gratifying and fulfill a need to prevent and control the greatest cause of acute respiratory disease at the recruit camps.

This achievement does indeed seem worthy to report in celebrating NAMRU-4's 20th anniversary.

I speak for all the members of NAMRU-4 when I express our gratitude to Dr. Chanock, Dr. Steinberg, Dr. Gutekunst, and Dr. Jackson for the roles they have played in making this a successful conference.

ROBERT O. PECKINPAUGH
CAPT MC USN
Officer in Charge

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A THUMBNAIL HISTORY OF NAMRU-4

31 May 1946	McIntire Research Unit (forerunner of NAMRU-4) was established at U.S. Naval Hospital, Dublin, Georgia (rheumatic fever convalescent center) with the mission of conducting research on rheumatic fever. A field unit was established at U.S. Naval Training Center, Great Lakes, Illinois.
4 June 1948	NAMRU-4 was established at Great Lakes with personnel and facilities transferred from Dublin, Georgia when U.S. Naval Hospital was turned over to Veteran's Administration. NAMRU-4 was housed in Building 2909 in the Farnsworth Housing area with field laboratory in Dispensary 1109 in Recruit Training Command. NAMRU-4 was under military command of the Commanding Officer, Administrative Command, U.S. Naval Training Center, Great Lakes, but under technical control and management of the Bureau of Medicine and Surgery. The mission was expanded to include research on control and prevention of acute communicable respiratory diseases due to bacterial and viral agents.
14 June 1961	NAMRU-4 was moved to Building 1-H and 43-H, U.S. Naval Hospital compound under military command of the Commanding Officer, U.S. Naval Hospital, Great Lakes, Illinois.
19 Sept 1962	Mycoplasma Research Laboratory was dedicated.
12 Oct 1964	Field Laboratory, Recruit Training Command was moved into the new recruit dispensary, 1017.
1 Jan 1965	All control and management was assigned to the Bureau of Medicine and Surgery and Area Coordination to Commandant of the Ninth Naval District.
1 Dec 1966	Nursing Research Division was established.

ORGANIZATION OF NAMRU-4

Scientific Department

Bacteriology Division
Biochemistry Division
Biometrics Division
Epidemiology Division
Immunology Division
Mycoplasma Research Division
Nursing Research Division
Virology Division

Administrative Department

Administrative Division
Fiscal and Supply Division
Operating Services Division

OFFICERS IN CHARGE

MC INTIRE RESEARCH UNIT

J.R. SEAL, LCDR MC USN
31 May 1946 to 28 June 1948

NAVAL MEDICAL RESEARCH UNIT NO. 4

J.R. SEAL, CDR MC USN
28 June 1948 to 14 July 1954

K.H. SESSIONS, CAPT MC USN
14 July 1954 to 13 October 1955

M.J. HANTOVER, CAPT MC USN
13 October 1955 to 4 January 1957

B.F. GUNDELFINGER, CDR MC USN
4 January 1957 to 4 August 1958

L.F. MILLER, CAPT MC USN
4 August 1958 to 16 May 1964

C.H. MILLER, CDR MC USN
16 May 1964 to 6 June 1964

R.O. PECKINPAUGH, CAPT MC USN
6 June 1964 to Present

IMMUNIZATION BY SELECTIVE INFECTION WITH TYPE 4 ADENOVIRUS GROWN IN HUMAN DIPLOID TISSUE CULTURE. I. SAFETY AND LACK OF ONCOGENICITY AND TESTS FOR POTENCY IN VOLUNTEERS*

Chanock, R.M., Ludwig, W., Huebner, R.J., Cate, T.R.,

and

Chu, L.

As a group, adenoviruses are important etiologic agents in pediatric respiratory tract disease. Vaccines for these viruses are difficult to evaluate, since no one adenovirus type causes enough disease in children to permit a successful vaccine trial. In the military, the situation is quite different. Here adenovirus infection constitutes a major cause of acute febrile respiratory disease, especially in recruits. Most recruit adenovirus infections are produced by type 4 virus and to a lesser extent by type 3 and 7 viruses. The impact of adenovirus infection on recruit populations is large in terms of morbidity, hospitalization, and costly disruption of military training. For these reasons, there is an urgent need for effective immunoprophylaxis.

The first experimental inactivated adenovirus vaccines were prepared in monkey kidney tissue culture and were extremely effective in preventing adenovirus disease. However, subsequent production lots of vaccine exhibited a variable degree of potency. In some instances very little protection was conferred. In addition, there has been considerable difficulty in producing adenovirus vaccines, which are free of contamination, with indigenous monkey viruses. The most troublesome of the simian viruses has been SV-40, a virus which induces tumors in suckling hamsters and neoplastic transformation of human cells in tissue culture. The problem of vaccine safety became more complex when it was discovered that a portion of the SV-40 virus genome can become incorporated into the adenovirus particle resulting in the formation of a virus hybrid which possesses the oncogenic potential of SV-40 virus.

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*Ref: JAMA 195:445-452, 1966 (Presented in part).

The difficulties with potency and simian virus contamination of adenovirus vaccines during the past few years suggested to us that other approaches to adenovirus immunization should be explored. For this reason, we have investigated an alternate technique which is based upon the observation that human adenoviruses exhibit a marked predilection for infection of the lower intestinal tract.

The virus used in these studies was a type 4 strain which was grown in human diploid fibroblast cultures (Wistar Institute - 26 or 38). This tissue culture system was chosen since these cells retain a normal karyotype after as many as 40 cell divisions, are free of demonstrable microbial contaminants, and can provide an extremely large yield of diploid cells at the 20th to the 30th passages from low passage seed stock stored in the frozen state. The virus strain of type 4 adenovirus was shown to be free of adventitious microbial contaminants and of oncogenic activity in newborn hamsters.

In preliminary experiments involving 40 volunteers, it was shown that this type 4 strain could selectively infect the lower intestinal tract when virus was administered in enteric-coated capsules which did not release virus until they passed beyond the stomach. In this manner, the nasopharynx and the respiratory tract, the usual sites of pathology, were by-passed. In addition, virus did not spread from the lower intestinal tract to the respiratory tract, nor could it be detected in the serum. Selective intestinal adenovirus infection stimulated moderately high levels of neutralizing antibody, but was not associated with any signs or symptoms of illness. Furthermore, type 4 virus did not spread from enterically infected volunteers to antibody-free individuals who were in close personal contact over a 30-day period. These findings suggested that the enteric capsule technique might be developed into an effective immunoprophylactic procedure for control of adenovirus infection, especially in military recruits.

EPIDEMIOLOGY OF ACUTE RESPIRATORY DISEASE IN MARINE RECRUITS

Paul Steinberg and Robert J. White

The epidemiology of adenovirus associated respiratory illness in Marine Corps recruits consists of yearly, sharply demarcated, winter epidemics with virtually complete absence during interepidemic periods and is distinct from the pattern of illness observed in Navy recruits at the Great Lakes Naval Training Center (1, 2, 3).

This paper describes the epidemiology of respiratory illness in recruits who presented themselves at dispensaries at Camp Lejeune, North Carolina and Parris Island, South Carolina. A detailed study of all recruits who were admitted to the Naval Hospital at Camp Lejeune for acute respiratory disease, including pneumonia, between April 1965 and January 1966 is presented. Finally, studies which relate possible environmental influences to the incidence of epidemic respiratory disease are described.

Study Population

The population was drawn from all recruits at Parris Island and from the First Infantry Training Regiment (ITR) at Camp Lejeune, North Carolina. Recruit training is a continuum between Parris Island, South Carolina where the first 11 weeks of training are spent and ITR where the men are transferred without leave and remain for 4 weeks in advanced stages of training.

Training is conducted at Parris Island at the platoon level (approximately 80 men). Each platoon is housed in a separate squad bay, 4 of which are adjoined and compose a company barracks. The recruit population of Parris Island varies between 3,000 and 10,000 men. At ITR, training is conducted at the company level (approximately 240 men). Each company is housed in 3 separate barracks containing about 80 men each. The ITR population fluctuated from just over 1,000 to more than 5,000 during the period of the study.

The studies described consist of acute respiratory illnesses in Marines reporting to the ITR and Parris Island dispensaries and in those ITR recruits admitted to the U.S. Naval Hospital, Camp Lejeune, North Carolina. X-ray confirmation was required for the diagnosis of pneumonia.

Recovery of Organisms and Serological Studies

Throat and anal swab specimens for virus isolation were inoculated into HEp-2 tissue cultures. Adenovirus isolates were identified by either the hemagglutination inhibition technique or the microneutralization test. Throat swab specimens for isolation of Mycoplasma pneumoniae were inoculated into diphasic culture media containing methylene blue. Identification was performed by hemadsorption with guinea pig erythrocytes, and by growth inhibition using filter paper discs containing specific antiserum. A complement fixation test for adenovirus 4 and M. pneumoniae was performed on acute and convalescent phase sera. All sera from the same recruits were tested on the same day.

Etiologic diagnoses were made by isolation of the agent and/or a serologic rise. Adenovirus 4 plus M. pneumoniae associated infections included instances where dual isolates and one serologic rise or dual isolates without serologic rises were present. There were no examples of dual isolates and dual serologic rises or dual serologic rises without isolates although these two circumstances have been observed in other studies. The non-adenovirus 4 or M. pneumoniae (unidentified) group contains neither an isolate nor a serologic rise to either agent.

RESULTS

The rates of acute respiratory illness in Marine Corps recruits for the years 1960-1965 are shown in Figure 1. At ITR, recruits experienced a sharp outbreak of respiratory disease each winter with rates varying from a low of 200 sick call visits to peak rates of 1,700 visits/1,000 men/month.

At Parris Island there were winter peaks of respiratory illness for the years 1960-1962 of considerably smaller magnitude, with rates varying from lows of 100 sick call visits to peak rates of 375 visits/1,000 men/month. Since Spring 1963, respiratory disease rates have leveled off at 100-150 visits/1,000 men/month with no recognizable seasonal variation. No consistent relationship between troop strength and illness rates could be seen either at Parris Island or at ITR.

For respiratory illness at Parris Island, the incidence of adenovirus positive specimens varied from a low of 2% to a high of 25%, with a tendency towards higher winter incidence. At Camp Lejeune, the incidence of specimens positive for adenovirus varied from 0% during the summer months (1960-1963) to 90% during the winter epidemic peaks. Since 1963, adenovirus associated illness has been endemic at low rates during the summer months at Camp Lejeune. Since 1963, only type 4 adenovirus has been isolated from Camp Lejeune and Parris Island. In previous years, type 7 was occasionally associated with acute respiratory illness.

For respiratory illnesses requiring hospitalization, the monthly rates are

given in Figure 2. Troop strength is shown as a dashed line (no increase occurred prior to March 1965 or after January 1966). A striking seasonal pattern was noted which reflected the ITR dispensary illness rates mentioned previously. Admission rates varied from 92/1,000/month during May to 5/1,000/month in August 1965. The per cent of total respiratory disease represented by pneumonia varied from 14% in April to 73% in August 1965. No relationship between illness rates and troop strength was observed.

The etiologic agents responsible for the respiratory illness in the hospital study are shown in Figure 3. The numbers in the bars of Figure 3 represent the percentage of the monthly acute respiratory illness associated with each agent. The striking seasonal pattern of adenovirus 4 associated illness is shown, comprising 77% of all the respiratory illness in April, decreasing markedly in June and July, absent from August through December and reappearing in January 1966. M. pneumoniae associated illness was endemic and ranged from an incidence of 4% in April to 43% in July. The adenovirus 4 plus M. pneumoniae associated illness was present from April to July and comprised 2-8% of the illness during these months. The unidentified illness, although undoubtedly due to more than a single etiologic agent, was endemic and made up 17% of all the illness in April to 93% in December.

An etiologic summary of the 794 cases of acute respiratory disease requiring hospitalization appears in Table 1. The unidentified fraction comprised 42% of the total. Of the identifiable fraction, the ratio of adenovirus 4 to M. pneumoniae was 4.6 to 1. In a small proportion of upper respiratory infections, a bacterial etiology was suspected clinically, but adequate laboratory confirmation was not available.

One striking finding seen in Figure 3 is that there was as much M. pneumoniae associated illness in the adenovirus epidemic months as in months when no adenovirus could be found. Evidence obtained in past years during more limited surveillance studies in epidemic periods led us to believe that M. pneumoniae was virtually "crowded out" by adenovirus 4 because of poor competitive ability. This concept has had to be abandoned and the inability to find M. pneumoniae in the past probably was due to studying only dispensary populations and obtaining smaller samples which included relatively more afebrile respiratory illness.

The etiologic agents associated with primary atypical pneumonia in the hospital study are shown in Figure 4. A seasonal variation in the incidence of pneumonia was noted, but was less striking than that of the total respiratory illness. The pneumonia rates varied from 12.4/1,000 in May to 3.5/1,000 in September. The incidence of adenovirus 4 associated pneumonia varied from 0% in June through December to 65% during April. M. pneumoniae associated pneumonia was endemic, varying from 11% of all pneumonia during April to 63% in June. Adenovirus 4 plus M. pneumoniae pneumonia incidence varied from 8% in April to 12% in July. The unidentified segment was endemic and varied from 13% in May to 88% during October and December.

An etiologic summary of the 233 cases of primary atypical pneumonia

appears in Table 2. The unidentified fraction comprised 52% of the total. Of the identifiable fraction, the ratio of M. pneumoniae to adenovirus 4 was 2.7 to 1.7.

For the etiologic categories described, the percent of the respective illness represented by pneumonia is shown in Table 3. Eleven percent of the cases hospitalized with adenovirus 4 infections had pneumonia (range 6-19%), while 78% of those with M. pneumoniae infections had pneumonia (range 50-100%). The average for the unidentified fraction with pneumonia was 87% with a very wide range from 8-100%, probably reflecting the heterogeneity of this group. Pneumonia made up 29% of all admissions for acute respiratory disease.

In 1961 Holland (4) showed by means of partial correlations of several outdoor weather parameters that acute respiratory disease admissions had "a significant negative correlation with temperature" and "a positive correlation with relative humidity". The population studied consisted of Royal Air Force troops stationed in three different parts of England. It has been noted by several authors in the past that these correlations could be found in both temperature and tropical regions. It is possible that abrupt changes in weather conditions rather than absolute measures are more important.

In an attempt to correlate several parameters of abrupt weather changes with rates of respiratory illness during epidemic periods, Figures 5 and 6 are presented. The weather data plotted were daily rainfall in inches averaged over a week, together with a horizontal line representing the mean rainfall over the 5-year period by the week. The temperature was plotted as the 24-hour maximum-minimum difference averaged over a week along with a horizontal line representing the mean difference over the 5-year period by the week.

The results are shown as the epidemic year along with the variations from the mean in the rainfall and temperature parameters described:

1961 - The temperature difference varied above average with the rainfall consistently below average.

1962 - The temperature difference was above average with the rainfall below average.

1963 - The temperature difference above average with shifting; rainfall below average, then above.

1964 - The temperature difference below average, rainfall considerably above average.

1965 - The temperature difference above average, then shifting, rainfall below average.

It appeared that during the start of the epidemics shown, either the temperature difference or the rainfall was above average. To be sure, this pattern can be found in non-epidemic periods, but not superimposed upon lower mean temperature base lines.

DISCUSSION

The reasons for the differences in patterns of adenovirus associated respiratory illness at Camp Lejeune and Parris Island and the striking seasonal changes at Camp Lejeune can only be speculated upon. The 80 men comprising a platoon train as a relatively isolated epidemiologic unit at Parris Island with little contact between platoons. At ITR, however, the epidemiologic unit is a company consisting of about 240 men with ample contact between companies.

The seasonal differences cannot be explained on the basis of training conditions which remain relatively constant throughout the year. There is inadequate information concerning correlation between seasonal temperature and humidity changes in the barracks and the occurrence of respiratory illness. No conclusive results can be drawn from the record of changes in the external environment presented earlier in this paper. Plotting weather data on a weekly basis, however, may be too long a time interval and mask any correlations present.

There is little experimental evidence which sheds light on the relationship between seasonal climatic variation and rates of acute respiratory disease.

The question of whether seasonal changes may alter the susceptibility of the population to naturally acquired infection, perhaps by altering mucous membrane susceptibility, or whether such changes may favor the transmission of the infective droplets, or both, has not been answered.

It is not known whether colder weather may decrease the rate of decline of infectivity of droplets containing virus or mycoplasma organisms or whether the number of infective particles may be increased by closing windows.

Does chilling or other environmental change associated with colder weather increase the amount of virus shed, perhaps by increasing the amount of nasal secretion?

We are in agreement with the findings of the Armed Forces Epidemiological Board which suggest that recruits become infected principally in the barracks and that the more important mode of infection is droplet contact rather than airborne droplet-nuclei (5). The microenvironmental temperature and humidity in the barracks which change rapidly when the number of men within increases and the probable greater number of infected particles present with closed windows appear to us more important in increasing the efficiency of transfer of the organisms than any seasonal alteration in the susceptibility of the population. The larger training units and increased contact at ITR, we believe,

favor propagation of any small epidemic to a much greater extent than occurs at Parris Island.

Galen's (130-200 A.D.) (6) interpretation of environmental factors was that "temperamental discord, producing humours ripe for putrefaction, or over-luxurious living, or an irregular mode of life, either or both, might render the body susceptible to the ill effects of inspiring air charged with putrid exhalation".

Although our current approach to the environmental problem has 18 centuries of precedence, it is hoped that the next 18 will answer a few more questions than the last.

SUMMARY

The epidemiology of adenovirus 4 associated acute respiratory disease in Marine recruits at Camp Lejeune, North Carolina, consists of sharply demarcated winter epidemics with dispensary visit rates as high as 1,700/1,000 men/month. Approximately 90% of the illness is adenovirus 4 associated in epidemic periods. At Parris Island, South Carolina, small winter outbreaks of adenovirus 4 respiratory illness have been noted in previous years with dispensary visit rates up to 375 visits/1,000 men/ month. Approximately 25% of the illness is adenovirus 4 associated in epidemic periods. At Parris Island, since 1963, there have been no recognizable seasonal variations to the illness patterns. No relationship between troop strength and illness rates at either training camp can be demonstrated. Type 4 has been the only adenovirus serotype isolated from recruit respiratory illness since 1963, prior to which type 7 was occasionally isolated. Recruits hospitalized for acute respiratory illness at Camp Lejeune from April 1965 to January 1966 were studied. Of the 794 hospitalized cases of respiratory illness, 29% had primary atypical pneumonia. Adenovirus 4 illness followed a winter and spring pattern, while M. pneumoniae and the unidentified fractions were endemic. For all acute respiratory illnesses, 42% was in the unidentified fraction, the remainder distributed in a ratio of adenovirus 4 to M. pneumoniae of 4.6 to 1. Fifty-two percent of the 233 cases of primary atypical pneumonia studied were in the unidentified group, the remainder distributed in a ratio of M. pneumoniae to adenovirus 4 of 2.7 to 1.7. The fraction of the total respiratory illness represented by pneumonia was 11%, 79%, 37% for adenovirus 4, M. pneumoniae and unidentified, respectively. No clear correlation between mean 24-hour maximum-minimum temperature differences and the mean 24-hour rainfall, both averaged over a week, and epidemic respiratory illness rates could be found. The possible relationships between seasonal climatic changes and the patterns of acute respiratory illness are discussed.

RELATIONSHIP BETWEEN RATES OF U.R.I. AND TROOP STRENGTH IN MARINE RECRUITS AT CAMP LEJEUNE AND PARRIS ISLAND

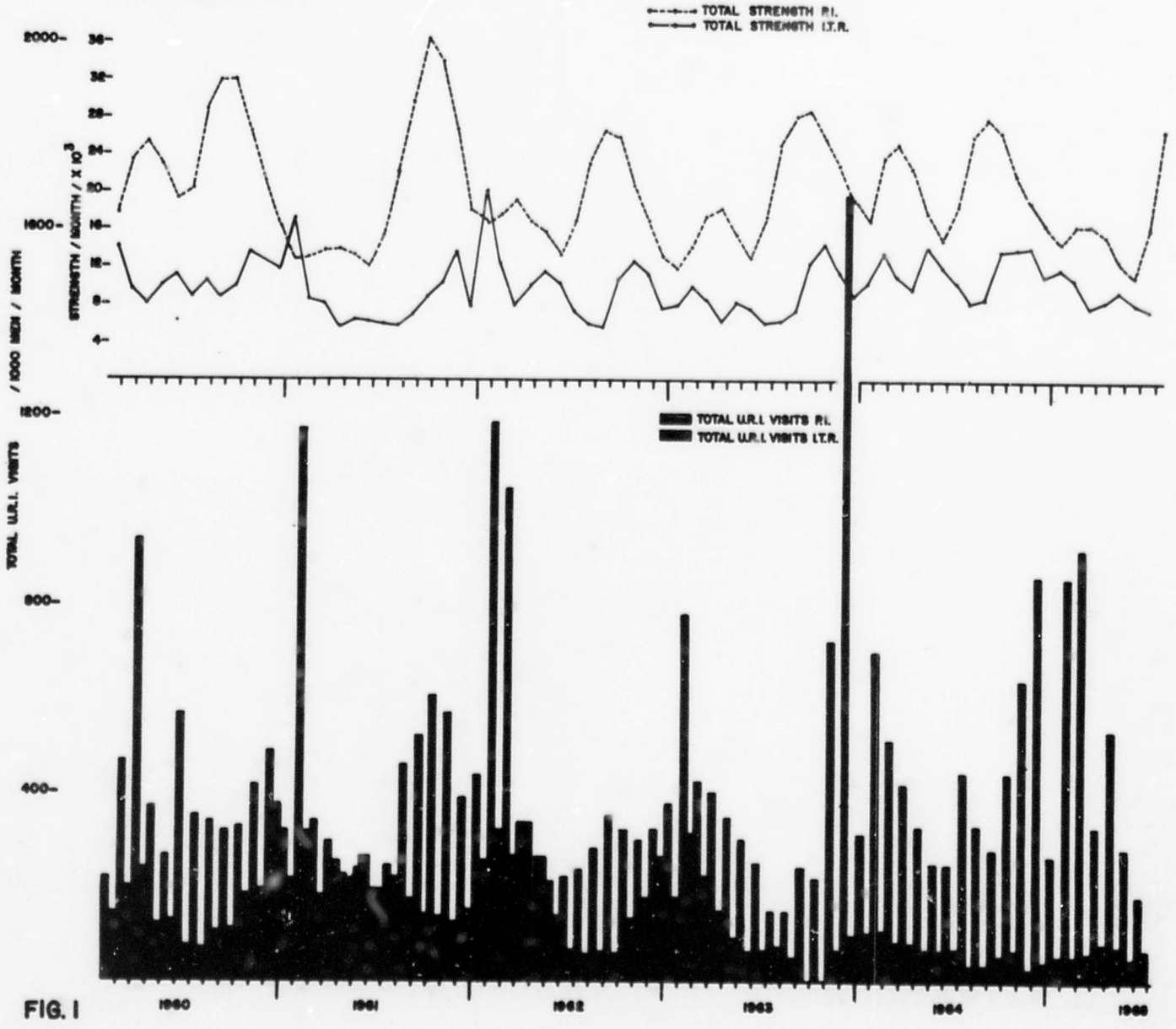

FIG. 1

RELATIONSHIP BETWEEN RECRUIT A.R.D. AND PNEUMONIA ADMISSION RATES TO NAVAL HOSPITAL, CAMP LEJEUNE AND TROOP STRENGTH.

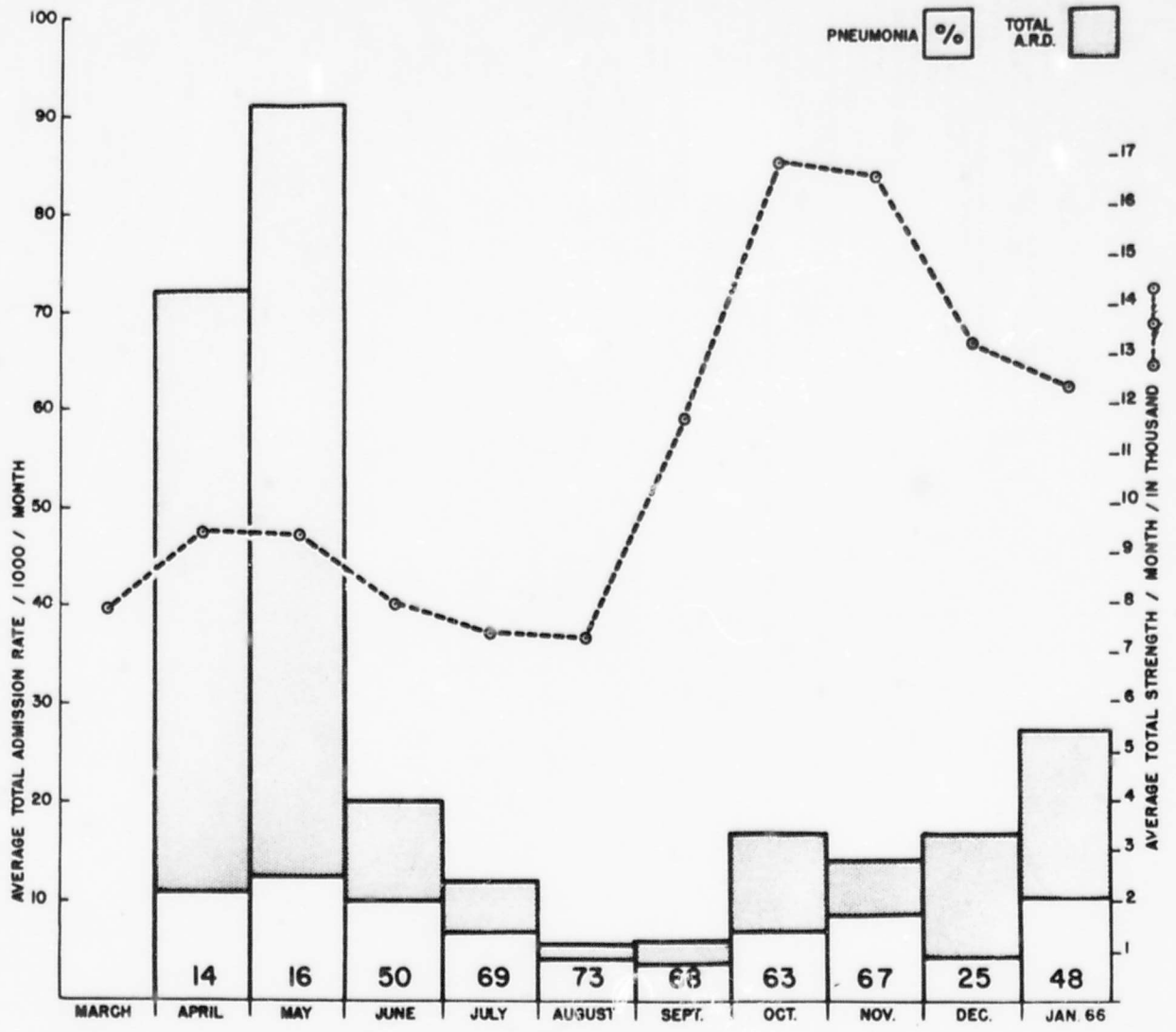

FIG. 2

ETIOLOGY OF A.R.D. IN MARINE RECRUITS ADMITTED TO NAVAL HOSPITAL CAMP LEJEUNE, N.C.

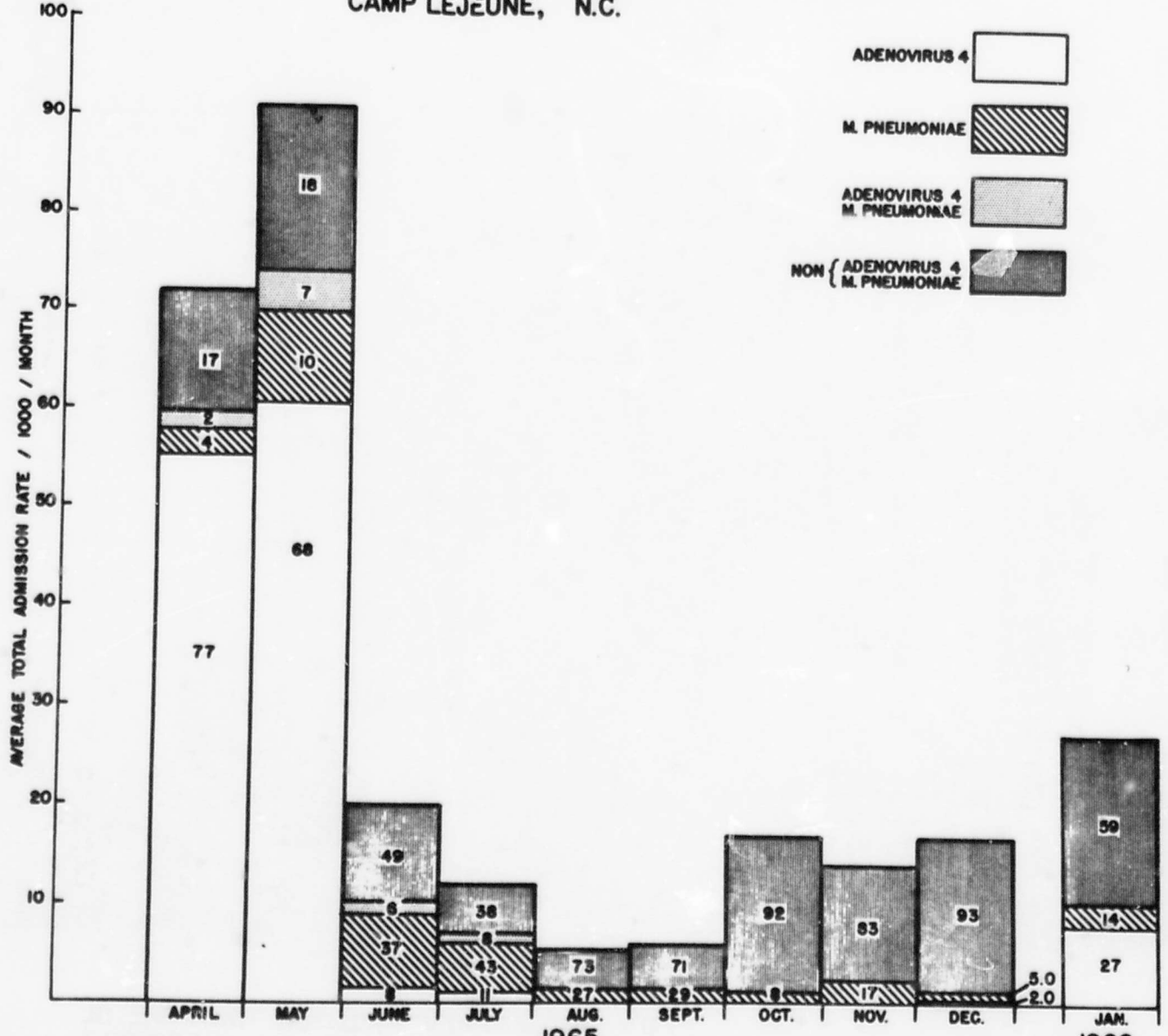

FIG. 3

ETIOLOGY OF PRIMARY ATYPICAL PNEUMONIA IN MARINE RECRUITS ADMITTED TO THE NAVAL HOSPITAL CAMP LEJEUNE, N.C.

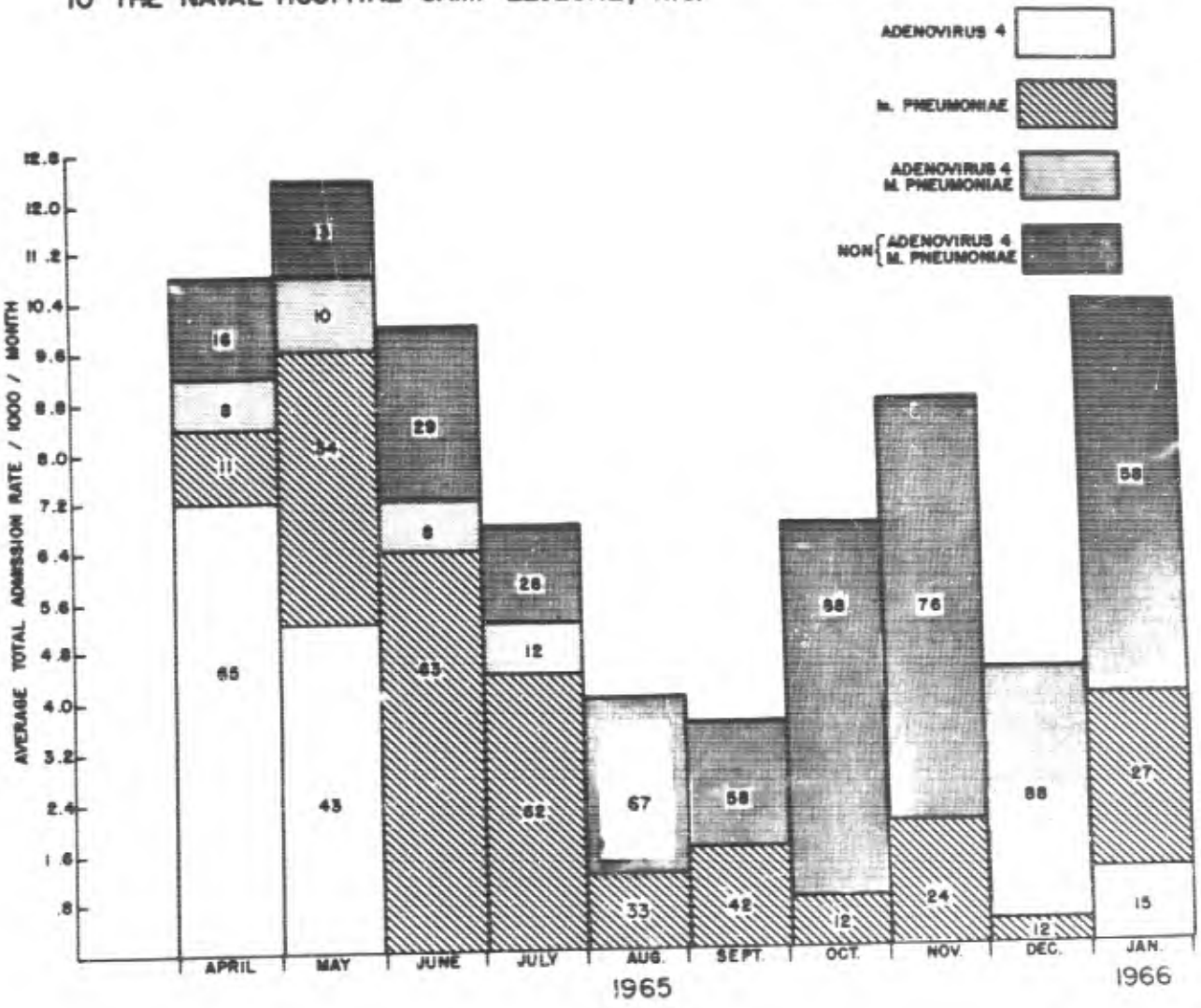

FIG. 4

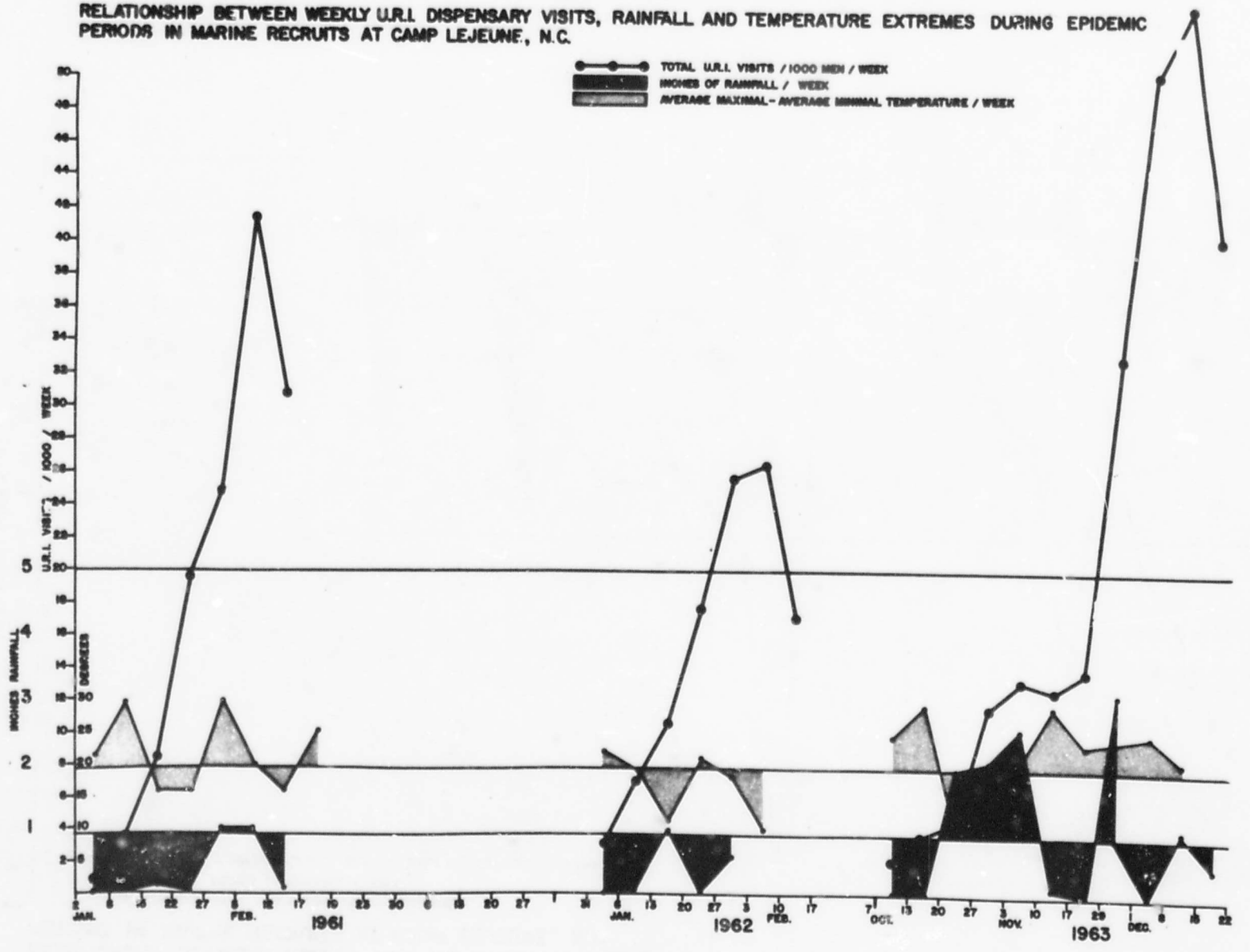
RELATIONSHIP BETWEEN WEEKLY U.R.I. DISPENSARY VISITS, RAINFALL AND TEMPERATURE EXTREMES DURING EPIDEMIC PERIODS IN MARINE RECRUITS AT CAMP LEJEUNE, N.C.
TOTAL U.R.I. VISITS / 1000 MEN / WEEK
INCHES OF RAINFALL / WEEK
AVERAGE MAXIMAL - AVERAGE MINIMAL TEMPERATURE / WEEK
U.R.I. VISITS / 1000 / WEEK
INCHES RAINFALL
DEGREES
JAN.
FEB.
1961
JAN.
1962
FEB.
OCT.
NOV.
1963
DEC.

FIG. 5

RELATIONSHIP BETWEEN WEEKLY U.R.I. DISPENSARY VISITS, RAINFALL AND TEMPERATURE EXTREMES DURING EPIDEMIC PERIODS IN MARINE RECRUITS AT CAMP LEJEUNE, N.C.

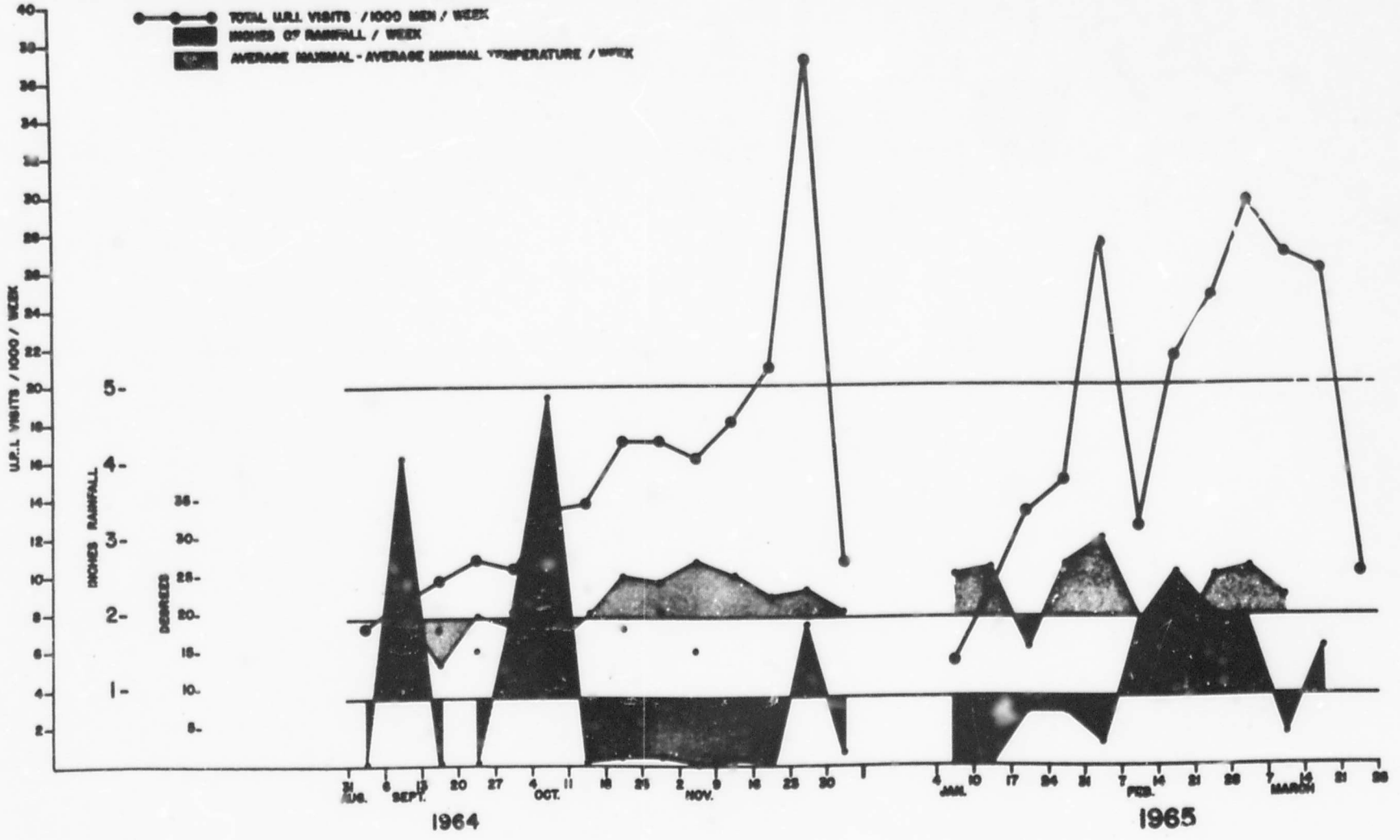

FIG. 6

TABLE 1

HOSPITAL ACUTE RESPIRATORY DISEASE SUMMARY

	No.	% Total
Adenovirus 4	363	46
M. pneumoniae	80	10
Adenovirus 4 + *M. pneumoniae*	19	2
Non-adenovirus 4 + *M. pneumoniae*	332	42
Total	794	100

TABLE 2

HOSPITAL PNEUMONIA SUMMARY

	No.	% Total
Adenovirus 4	39	17
M. pneumoniae	62	27
Adenovirus 4 + *M. pneumoniae*	10	4.0
Non-adenovirus 4 + *M. pneumoniae*	122	52
Total	233	100

TABLE 3

HOSPITAL PNEUMONIA
ACUTE RESPIRATORY DISEASE
ADMISSIONS SUMMARY

		Range
Adenovirus 4	11%	6-19%
M. pneumoniae	78%	50-100%
Adenovirus 4 + *M. pneumoniae*	53%	50-100%
Non-adenovirus 4 + *M. pneumoniae*	37%	8-100%
Total	29%	

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SUMMARY REPORT OF LIVE TYPE 4 ADENOVIRUS VACCINE FIELD TRIALS CONDUCTED FROM 1964-1966 AT PARRIS ISLAND, SOUTH CAROLINA AND CAMP LEJEUNE, NORTH CAROLINA

Richard Ralph Gutekunst

The purpose of this report is to summarize the results of vaccine field trials conducted for the last three years at the Marine Corps Recruit Training Depot, Parris Island, South Carolina and at the Infantry Training Regiment, Camp Geiger, Camp Lejeune, North Carolina, and represents the united efforts of Doctors Chanock, Edmondson, Gundelfinger, White, Fuld and Steinberg, and some hard-working hospital corpsmen. Recipients of the vaccine were young healthy male marine recruit volunteers undergoing training at Parris Island. While at Parris Island, the recruits train in 75-man platoons and very little contact with personnel outside their training platoon is permitted. This location was chosen for the administration of the vaccine since adenovirus infection occurs infrequently at Parris Island. For this reason, safety, antigenicity and communicability of the enterically administered adenovirus could be investigated without interference from naturally occurring adenovirus infection.

Upon completion of recruit training, the majority of the trainees are transferred without intervening leave to Camp Lejeune for an additional training period. While at Camp Lejeune, the men train in 240-man companies and there is ample opportunity for contact between men in the various training units. Sharp extensive epidemics of type 4 adenovirus infection occur at Camp Lejeune during the winter and early spring. The ecology of adenovirus infection at the two training bases thus made it possible to sequentially evaluate the safety and antigenicity of the virus vaccine at Parris Island and the protective effect of prior enteric infection when the same recruits were subsequently challenged with naturally occurring adenovirus during a type 4 virus epidemic at Camp Lejeune.

Virus Strain and Vaccine Preparation

The strain of type 4 adenovirus incorporated into the vaccine was isolated in human embryonic tissue culture from the throat swab specimen of a marine recruit ill with a febrile respiratory disease. Prior to lyophilization and encapsulation, the virus had been transferred 11 times in human diploid fibroblast tissue culture (WI-38). The virus preparation employed in the 1964 and 1965 studies was encapsulated following lyophilization. The lyophilized virus

suspension was mixed with lactose so that a No. 3 hard gelatin capsule, when filled, received 60 mg of lyophilized virus suspension, an amount of virus equivalent to 1 ml of original tissue culture fluid. The No. 3 capsule was enclosed in a No. 2 opaque capsule which was then coated with a standard enteric phthalate film. Infectivity titrations in human embryonic kidney were performed on several of the virus containing capsules and the 50% tissue culture infectious dose ($TCID_{50}$) for the 1964 preparation was calculated to be 10^{-6} and for the 1965 vaccine preparation to be $10^{-7.2}$. Placebo capsules containing lactose instead of virus were prepared in the same manner as virus capsules. The vaccine preparation employed in the 1966 study was a press-coated tablet or "tablet within a tablet". The tablets were press-coated by compressing about the virus tablet a mixture of lactose, magnesium stearate and microcrystalline cellulose. The tablets were then coated with approximately 25 mg standard enteric phlate coating. Each tablet contained 19.1 mg of lyophilized solid or the equivalent of 1/3 cc of the original fluid. The approximate titer of virus in the tablet was $10^{-6.5}$. $TCID_{50}$. Safety tests performed on all vaccine preparations revealed that the virus suspensions incorporated into the vaccines were free of contaminating adventitious agents and extensive tests in newborn hamsters failed to demonstrate any evidence of oncogenicity.

Virus Recovery and Serological Studies

Throat and anal swab specimens collected for virus isolation were stored at -70°C for varying periods of time prior to inoculation into HEp-2 tissue cultures. All inoculated tissue cultures were observed for a minimum of 30 days before being discarded as negative. Adenovirus isolates were identified by either the hemagglutination inhibition technique or the micro-neutralization test. Serum neutralization tests were performed in embryonic human kidney employing 10-32 $TCID_{50}$ of vaccine virus. All sera from the same individual were tested on the same day. Antibody titers are expressed as the final dilution of serum.

1964 Field Trial

In this first vaccine field trial, 276 recruit volunteers in the 8th week of training at Parris Island were randomized by serial number into two groups. One hundred thirty-four men were administered vaccine and 142 men were fed a placebo capsule. At the time the recruits were fed either a virus or placebo capsule, a medical history was taken, oral temperature was recorded, and a physical examination of the ears, nose, throat and chest was done. In addition, throat and anal swab specimens for virus recovery were taken. These procedures were repeated at 2- to 3-day intervals for the duration of the 13-week study. Fifty-two men from the virus capsule group and an equal number of placebo controls were randomly selected and all of their throat and anal swab

specimens were tested in tissue culture for the presence of adenovirus. In addition, each of these recruits was tested for the development of type 4 adenovirus neutralizing antibody 4 weeks after the capsules were given. The 104 men tested in this manner are referred to as the intensive surveillance group. Specimens were also collected from all recruits with a respiratory illness and were tested in tissue culture.

The neutralizing antibody response of 52 vaccinees is shown in Table 1. All recruits who lacked detectable neutralizing antibody became infected following administration of the vaccine. Type 4 adenovirus neutralizing antibody developed in each of these men and type 4 adenovirus was recovered from anal swab specimens of all but one of these recruits. Five of the six recruits with low levels of pre-existing antibody were infected by the enteric route, but only two of nine recruits with titers of 1:32 or greater became infected. These latter two individuals, however, shed type 4 virus for approximately the same interval as did antibody-free recruits or men with low levels of pre-existing antibody.

The virus excretion pattern of the 37 men who initially lacked detectable neutralizing antibody is shown in Figure 1. Virus was detected in the anal swab specimen of only one man on the 2nd day following administration of the vaccine. Approximately one-third of the recruits shed virus on the 4th day and excretion reached its peak on the 10th day, by which time 97% of the group had excreted type 4 virus. Virus excretion could not be detected after the 18th day.

Table 2 illustrates the neutralizing antibody response of the 37 seronegative recruits in the intense surveillance group. Each of the recruits developed a 4-fold or greater rise in specific neutralizing antibody by one month following vaccination. The level of neutralizing antibody which developed ranged from 1:8 to 1:256 with a mean level of 1:32. For comparison, the antibody response of 20 men naturally infected at Camp Lejeune is shown on the lower line. The median neutralizing antibody level of the vaccinated recruits was 1:32, whereas, those naturally infected had a mean titer of 1:64, only a 2-fold difference.

One of the most significant findings of this first field trial was the failure of type 4 virus to spread to susceptible contacts who were in close personal contact with the vaccinees throughout recruit training. Table 3 shows that although men fed virus capsules excreted type 4 virus over a 16-day interval, infection was not acquired by the antibody-free men in the placebo group. Type 4 virus was not recovered from any man in the placebo group, nor did neutralizing antibody develop in any man in this group. In addition, among the vaccinees type 4 virus was recovered only from anal swab specimens and never from throat swab specimens. This indicated that infection did not spread to the upper alimentary tract or to the respiratory

tract from the lower intestinal tract. In this sense, infection was selective for the lower intestinal tract.

Data on the safety of the vaccine preparation was obtained by observing the illness experience of the 279 recruits during the 30-day interval following administration of either a virus or placebo capsule. No significant difference in the illness patterns among vaccinees and placebo controls was noted. No signs or symptoms referable to the central nervous system developed in any of the recruits. An 11-month follow-up of the men in this study was done by reviewing health records. The records of 103 men in the virus capsule group and 110 men in the placebo group were available for this review. Although men from each group were hospitalized or seen in the clinic during a 7-month interval following the end of post-recruit training at Camp Lejeune, no specific illness pattern was noted in either the virus capsule or placebo control groups.

The specific protective effect of the type 4 enteric infection was demonstrated when 253 of the recruits in the study were transferred to Camp Lejeune where type 4 adenovirus infection was epidemic. A specific protective effect of type 4 virus enteric infection was evident when the hospital admissions for type 4 adenovirus febrile acute respiratory tract disease were compared for the virus and placebo control groups (Table 4). None of the 125 men in the virus capsule group was hospitalized with an adenovirus-associated illness, whereas, 32 of 128 placebo capsule men were hospitalized with an adenospecific illness. A similar protective effect of the vaccine virus was seen when total adenovirus respiratory tract disease was compared for the virus and placebo groups. A total of 42 adenovirus-associated febrile respiratory tract illnesses occurred among the 128 men in the placebo group, whereas, only one febrile illness, not associated with respiratory symptoms, occurred among the 125 men in the virus capsule group. A definite, but lesser protective effect against afebrile respiratory tract disease was also observable. The correlation of vaccine-induced neutralizing antibody, with resistance to subsequent infection with naturally occurring virus, is shown in Table 5. Recruits in whom neutralizing antibody developed following administration of the virus capsule were infected significantly less often than were men in the placebo group who lacked neutralizing antibody. All 36 men in the placebo group without antibody became infected. Virus was recovered from 10 to 34 vaccinees who developed specific neutralizing antibody following vaccination, indicating reinfection can occur, but such reinfections were not associated with febrile illness.

1965 Field Trial

The purpose of the 1965 vaccine field trial was 2-fold: 1) we were interested in determining whether we could confirm the protective effect demonstrated in the 1964 study, and 2) we were interested in determining the quantity of type 4 virus required to infect the lower intestinal tract of

man. For Phase I of this study, 360 men were fed a virus capsule containing approximately 10 million $TCID_{50}$ of type 4 adenovirus and 365 recruits were fed a placebo control. Oral temperatures were recorded on all men prior to feeding the capsule, and blood specimens were collected from each recruit included in the study. Additional blood specimens were collected 4 weeks after administration of the vaccine when the recruits arrived at Camp Lejeune.

One hundred thirty-four recruits were selected for an extensive follow-up study at Parris Island. After an initial interview and physical examination, each man was observed 3 times weekly for 4 weeks. In addition to a physical examination, anal swab and throat swab specimens for virus isolation were collected at each interview period. A sick call surveillance was also maintained at the ITR dispensary at Camp Lejeune and throat washings and anal swab specimens were collected on all men in the study who reported to sick bay with a respiratory illness. Specimens were also collected from all men admitted to the hospital with an acute respiratory disease. Acute and convalescent phase sera were collected on all individuals studied. Analysis of the specimens collected on the day of feeding revealed that natural adenovirus infection was occurring in the recruits selected for study. This observation made it impossible to determine the communicability of the vaccine virus among vaccines and placebo controls. It did not interfere, however, with observations designed to answer the question of the safety of the vaccine preparation, or its protective effect when vaccinees were subsequently challenged with naturally occurring type 4 adenovirus.

Serum neutralizing antibody titers and rectal excretion patterns were determined on 60 vaccinees and 32 placebo controls who were devoid of detectable neutralizing antibody at the time of administration of the capsule. All recruits given virus capsules developed a 4-fold or greater rise in specific neutralizing antibody with a mean geometric titer of 1:52. One hundred percent of the vaccinees shed virus by the 13th day after administration of the vaccine. The mean number of days excretion was 8. Only one recruit in the placebo group developed a rise in neutralizing antibody and only two men shed virus. Infection in the placebo group probably represents naturally acquired type 4 infection. At no time was type 4 adenovirus isolated from the throat of either a vaccinee or placebo control.

The illness experience of all recruits in the study is compared in Table 6 and covers a period of 19 to 24 days following administration of the vaccine. Casual surveillance includes all dispensary visits for each of the 716 men studied and the longitudinal surveillance notes only those illnesses present in the 134 recruits examined 3 times weekly. The number and types of illnesses in the vaccine and placebo groups were similar. No specific excess illness relative to the administration of the virus capsule was noted. Although there was a tendency for more respiratory disease to occur in the men receiving the placebo capsule, the difference from the vaccine group is not statistically significant. As was noted in the 1964 field trial, signs and symptoms referrable to central nervous system were not noted.

Six hundred seventy-six men from the study group were transferred to Camp Lejeune for infantry training and remained here for varying periods from late February through late May, during which time natural type 4 adenovirus disease was present.

The specific protection afforded vaccinees during their stay at Camp Lejeune is shown in Table 7. Forty-five of 337 recruits receiving the placebo capsule were hospitalized with an acute respiratory disease associated with the isolation of type 4 adenovirus. Forty-two of the hospitalizations occurred in men initially free of neutralizing antibody. In addition, 10 other febrile illnesses which occurred in the placebo group were associated with the isolation of type 4 adenovirus. Seven of these illnesses were in men devoid of antibody. None of the 339 recruits receiving the virus capsule was hospitalized with an adenovirus illness and none experienced a febrile illness associated with this virus. The association of the isolation of adenovirus with an afebrile respiratory illness was similar for both the vaccine and placebo groups.

The second phase of the 1965 vaccine program was designed to determine the limiting dilution of adenovirus capable of establishing an infection in the lower intestinal tract. This study was conducted at Parris Island during the summer of 1965 when natural infection with type 4 adenovirus could not be detected. Recruit volunteers free of specific neutralizing antibody were divided in four groups and fed capsules containing either $10^{-6.5}$, 10^{-2} and $<10^{-1}$ $TCID_{50}$ of type 4 adenovirus. The results of this study are shown in Table 8. All or almost all men given 10^{-4} to $10^{-6.5}$ $TCID_{50}$ of virus were infected. The 50% infectious dose for the lower intestinal tract appears to be in the 10^{-1} to $10^{-2.7}$ range. The antibody response did not appear to differ significantly from men given different quantities of virus. It should be noted that in men infected by the enteric route, virus was recovered only from the rectal swab specimens and never from throat swab specimens. This finding confirms observations made during 1964 which indicated that enteric type 4 infection remains localized in the intestinal tract. Furthermore, infection did not spread to 24 placebo controls who were free of detectable neutralizing antibody and who were in the same training units containing recruits who received varying quantities of type 4 virus by the enteric route. Eighty-eight of 129 recruits were successfully infected following administration of type 4 virus capsules, but despite this extent of infection, unvaccinated susceptibles did not acquire type 4 virus infection. This finding confirms the lack of communicability of enteric type 4 infection in marine recruits. The rectal excretion patterns for all groups in the dilution study are shown in Figure 2. The pattern for each group is similar irrespective of the amount of virus in the capsule. The maximum number of men shed virus between the 10th and 13th day.

1966 Field Trial Study

The present vaccine study currently under evaluation involved the

administration of a new type of vaccine preparation. Whereas all previous vaccine field trials were conducted with an encapsulated virus vaccine, the present preparation is a "tablet within a tablet" preparation. Preliminary studies showed this preparation to be extremely immunogenic; all antibody-free recruits developing neutralizing antibody 11 days after administration of the pill with an additional rise in antibody between day 11 and day 15 (Table 9). The appearance of antibody by day 11 is similar to the antibody response observed in the 1964 field trial. It is of interest that the smaller tablet tested at this time did not elicit the antibody response noted with the larger tablet, nor was it 100% effective in immunizing all antibody negative recruits. For this reason, the large tablet was selected for production and evaluation in a large-scale field trial.

The 1966 field trial was initiated at Parris Island on 6 February 1966 and coincided with an extensive type 4 adenovirus outbreak at Camp Lejeune. Recruits were immunized during their 5th week of training and were transferred to Camp Lejeune 3 weeks later. Recruits were randomized by serial number and approximately 50% of all men in each series received an enteric-coated tablet and the remainder received nothing and served as controls. Administration of the vaccine was terminated at Parris Island on 11 April 1966. During this interval, 6884 recruits were given enteric-coated virus tablets and 6972 served as controls. Vaccinees began arriving at Camp Lejeune on 23 February and remained here for varying periods of 2 to 4 weeks. The last vaccinees arrived in late April.

Analysis of hospitalized illnesses which occurred in the study group following vaccination is shown in Table 10. A total of 46 recruits were hospitalized, but as noted in the two previous field trials, no specific excess illness was noted in the vaccine group. There were no admissions for central nervous system illness.

Data on the protective effect of the vaccine was obtained when personnel in the study group were transferred to Camp Lejeune. Forty-one men in the placebo group and 11 vaccinees became ill with a febrile respiratory illness associated with the isolation of type 4 virus (Table 11). No surveillance was maintained for afebrile respiratory disease. Three vaccinees were hospitalized with a febrile illness and 8 were placed on no duty after reporting to sick call with a febrile respiratory illness. Among the placebo group, 34 recruits were hospitalized with a type 4 adenovirus illness and 39 were placed on no duty with an illness associated with type 4 virus.

The protective effect observed in each of the three vaccine field trials is also summarized in Table 11. Since 1964, seven thousand three hundred forty-seven marine recruits have been immunized and of this group, 4 have been hospitalized with an adenovirus illness and 15 experienced a type 4 febrile respiratory illness which did not require hospitalization. In comparison, of 7437 placebo controls, 111 were hospitalized with an adenovirus illness and an additional 59 sought medical attention at sick call for an adenovirus-associated

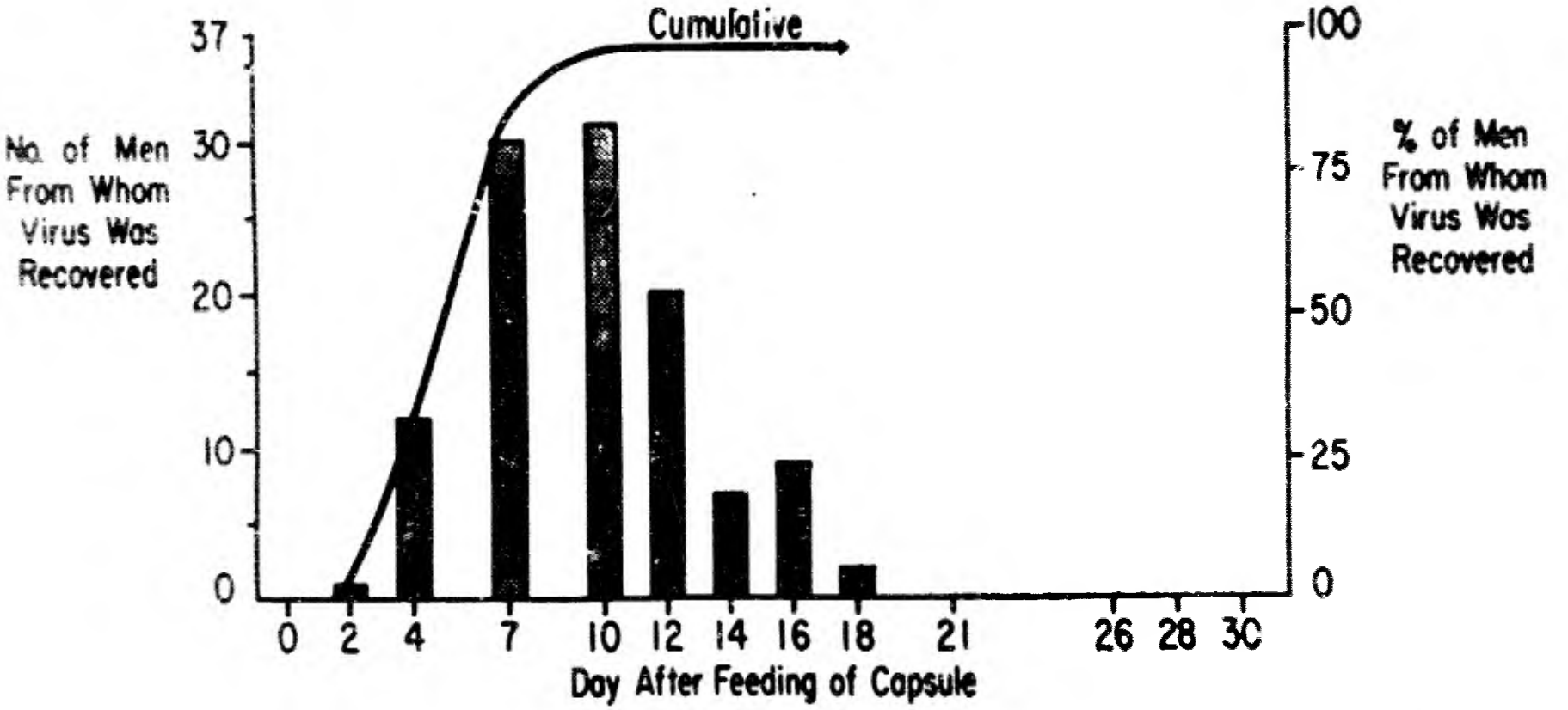
febrile respiratory illness.

Thus, this live type 4 adenovirus vaccine which selectively infects the lower intestinal tract has been shown to be 93% effective in protecting marine recruits against a type 4 febrile respiratory illness and 85% effective in protecting against both a febrile and afebrile respiratory disease associated with type 4 adenovirus.

SUMMARY

Adenovirus infections, which constitute a major cause of acute respiratory disease in military recruits, are best controlled by vaccination. For a vaccine to be acceptable, however, the following criteria must be satisfied: (a) the vaccine must produce a level of immunity which will protect the susceptible individual; (b) no increased illness can be associated with the administration of the vaccine; and (c) if the vaccine is a live attenuated virus, there should be no spread of vaccine virus between vaccinees and placebo controls. All these criteria were satisfied by the live type 4 adenovirus vaccine which was administered to healthy marine recruits for the last 3 years.

FIGURE 1



1. Recovery of type 4 adenovirus from anal swab specimens following administration of enteric-coated capsules to Marine recruits free of neutralizing antibody (<1:4).

FIGURE 2

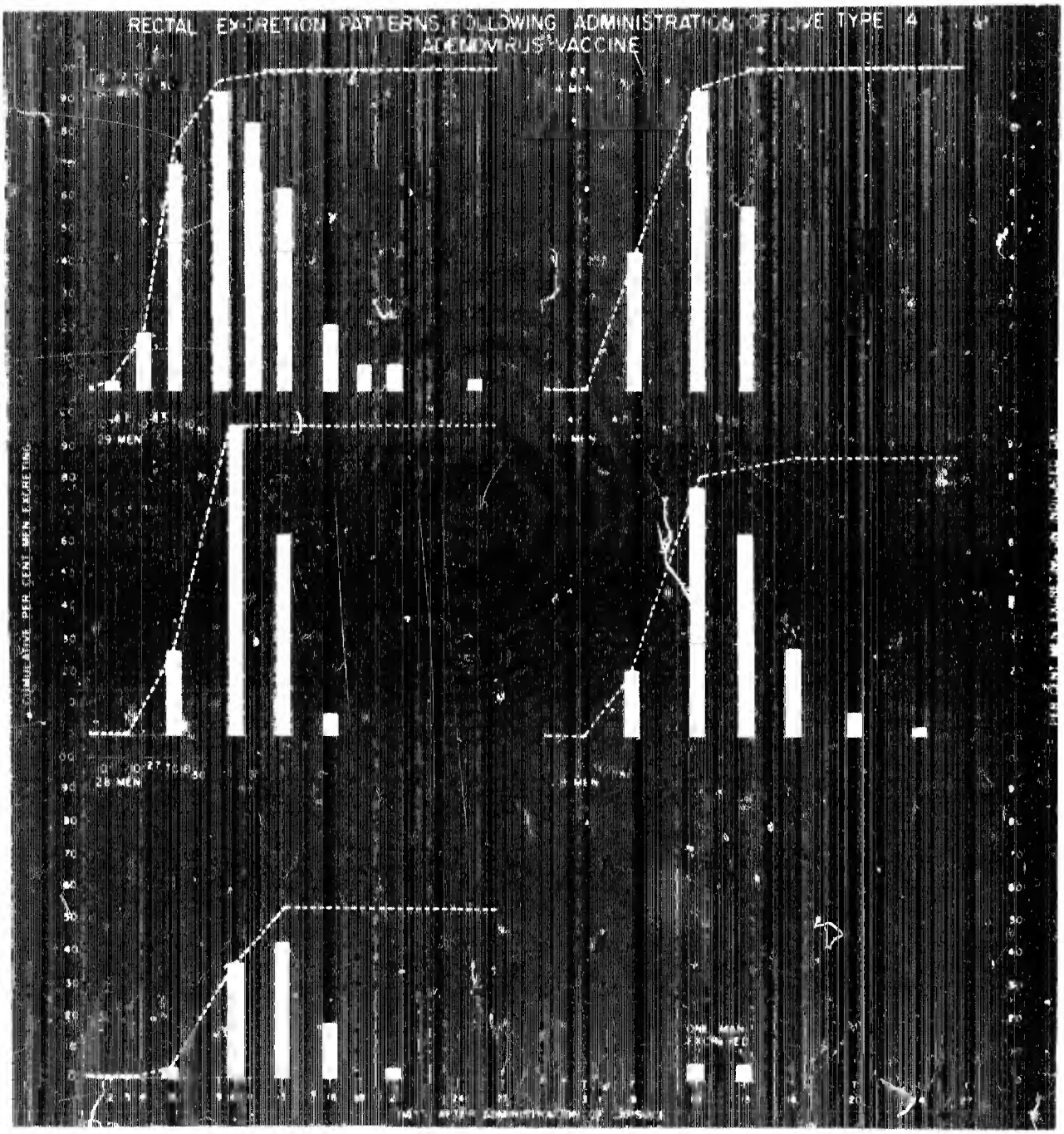

RECTAL EXCRETION PATTERNS FOLLOWING ADMINISTRATION OF LIVE TYPE 4
ADENOVIRUS VACCINE
CUMULATIVE PER CENT MEN EXCRETING

TABLE 1

EFFECT OF PREEXISTING NEUTRALIZING ANTIBODY UPON INFECTION OF RECRUITS WITH TYPE 4 ADENOVIRUS ADMINISTERED BY ENTERIC CAPSULE

Reciprocal of neutralizing antibody for type 4 adenovirus prior to feeding of capsule	Number of recruits in group	Number of men from whom virus recovered* from anal swab specimen	Duration of excretion, days		Number of men with rise in antibody neutralizing	CF**	Total number of men infected
			range	mean			
<4	37	36	1-13	7	37	15	37
4-16	6	5	1-10	5	5	2	5
32 or >	9	2	4-8	6	1	1	2

*During 30-day period of surveillance. Twelve tests for virus done during this interval.

**Complement fixing.

TABLE 2

NEUTRALIZING ANTIBODY RESPONSE OR SERONEGATIVE (<1:4) RECRUITS TO ENTERIC TYPE 4 ADENOVIRUS VACCINE -- COMPARISON WITH THAT OF NATURALLY INFECTED SUBJECTS

Location	Group	Number of men	Number of men with indicated level of neutralizing antibody (reciprocal) 4 weeks after feeding of capsules or 2 to 4 weeks after onset of symptoms							
			<4	4	8	16	32	64	128	256 or >
Parris Island	Virus capsule, placebo	37	-	-	4	10	8	6	6	3
	capsule	36	36		-	-	-	-	-	-
Camp Lejeune	Natural infections*	20	-	-	-	-	4	8	7	1

*Neutralizing antibody-free (<1:4) recruits from placebo capsule group who subsequently acquired natural infection with type 4 adenovirus at Camp Lejeune.

TABLE 3

LACK OF COMMUNICABILITY OF TYPE 4 ADENOVIRUS SHED DURING SELECTIVE INFECTION OF LOWER INTESTINAL TRACT

Group	Preexisting neutralizing antibody (1:4 or >)	Number of men	Number from virus recovered*		4-fold or greater rise in neutralizing antibody**
			Throat swab	Anal swab	
Virus capsule	No	37	0	36	37
	Yes	15	0	7	6
Total		52	0	43	43
Placebo capsule	No	36	0	0	0
	Yes	16	0	0	0
Total		52	0	0	0

*Thirty-day period of surveillance. Twelve tests for virus done during this interval.

**Twenty-eight days following administration of capsules.

TABLE 4

SPECIFIC PROTECTIVE EFFECT OF ENTERIC TYPE 4 ADENOVIRUS VACCINE AGAINST FEBRILE RESPIRATORY TRACT DISEASE REQUIRING HOSPITALIZATION, CAMP LEJEUNE, FEBRUARY AND MARCH 1964

Group	Neutralizing antibody prior to feeding of capsule	Number in group	Number men admitted to hospital for febrile respiratory tract illness	
			Adenovirus recovered*	Adenovirus not recovered
Virus capsule	<1:4	89	0	4
	1:4 or >	36	0	2
Total		125	0	6
Placebo capsule	<1:4	90	32	7**
	1:4 or >	38	0	1
Total		128	32	8

*Adenovirus recovered before or at the latest two days after onset of illness for which recruit was hospitalized.

**Three of these recruits were hospitalized for an adenovirus positive illness at another time during the study interval.

TABLE 5

EFFECT OF NATURALLY ACQUIRED OR ENTERIC-CAPSULE-INDUCED TYPE 4 ADENOVIRUS NEUTRALIZING ANTIBODY ON TYPE 4 VIRUS INFECTION DURING AN OUTBREAK AT CAMP LEJEUNE

Group	Reciprocal of neutralizing antibody		Longitudinal Study Population							Total Study Population	
			Number	Number from whom virus recovered			Mean-duration of virus excretion, days				
	Parris Island prior to feeding of capsule	Camp Lejeune prior to start of outbreak	in group	Throat swab	Anal swab	Total no. of men	Throat swab	Anal swab	Total	CF* Antibody rise, no.	No. in group	No. with CF antibody rise
Virus capsul	<4	4 or >	34	9	4	10 (29%)	3	1	3	0	89	2
	4 or >	4 or >	15	1	0	1 (7%)	3	-	3	0	36	1
Total			49	10	4	11 (22%)	3	1	3	0	125	3 (2%)
Placebo capsule	<4	<4	36	36	36	36 (100%)	5	8	10	32 (80%)	90	82
	4 or >	4 or >	13	3	4	4 (31%)	8	4	7	2 (15%)	38	9
Total			49	39	40 (82%)	40	6	7	10	34 (67%)	128	91 (71%)

*CF indicates complement fixing.

TABLE 6

COMPARISON OF ILLNESS WHICH OCCURRED AT PARRIS ISLAND FOLLOWING ADMINISTRATION OF VIRUS AND PLACEBO CAPSULES

Type of illness surveillance	Group	Number of recruits	Number of Illnesses: Respiratory Tract Illness: Afebrile	Febrile	Pneumonia	Gastro-enteritis*	Other
Longitudinal	Virus capsule	81	2	9	0	0	Otitis media 1 Cervical adenitis 1 Herpes Zoster 1
(Recruits examined 3 times a week)	Placebo capsule	53	3	7	0	0	
Casual	Virus capsule	360	18	3	0	6	Otitis media 1 Scarcoidosis 1
(Dispensary visits)	Placebo capsule	356	30	11	3	11	Otitis media 1

*Includes diarrhea, stomach cramps, nausea and vomiting.

TABLE 7

SPECIFIC PROTECTIVE EFFECT OF ENTERIC VACCINE AGAINST TYPE 4 ADENOVIRUS

Group	Neutralizing antibody prior to feeding of capsule	Number in group	No. Men Admitted to Hospital for Febrile Respiratory Illness		No. Men with Febrile Respiratory Illness Not Requiring Hospitalization		No. Men with Afebrile Respiratory Illness	
			Type 4 adenovirus recovered	Type 4 adenovirus not recovered	Type 4 adenovirus recovered	Type 4 adenovirus not recovered	Type 4 adenovirus recovered	Type 4 adenovirus not recovered
Virus capsule	1:4	246	0	12	0	2	9	48
	1:4 or >	93	0	4	0	0	1	14
Total		339	0	16	0	2	10	62
Placebo capsule	1:4	236	42	10	7	5	16	31
	1:4 or >	101	3	3	3	6	3	13
Total		337	45	13	10	11	19	44

TABLE 8

RESPONSE OF NEUTRALIZING ANTIBODY-FREE (<1:4) RECRUITS TO DIFFERENT QUANTITIES OF TYPE 4 ADENOVIRUS ADMINISTERED BY ENTERIC CAPSULE

Quantity of type 4 virus in capsule (Log_{10} TCD_{50})	Number in group	Number of Recruits Infected as Evidenced By Virus Recovery from Rectal but Not throat swab specimens* Associated with a rise in neutralizing antibody	Not associated with a rise in neutralizing antibody	Rise in neutralizing antibody-virus not recovered	Total	Geometric mean titer of neutralizing antibody post infection† (reciprocal)
6.5	14	14	0	0	14(100%)	63
6.2-6.4	29	28	0	1	29 (100%)	42
4.2-4.7	30	24	2	2	28 (93%)	27
1.0-2.7	28	13	2	1	16 (57%)	32
<1.0	28	1	0	0	1 (3%)	(32)
Placebo	24	0	0	0	0	-

*Virus was never recovered from throat swab specimens.

† 30 days following administration of capsules.

TABLE 9

NEUTRALIZING ANTIBODY RESPONSE FOLLOWING ADMINISTRATION OF LIVE TYPE 4 ADENOVIRUS CONTAINED IN AN ENTERIC-COATED TABLET

Type tablet	Initial Neutralizing antibody titer	Number men	Number with 4-fold rise in neutralizing antibody		Geometric mean titer	
			11 days	15 days	11 days	15 days
Press coated*	<4	13	13 (100)	13 (100)	19.8	115.4
	4 or >	11	4 (36)	7 (64)	16.0	141.1
Non-press† coated	<4	20	10 (50)	14 (70)	10.6	26.2
	4 or >	4	1 (25)	1 (25)	32	>256

* "Tablet within a tablet" = press-coated by compressing about virus tablet a mixture of lactose, magnesium stearate and microcrystalline cellulose. Each tablet then coated with approximately 25 mg enteric coating material. Overall tablet size: 0.375" diameter and 0.212" high.

† Each virus tablet coated with 6 mg enteric coating material.

TABLE 10

ADENOVIRUS STUDY POPULATION ADMISSIONS TO NAVAL HOSPITAL, BEAUFORT, SOUTH CAROLINA FROM 6 FEBRUARY to 25 APRIL 1966

	Number	V	P
Abdominal pain, unknown etiology	1		1
Cellulitis of jaw due to wisdom tooth with penicillin reaction	1		1
Indirect inguinal hernia	3	2	1
Traumatic effusion, right knee	1		1
Contusion, left foot	1		1
Torn left tibia fibula ligaments	1		1
Fractures, traumatic, all types	8	7	1
Schistosomiasis	6	4	2
Varicella	3	1	2
Impetigo contagiosum	1		1
Infectious mononucleosis	2	1	1
Cellulitis, left ankle	1		1
Frontal sinusitis	1	1	
Abscess right inguinal area due to staphylococcus	1	1	
Abscess, face	1	1	
Abscess, right groin	1		1
Viral URI	1		1
Bronchitis	1	1	
Pneumonia, non-bacterial	8	5	3
Pneumonia, bacterial	1	1	
Elective bronchography	1		1
Elective tonsillectomy	1		1
Total	46	25	21

TABLE 11

SUMMARY OF PROTECTIVE EFFECT OF LIVE TYPE 4 ADENOVIRUS VACCINE

Year	Group	Neutralizing antibody titer prior to feeding of capsule	Number in group	No. Adenovirus Associated Illnesses at Camp Lejeune			Total
				requiring hospitalization	febrile	afebrile	
1964	Virus capsule	<1:4	89	0	1	8	9
		1:4 or >	36	0	0	4	4
	Placebo capsule	<1:4	90	32	10	27	69
		1:4 or >	38	0	0	5	5
1965	Virus capsule	<1:4	246	0	0	9	9
		1:4 or >	93	0	0	1	1
	Placebo capsule	<1:4	236	42	7	16	65
		1:4 or >	101	3	3	3	9
1966	Virus capsule	-	6883	4	14	*	18
	Placebo capsule	-	6972	34	39	*	73
Total	Virus capsule		7347	4	15	22	41
	Placebo capsule		7437	111	59	51	221

*No surveillance maintained for afebrile respiratory illness.

PROPHYLAXIS OF RECRUIT ACUTE RESPIRATORY DISEASE WITH LIVE ADENOVIRUS VACCINE

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Acute respiratory disease (ARD) is dispropotionately prevalent in military recruits (1-5). Although the navy recruit population represents less than 5% of the entire navy population, nearly 50% of all the ARD in the Navy is attributed to recruits (6).

The Naval Medical Research Unit No.4 (NAMRU-4) at Great Lakes, Illinois has continually collected, since 1949, illness data on the Great Lakes navy population. When one views the ARD admission rates of recruits throughout these years, certain patterns become apparent (Fig. 1). Epidemics of specific etiology can generally be recognized, for example, the streptococcal outbreak in 1955, and the Asian influenza epidemic of 1957. Generally, the typical seasonal increase in ARD is observed during the cold months. However, there are times when the highest illness rates occur in the summer and the fall. This is particularly true in 1961. The most consistent pattern of ARD that has emerged is the relationship of the prevalence of ARD to time in training (Fig. 2). This pattern has remained so constant throughout this time period that we have labeled it the "fingerprint" of ARD. Nearly 90% of the ARD in Great Lakes recruits occurs in the first half of training. The illness experience in the latter half of training is not greatly different than that of the other more seasoned personnel at Great Lakes.

Adenovirus infections have been known to be prevalent in the navy recruit population at the Naval Training Center, Great Lakes, since at least 1954 (7-12). Fifty to eighty percent of the recruits experience infection with this agent during recruit training. Most of these infections occur in the first one-half of the 9-week training period, which coincides with the peak admission rate for acute respiratory disease. Adenovirus type 4 has been, throughout the years, the type most frequently observed, while types 2, 3 and 7 have been sporadically isolated from this population (8-12, 14-16).

Vaccines containing killed adenovirus prepared in monkey kidney tissue culture have repeatedly reduced admissions for respiratory disease in recruits. However, the extent of protection afforded by these vaccines has varied (17-33). Since its first adenovirus vaccine study in December of 1955 (17) through May of 1963, NAMRU-4 has evaluated adenovirus vaccines in some 20 different studies. More than 100,000 recruits have been involved in these trials. To summarize our experience, the vaccines generally reduced acute respiratory disease admissions about 50%.

The development of the live adenovirus vaccine (34) led NAMRU-4 to new studies. A trial of the same live adenovirus vaccine used in marine recruits at Parris Island, South Carolina (35), was undertaken in the Great Lakes navy recruits from February to June of 1965. The epidemiological setting at Great Lakes afforded a different type of challenge for the vaccine. Recruits would be immunized and become enterically infected at the same time of exposure to natural adenoviral infection. This would be a common situation for the routine use of these vaccines. This environment did, indeed, provide a critical test for this vaccine. The objectives of the study were to confirm and extend the results of the Parris Island study of the live adenovirus vaccine by using it in the recruit population at Great Lakes. More specifically, these objectives were to determine the effectiveness of the live adenovirus vaccine in the prevention of recruit acute respiratory disease, to compare its effectiveness with an inactivated adenovirus vaccine, and to determine if there were any adverse reactions to the vaccine, and to determine if the vaccine virus spread to the placebo control.

PHASE II:

MATERIALS AND METHODS

The recruit population at the Naval Training Center, Great Lakes, Illinois, is made up of young adult males, primarily 17 to 20 years of age, who originate principally from the Eastern half of the United States. Recruits arrive daily in varying numbers from the various recruiting stations.

Normally about 4 days are required to administratively process, classify, examine, inoculate and outfit a recruit. More than 10% of the recruits are delayed in the processing area for various administrative or medical reasons. At the completion of the processing period, the recruits are trained in companies of 75 to 80 men for 9 weeks. All recruits not in training are housed in the same area. After processing, the training company is moved to another camp for the primary phase of training. After spending 3 weeks in this area, recruits are assigned work details and are housed and work throughout the entire recruit training complex for 1 week, including the processing area. Upon completion of this workweek, the recruit companies are again physically moved to another camp for the final 5 weeks of advanced training.

Sick call is held 3 times a day. A recruit may seek treatment at his own discretion at any of these times. If treated as an outpatient, an individual permanent record is made of each visit noting such data as symptoms, temperature and disposition. Daily official records indicate those men admitted to the sick list for inpatient care, as well as the admission diagnosis. Such illness data on each recruit in training is abstracted and transcribed to machine punched cards.

Throughout the calendar year of 1964, the recruit population at Great Lakes experienced relatively low admission rates for acute respiratory disease. All recruits received the standard polyvalent influenza vaccine prior to the commencement of training. Benzathine penicillin (1.2 million units) was given during the second week of training to all recruits not giving a history of penicillin hypersensitivity. All recruits received bivalent type 4 and 7 inactivated adenovirus vaccine during 1964 and until January 28, 1965. The adenovirus infection rates were low during the summer months. However, despite the administration of the inactivated vaccine, a definite increase in adenovirus infections, as well as a sharp increase in the ARD admission rates, was noted in recruits training from October 1964 to January 1965 (Fig. 3).

Forty-five recruit companies, approximately 3500 men, were randomly assigned to be treated as shown in Figure 4. Half of the men in each of these study companies were treated with either an adenovirus vaccine or a placebo by the appropriate route. The vaccines used were the live oral monovalent type 4, an inactivated parenteral monovalent type 4, and an inactivated parenteral bivalent type 4 and 7 vaccine. As each company contained both recruits who had received a specific vaccine or placebo, this study design allowed a comparison of the placebo control groups to detect any abnormal incidence of disease in individuals who were contact cohorts of men fed live adenovirus vaccine. The vaccines were administered from 19 February to 16 April 1965. The period of observation in this study population was from 19 February to 20 June 1965.

All men in the study companies who received the live and inactivated monovalent vaccine, and all men from 10 companies, selected at random, who received the bivalent vaccine were studied extensively. A prevaccination, 35- and 65-day postvaccination blood specimen was collected from all of these men. Throat and rectal specimens were collected for virus isolations, as well as an acute and 21-day convalescent blood specimen from all of these recruits who were admitted for inpatient care. All men from these companies, who were admitted for inpatient care, were seen by a medical officer who obtained a history, conducted a physical examination, and evaluated the illness. A 14 X 17 chest film was obtained on all of the admitted men. Further, all men in these companies were seen by a team of medical examiners, which was headed by a medical officer, before vaccination and each week thereafter for 3 weeks, and then every 2 weeks until their departure from the training center. This team recorded oral temperatures and listed disease complaints.

All vaccines were administered on or about the 4th day after arrival at Great Lakes, after all the processing procedures had been completed, to preclude giving the live adenovirus vaccine to a recruit who may be held in the processing area or returned to his home.

Double-blind technics were carefully followed so that neither the recruit, personnel responsible for training, nor medical personnel responsible for observations or treatment had any knowledge of the treatment group to which any recruit or company was assigned.

All recruits received the standard polyvalent influenza vaccine during the processing period. Further, all men not hypersensitive to penicillin received 1.2 million units of benzathine penicillin in the second week of training for the prophylaxis of streptococcal disease. Live poliovirus vaccine was withheld from all of the 45 study companies during their training.

RESULTS

Although the treatment which a company or a recruit received was assigned randomly, an analysis of certain attributes was made to determine the comparability of the relatively small numbers of subjects assigned to each treatment group. By parameters of similar age, geographic origin, swimming ability, extent of adenovirus infection in the placebo-treated men in a company, all groups were indeed comparable. This held true, likewise, for the recruit population as a whole.

The effect the vaccines had on recruit acute respiratory disease admissions is displayed in Table I. The reduction of disease associated with each vaccine when compared to its placebo, is statistically significant. None of the differences observed among vaccine groups or among placebo groups are statistically different.

A slight protective effect of the live adenovirus vaccine became apparent by the 8th day postvacciation (Fig. 5). All vaccines were definitely effective by the 11th day postvaccination.

The amount of non-respiratory disease admissions from each vaccine or placebo group was the same. The vaccine had little effect on the length of time spent on the sick list or the number of readmissions for ARD. Each vaccine did reduce the number of men requiring treatment as an outpatient for febrile respiratory disease, and, likewise, reduced the number of visits. More men from each of the vaccine groups were treated as outpatients for afebrile disease. This is a finding that has frequently been observed in adenovirus vaccine studies in this population and has been described as an ameliorative effect of the vaccine.

No difference in the number of men with symptoms or number of individual symptoms, or number of men who were febrile on each visit could be detected in any vaccine group in the barracks surveys. The number of symptoms elicited from recruits in each vaccine group in every company was remarkably similar. The number of men who were asymptomatic on every interview was small and these were distributed equally throughout all study companies and treatment groups.

In an attempt to determine the effect the vaccine had on specific adenoviral disease, the criteria of infection were isolation of adenovirus type 4

from the throat and a 4-fold or greater complement-fixing antibody rise. The results of this analysis are displayed in Table II, utilizing both the conventional specific reduction and the intrinsic vaccine efficacy method of Stille (36). While this analysis does tend to show an increased efficacy of each vaccine, the total reduction does not approach that which was previously observed in marine recruits, receiving the live vaccine, at Camp Lejeune, North Carolina (35). This result was certainly not surprising as the epidemiological conditions of the two trials were remarkably different. Recall, that the navy recruits were vaccinated 4 days after arrival at Great Lakes, while the marine recruits had more than 3 weeks to develop protective antibodies before being challenged with naturally occurring adenovirus disease. Two weeks after vaccination of the navy recruit, one-half of the respiratory disease admissions had already occurred. It would appear that these naval recruits were being challenged to adenovirus infection at the time of, or shortly after, vaccination. One explanation of the lesser effect of these vaccines would be the early natural challenge before the development of protective antibody. Another plausible explanation of the diminished effectiveness shown in this trial may lie in the multiple etiology of respiratory disease in this population. If the vaccine prevents all of its homologous illness, and this vaccine is capable of this (35), then any remaining illness must be of another etiology. There was laboratory evidence of multiple infections during the time of this study.

Prompted by the results of the 1965 study, the 1966 live adenovirus vaccine study was designed to test the efficacy of the live adenovirus vaccine to interrupt and contain a high rate of acute respiratory disease in the Great Lakes naval recruit population. The design called for the immunization en mass, on one day, of all recruits who had not completed the primary phase of training --- the first 3 weeks, and the immunization of all subsequently arriving recruits with 24 hours after arrival.

The placebo control population in this study represented only 10% of the entire population. The placebo was distributed in one-half of 20% of the companies. The other half of these companies received either the live vaccine or an inactivated bivalent adenovirus 4 and 7 preparation. In other words, all recruits in 8 of 10 companies received the live vaccine. Fifty percent of the men in one of 10 companies received the live vaccine, and the remaining men an oral placebo. Fifty percent of the men in one of 10 companies received the inactivated bivalent 4 and 7 vaccine, and the remaining men a saline placebo.

The mass immunization phase of this study was accomplished on 14 February 1966. Because complete cooperation was given by all commands concerned, 98.6% of more than 5800 men designated to be immunized on this day were, indeed, vaccinated. The majority of those omitted were contacted and vaccinated on the following day. Table III shows the number of companies and men involved in the entire study. Input into the study was terminated on 3 June 1966.

The illness rates during the Fall of 1965 were moderate (Fig. 6). However, during January 1966 these rates fell to an exceptionally low level. Three weeks before the mass immunization was undertaken, the ARD rates again increased. On the week ending 12 February, the rates were approximately 30 per 1,000 per week. Based on the previous vaccine study, it was anticipated that the mass immunization would markedly reduce ARD admissions. However, such a swift and dramatic effect was not expected. The total ARD rates halved the first week following treatment and halved again the second. A day-to-day plot of illness admissions indicated that this sharp reduction in illness occurred as early as 4 days following vaccination.

In an attempt to determine if this reduction of acute respiratory disease was due primarily to the vaccine or the result of a fortuitous reduction in ARD for some other reason, the following analyses were made. First, as shown in Figure 7, was an analysis of the illness experience of the cohorts of companies in various weeks of training when immunized en mass on 14 February. The top curve represents the weekly ARD admissions of that cohort of companies that was in the 4th week of training on 14 February, when the mass immunizations were given. This group of companies did not receive the benefit of vaccination. The next lower curve is the acute respiratory disease experience of those companies who were vaccinated in the 3rd week of training. The broken line represents the illness that occurred subsequent to vaccination. From this analysis, it is quite obvious that at the time of the mass immunization, a marked build-up of acute respiratory disease admissions was occurring in this population as evidenced by the step-wise increase in the admission rates in the early weeks of training. In each week's cohorts of vaccinated companies, there was the same marked reduction in illness 1 week following vaccination. It would appear from these data that the illness reduction following mass immunization was truly the effect of vaccination.

Further substantiation that the reduction of acute respiratory disease following vaccination was not a chance fluctuation of disease rates is shown in Figure 8. The light lines represent one standard deviation on each side of the mean illness rate for each calendar week for the years of 1957 to 1965. The heavy line is 1966 illness rate. Note the week following the mass immunization, the illness rates dropped below one standard deviation from the mean rate and remained below for 15 of the 16 subsequent weeks. Several points on this curve set an all-time low for ARD for that particular calendar week.

The total respiratory disease admissions from each of the treatment groups that were admitted are shown in Table IV. The lowest rate seen was in the live vaccine recipients in the solidly immunized companies. The difference between the two live vaccine groups is statistically significant. The inactivated vaccine in this instance did not appear to be highly effective. It would be tempting to speculate, however, that the differences between the two live vaccine groups was due to a herd immunity response.

The preliminary data of this year's vaccine study indicates that the

mass immunization employing the live adenovirus vaccine was effective in interrupting and containing an epidemic of acute respiratory disease in a naval recruit population. Further, the vaccine effect was apparent less than 1 week following vaccination, even when administered at the time of naturally occurring adenoviral disease. The vaccine was easily administered and there were no obvious adverse effects. Thus, the vaccine appears to have great promise in its role of a prophylactic agent of recruit acute respiratory disease.

TABLE 1

ACUTE RESPIRATORY DISEASE ADMISSIONS IN VARIOUS ADENOVIRUS VACCINE AND PLACEBO TREATED RECRUITS, NTC, GREAT LAKES, ILL., 1965

	Live	Placebo	Monovalent	Placebo	Bivalent	Placebo
Population	386	386	375	391	963	986
No. ARD admissions	75	139	61	134	160	332
Rate/1000	194	360	163	343	166	337
% Relative reduction	46		53		51	

TABLE II

ESTIMATE OF ADENOVIRUS VACCINE EFFECTIVENESS
UTILIZING "SPECIFIC" ILLNESS REDUCTION AND IVE

	Live Monovalent 4 vs. Placebo	Inactivated Monovalent 4 vs. Placebo	Inactivated Bivalent 4, 7 vs. Placebo
"Specific Reduction"	69	83	72
IVE	75	78	74

Extent of infection based on virus recovery from throat specimens and 4-fold or greater complement fixation response.

TABLE III

POPULATION LIVE ADENOVIRUS VACCINE STUDY,
NTC, GREAT LAKES, ILL., 1966

Date of vaccination		100% live vaccinated companies live	50% live vaccinated 50% placebo vaccinated live	placebo	50% inactivated vaccinated 50% placebo vaccinated inactivated	placebo
2/14/66	No. comp.	50	5		5	
	No. men	4936	225	224	223	250
2/15/66 to 6/3/66	No. comp.	199	22		22	
	No. men	16,761	1,093	1,085	1,099	1,070
Total study	No. comp.	249	27		27	
	No. men	21,697	1,318	1,309	1,322	1,320

TABLE IV

ACUTE RESPIRATORY DISEASE ADMISSIONS IN VARIOUS ADENOVIRUS VACCINE AND PLACEBO TREATED RECRUITS, NTC, GREAT LAKES, ILL., 1966

	Live (100% Co's)	Live (50% Co's)	Oral placebo (50% Co's)	Inactivated bivalent (50% Co's)	Saline placebo (50% Co's)
Population	21,697	1,318	1,309	1,322	1,320
No. ARD adm.	1,300	103	160	123	153
Rate/1000	59.9	78.1	122.2	93.0	115.9
% relative reduction	To combined placebo				
	50	36		20	

FIGURE 1

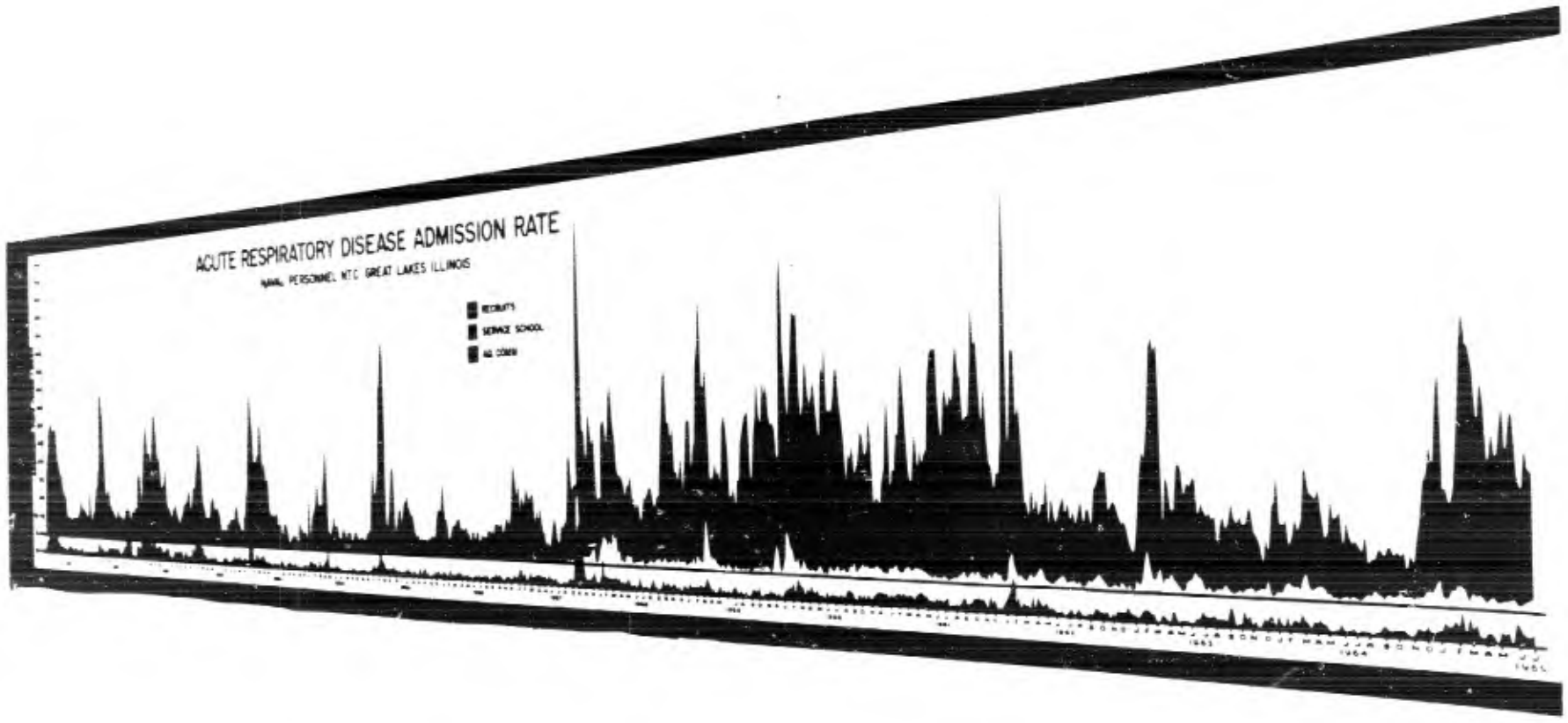

ACUTE RESPIRATORY DISEASE ADMISSION RATE
NAVAL PERSONNEL NTC GREAT LAKES ILLINOIS
RECRUITS
SERVICE SCHOOL

FIGURE 2.

THE FINGERPRINT OF RECRUIT ACUTE RESPIRATORY DISEASE

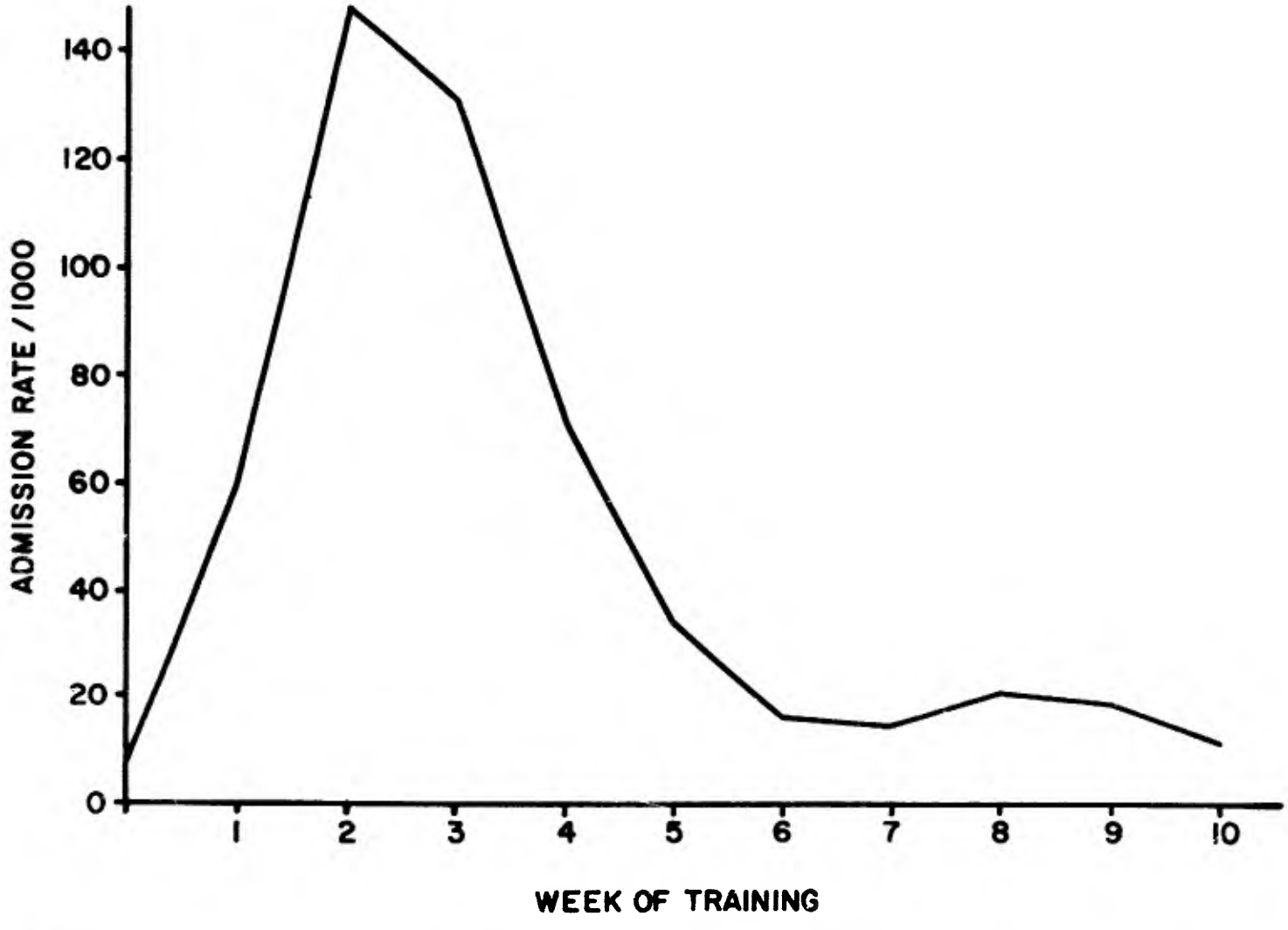


FIGURE 3

ACUTE RESPIRATORY DISEASE ADMISSION RATE/1000/WEEK
NAVAL RECRUITS, GREAT LAKES, ILLINOIS, 1964-65

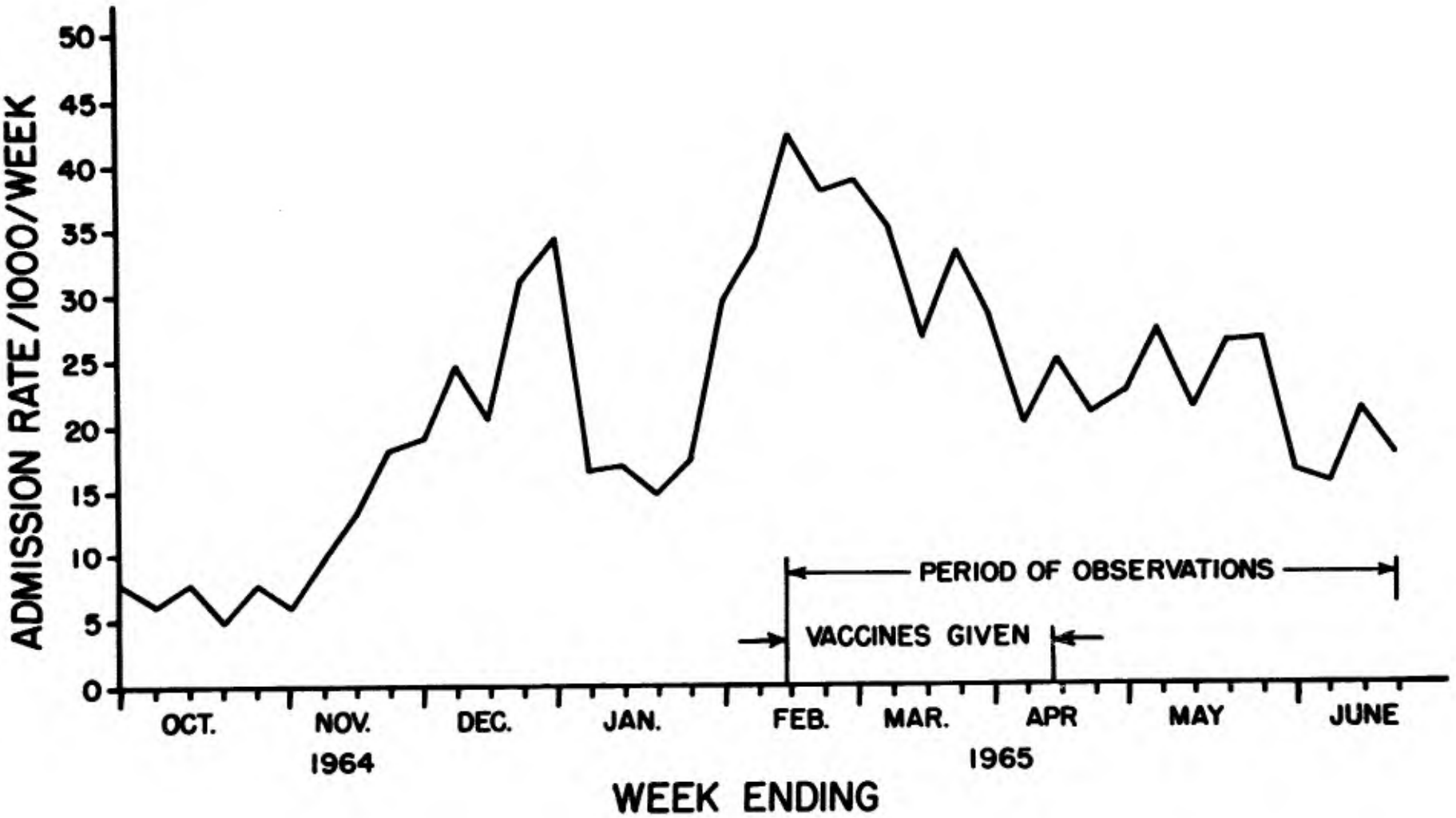


FIGURE 4

STUDY POPULATION LIVE ADENOVIRUS VACCINE NAVAL RECRUITS, GREAT LAKES, ILLINOIS, 1965

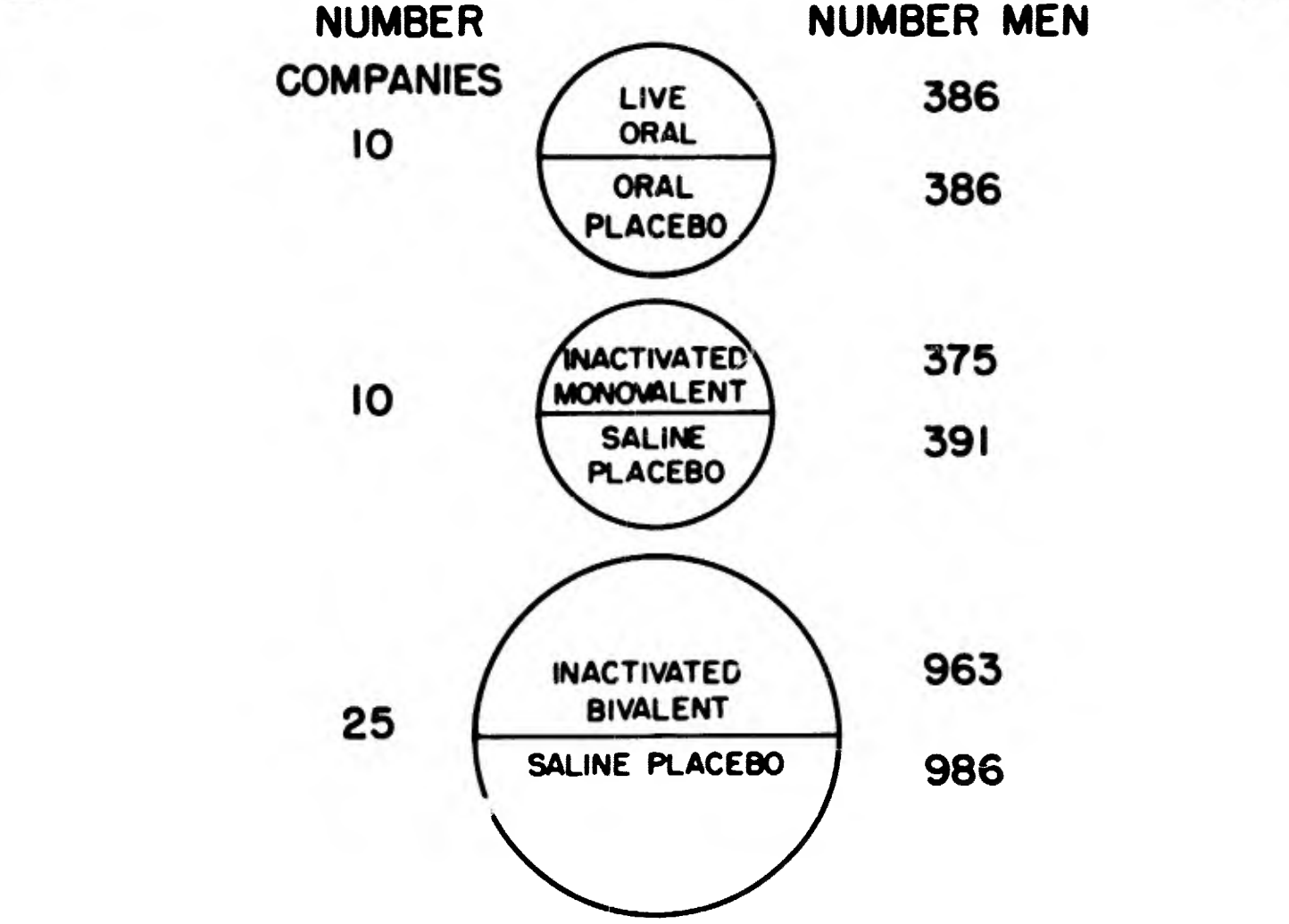


FIGURE 5

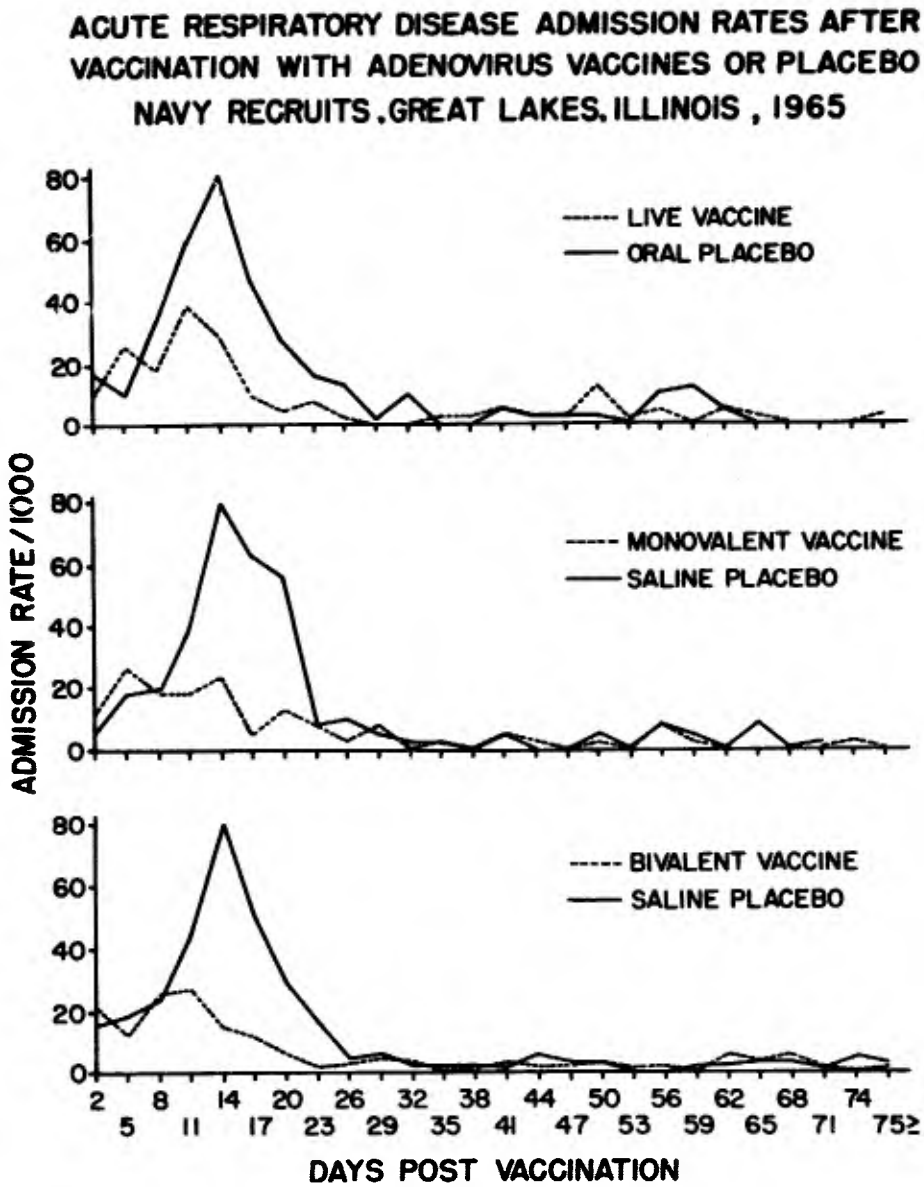

ACUTE RESPIRATORY DISEASE ADMISSION RATES AFTER VACCINATION WITH ADENOVIRUS VACCINES OR PLACEBO
NAVY RECRUITS, GREAT LAKES, ILLINOIS, 1965
ADMISSION RATE/1000
LIVE VACCINE
ORAL PLACEBO
MONOVALENT VACCINE
SALINE PLACEBO
BIVALENT VACCINE
SALINE PLACEBO
0
20
40
60
80
2 5 8 11 14 17 20 23 26 29 32 35 38 41 44 47 50 53 56 59 62 65 68 71 74 75
DAYS POST VACCINATION

FIGURE 6

ACUTE RESPIRATORY DISEASE ADMISSION RATE/1000/WEEK NAVAL RECRUITS. GREAT LAKES, ILLINOIS, 1965-66

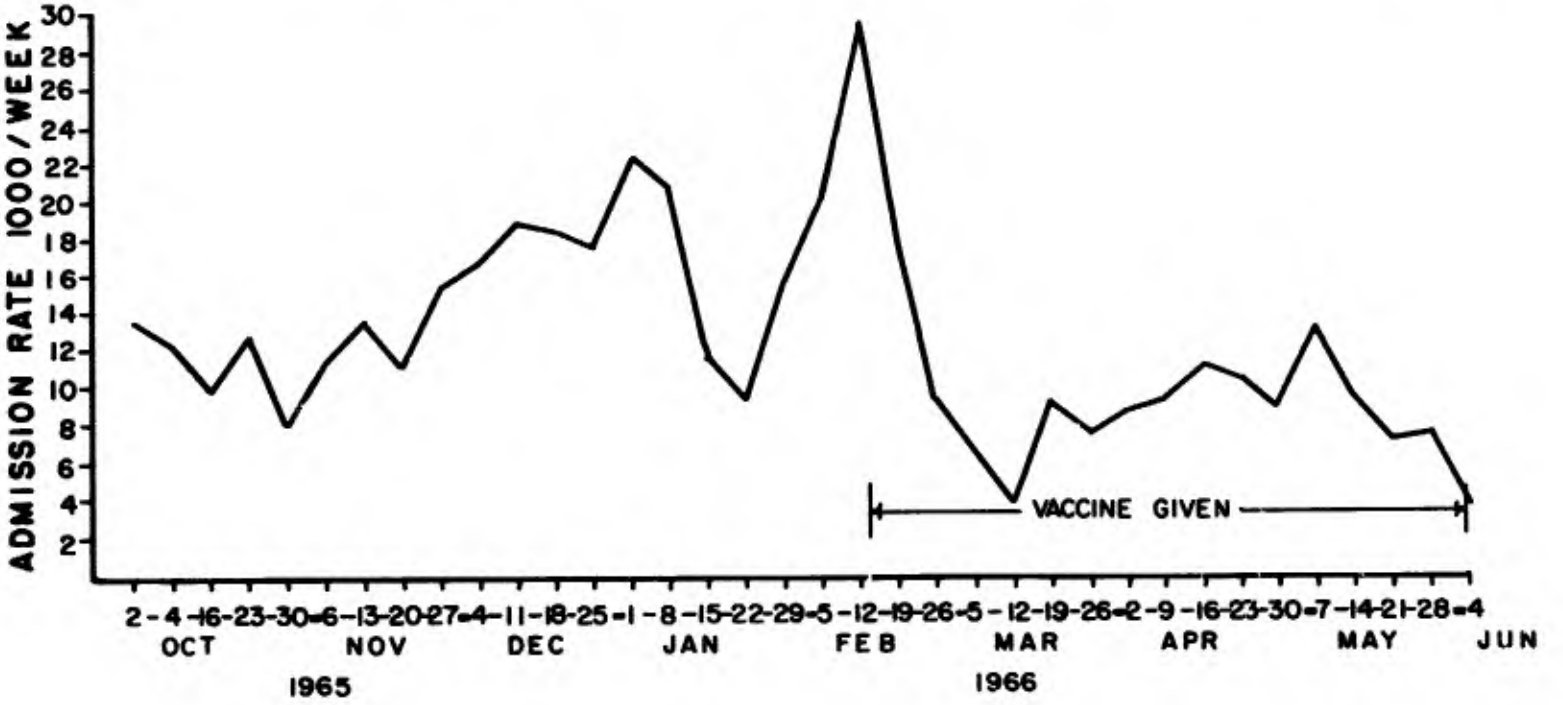


FIGURE 7

ARD ADMISSION RATES, NAVY RECRUITS, BEFORE AND AFTER RECEIVING LIVE ADENOVIRUS VACCINE. GREAT LAKES, ILL. 1966

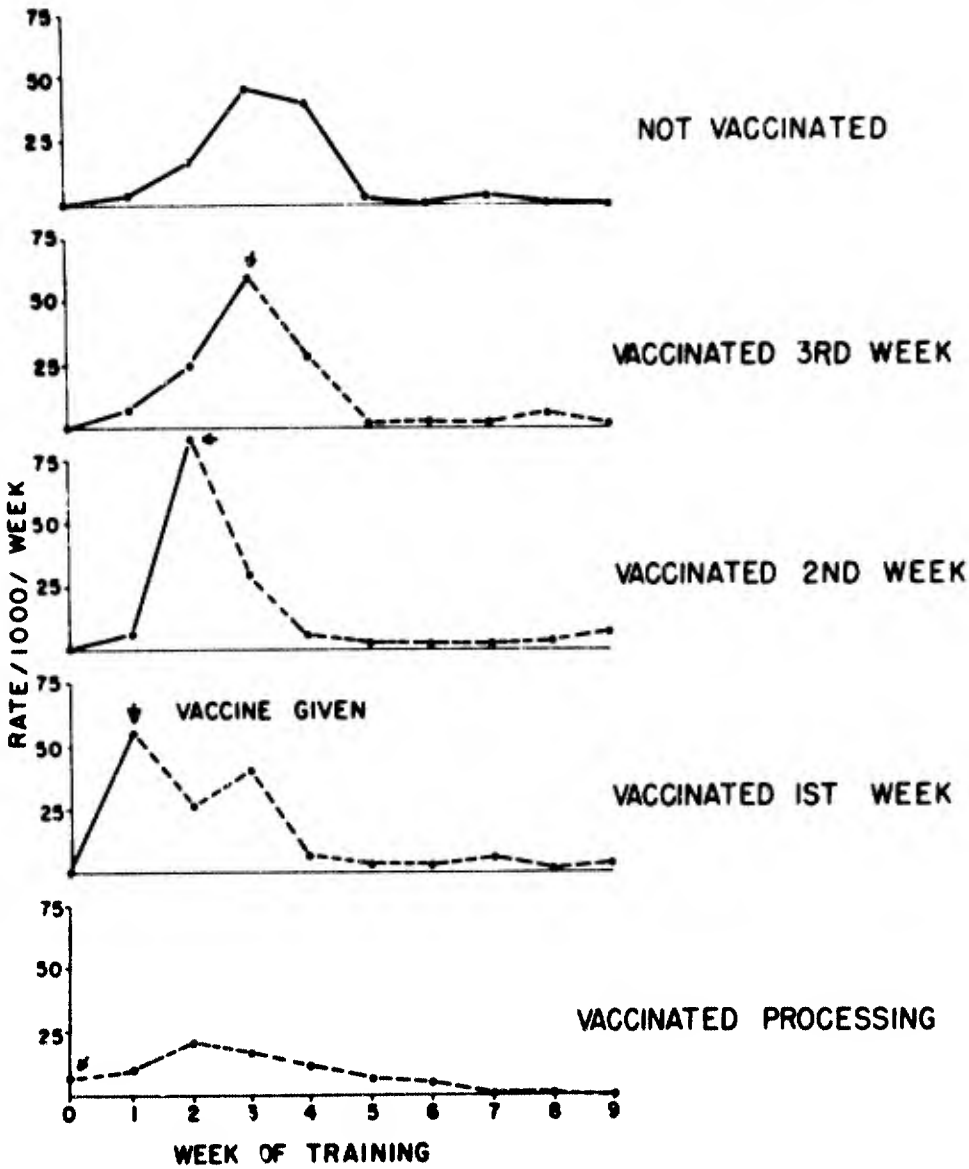
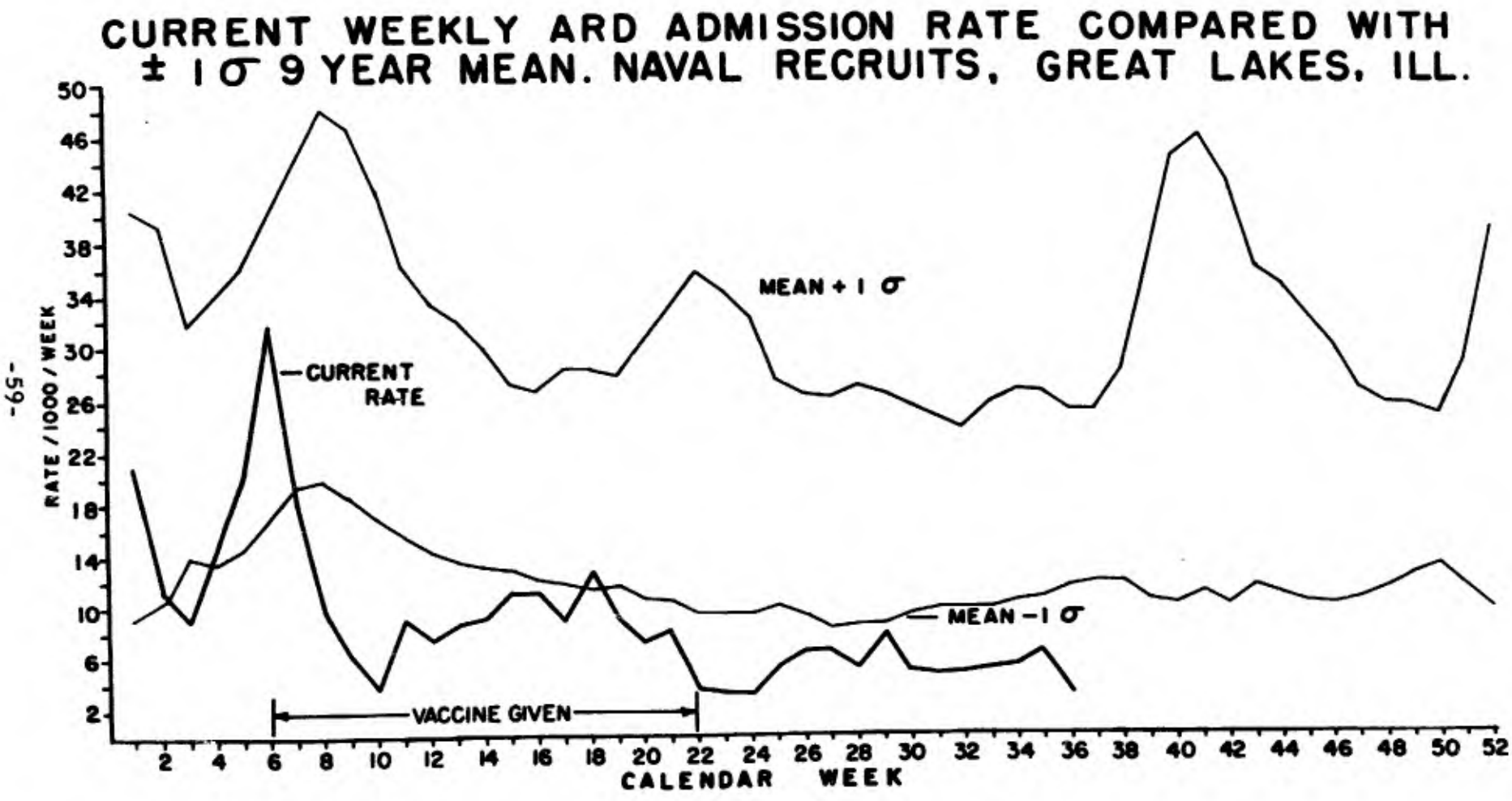


FIGURE 8


CURRENT WEEKLY ARD ADMISSION RATE COMPARED WITH ± 1σ 9 YEAR MEAN. NAVAL RECRUITS, GREAT LAKES, ILL.
RATE / 1000 / WEEK
50
46
42
38
34
30
26
22
18
14
10
6
2
MEAN + 1 σ
CURRENT RATE
MEAN - 1 σ
VACCINE GIVEN
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52
CALENDAR WEEK

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KINETICS OF ADENOVIRUS INFECTIONS IN MILITARY RECRUITS IN CONNECTION WITH ADENOVIRUS VACCINATION

Rosenbaum, M.J., DeBerry, P., Sullivan, E., Olszak, H., Frazier, W.E., Mueller, R., Howard, D.,

and

Peckinpaugh, R.O.

The recruit training center at Great Lakes, Illinois, has long been known as a "hotbed" of acute respiratory disease. A large share of this disease has been attributed to adenovirus infections in well documented studies carried on over the past 10 years (1-8).

The successful development and use of live oral adenovirus vaccine reported by Chanock et al (9) and its effective use in marines at Parris Island and Camp Lejeune (10) encouraged the testing of such vaccines here at Great Lakes.

Several studies were designed and are described in Table I.

PHASE I: Studies on Live Adenovirus Vaccine, NTC, Great Lakes, Illinois, February 1965.

MATERIALS AND METHODS

Phase I was a small study to determine virus excretion patterns and possible markers to differentiate vaccine from "wild" adenoviruses and to study the antibody response to oral live and parenteral inactivated adenovirus vaccines. The design of Phase I and number of subjects involved are shown in Table II. A company of naval recruits (83 men) commencing training was selected and randomly divided into three vaccine and one placebo groups. Men in the live adenovirus vaccine (LAV) group received an enteric-coated capsule containing approximately 10^6 tissue culture (HEK) doses of lyophilized adenovirus type 4 prepared by Wyeth Laboratories (9). Placebo (PLAC) controls received a similar, but inert capsule. The remaining subjects received either the standard inactivated parenteral bivalent adenovirus preparation containing adenovirus types 4 and 7 (BAV), or a special monovalent adenovirus type 4 vaccine (MAV) obtained from Dr. Anthony Morris of the Division of Biological Standards, National Institutes of Health, Bethesda, Maryland.

Throat and rectal specimens for virus isolation were obtained from all

subjects at the indicated times, and bleedings for serum were made at weekly intervals for the determination of adenovirus antibody titers. Virus isolates were identified by microplate neutralization tests (11).

The results of the serological studies will be described elsewhere (12).

DISCUSSION OF RESULTS

As yet, no definite virus markers to separate "wild" from vaccine adenoviruses have been recognized. However, some interesting data were obtained regarding adenovirus excretion patterns and protection. The cumulative curves depicting the percent of men who became infected as indicated by isolation of adenovirus, rectally is shown in Figure 1. The first curve was derived from data on subjects in the LAV group. The exponential character of this curve is indicative of simultaneous infection with vaccine virus. The slight tilt of slope is thought to be due to variation in onset of individual excretion of virus and the presence of mitigating, pre-existing adenovirus antibody. Fifty percent of these subjects were infected within 6 days after administration of vaccine virus. Shedding of vaccine virus was virtually terminated by the 21st day.

The second curve represents adenovirus infection in subjects who received placebo. It can be seen that the slope of this curve is similar to the men simultaneously infected with vaccine virus and indicates the explosive spread of adenovirus in this group. The fact that onset of adenovirus infection (circa day 9) in placebo subjects occurs when men in the LAV groups are heavily shedding vaccine virus may be coincidental, but may indicate spread of vaccine virus to the contact cohorts. This point will remain moot until markers to discriminate wild from vaccine virus have been obtained.

The remaining curves showing infections in subjects treated with killed vaccines are similar in onset to the placebo, but differ in rate and total percent of men being infected with adenovirus (25% less). These differences are attributed to inhibition of virus by antibody stimulated by parenteral vaccination. This protective effect is apparent 2 weeks after immunization.

The LAV was equally protective against throat adenovirus infection and the evidence is shown in Figure 2. It can be seen that all vaccine groups differed significantly from the placebo in percent of men infected.

Protection is apparent beyond the 19th day, with the bivalent parenteral vaccine protective effect commencing somewhat earlier.

SUMMARY AND CONCLUSIONS

Fifty percent of men fed LAV excreted adenovirus rectally by day 6. A

similar excretion rate for placebo subjects was observed, although the 50 percentile was not attained until day 13.

Although these studies at Great Lakes have thus far failed to uncover any reliable markers to distinguish vaccine virus from wild, and, thus could not unequivocally indicate whether vaccine virus had spread to contacts, they showed that all vaccines (oral and parenteral) were effective in reducing adenovirus infections by 25-50% as compared to placebo. It is conceivable that had the vaccines been given earlier, or had natural infections occured later, this reduction would have been even greater.

PHASE III: Studies on Live Adenovirus Vaccine, Marine Corps Recruit Depot and Naval Training Center, San Diego, California, November 1965.

Because endemic adenovirus infections at Great Lakes precluded a definitive study on vaccine virus spread, other military areas with less adenovirus disease were considered. The marine and naval recruit training centers at San Diego, California appeared to be a logical choice. It was felt, that there the following objectives could be attained: 1) Viral spread study in two different military populations situated in the same geographical location, and climatic and ecological environment, yet differing in training procedures and in illness patterns (Fig. 3); 2) Confirmation of the virus shedding patterns and protection data previously obtained in the Phase I Great Lakes study.

The design of the San Diego study (known as Phase III) is shown in Table III. Four groups of military recruits were selected --- 2 marine platoons and 2 navy companies were selected. Each group was randomly divided into live adenovirus vaccine (LAV) or parenteral inactivated components (IAV) with their respective controls, either oral placebo (PLAC) or injectible saline (SAL). Laboratory specimens were collected on the days shown. Sampling and laboratory procedures were similar to the Great Lakes, Phase I, program except viral specimens were collected only until the 27th day after immunization and medical interviews were made randomly with subjects in each group (25%) weekly for 4 weeks. The LAV enteric-coated capsules were similar to those used in the Great Lakes study, but only a single inactivated parenteral trivalent adenovirus vaccine, which was different in make-up and origin from that used in Phase I, was employed.

DISCUSSION OF RESULTS

Unexpectedly, the LAV marine platoon and its placebo cohorts were found to be seeded with adenovirus infections prior to administration of vaccine virus (4 men excreted adenovirus type 4 on day zero), but the remainder of the marine and navy study groups stayed relatively free of such infections for the duration of the study period. The rate of vaccine virus rectal excretion, shown in Figure 4, was quite similar to the value obtained in the Phase I study, however, the placebo contacts in this company did not

exhibit linear infection rates until beyond the 21st day after the study was initiated. This is at considerable variance with the Great Lakes study, and would have occurred beyond the time when maximal vaccine virus was shed. These data appear to refute the hypothesis that vaccine virus spreads to a significant extent.

Further proof against such spread is obtained in Figure 5. Here shown are cumulative infection patterns of Navy recruits. It can be seen again that the men given the LAV exhibit the same rate of adenovirus excretion as previously shown in the marines, and in Phase I, Great Lakes study. However, here there is only little involvement of the placebo cohorts, and only beyond the 24th day. Also, neither the IAV vaccine groups nor their saline placebo controls show any evidence of extensive "wild" adenoviral challenge.

The slight protective effect of the LAV as measured by throat virus infections is shown in Figure 6.

Although the amount of challenge was minimal during the observation period, less LAV subjects (approximately 12%) were infected than placebos.

Figure 7 shows, even more striking, the LAV protection in the marine studies. It can be seen that the LAV platoon was already accumulating adenovirus infections at the initiation of the study. Despite this seeding, the protection afforded by the LAV is evident at day 17 and is dramatic at day 21. Extrapolation of the curves beyond the 27th day would indicate an even more favorable protective effect.

SUMMARY AND CONCLUSIONS

It is apparent from the San Diego studies (Phase III) that spread of vaccine virus to susceptible cohorts does not readily occur, but even more important, both the Great Lakes and San Diego study show that LAV is capable of affording effective protection even when given amidst a considerable outbreak of wild adenoviral infections. The data also suggest, that best protective effect is attained when inactivated or live vaccines were administered at least two weeks prior to an adenovirus outbreak.

TABLE I

NAMRU-4
STUDIES ON LIVE ADENOVIRUS VACCINES IN MILITARY RECRUITS

	Study	Vaccines	Doses	Study population	Objective
I	Great Lakes Feb. 1965	Oral live	20	84 Navy recruits	Antigenicity Spread
		Parenteral killed			
		monovalent	21		
		bivalent	22		
		Placebo	21		
II	Great Lakes Feb.-June 1965	Oral live	386	3487 Navy recruits	Protection Acceptability
		Parenteral killed			
		monovalent	375		
		bivalent	963		
		Placebo	1763		
III	San Diego Nov.-Dec. 1965	Oral live	81	194 (live 45) Navy recruits	Spread
		Parenteral killed			
		trivalent	80		
		Placebo	168	155 (live 36) Marine recruits	
IV	Great Lakes Feb.-June 1966	Oral live	23015	26,966 Navy recruits	Protection
		Parenteral killed			
		bivalent	1322		
		Placebo	2629		

Total doses of live adenovirus vaccine administered = 23,502

TABLE II

STUDY DESIGN FOR USE OF ADENOVIRUS VACCINE IN A NAVAL RECRUIT COMPANY, NTC, GREAT LAKES, ILLINOIS, 1965

Vaccine group	Subjects	Day of specimen collection		
		Throat	Rectal	Serum
Live adenovirus vaccine (LAV-type 4)	20	0	0	0
		3	3	
		5	5	
		7	7	7
Oral placebo (PLAC)	21	9	9	
		13	13	
		14	14	14
		16	16	
Inactivated bivalent vaccine (BAV-types 4 & 7)	21	19	19	
		21	21	21
		28	28	28
Inactivated adenovirus vaccine (MAV-type 4)	21	40	40	40
		49	40	
		57	57	
		62	62	62

TABLE III

STUDY DESIGN FOR LIVE ADENOVIRUS VACCINE IN MARINE AND NAVY RECRUITS, MCRD AND NTC, SAN DIEGO, CALIF., 1965

Vaccine group	Subjects		Day of specimen collection			Medical interviews (25% sample) Week
	Marine	Navy	Throat	Rectal	Serum	
Live adenovirus vaccine (LAV-type 4)	36	45	0	0	0	
			2	2	7	
			4	4	14	1
Oral placebo (PLAC)	39	42	6	6	21	2
			8	8	28	3
			10	10	35	4
Inactivated trivalent vaccine (IAV-types 3,4 &7)	36	44	13	13	42	
			15	15		
			17	17		
Saline placebo (SAL)	44	43	20	20		
			24	24		
			27	27		

FIGURE 1

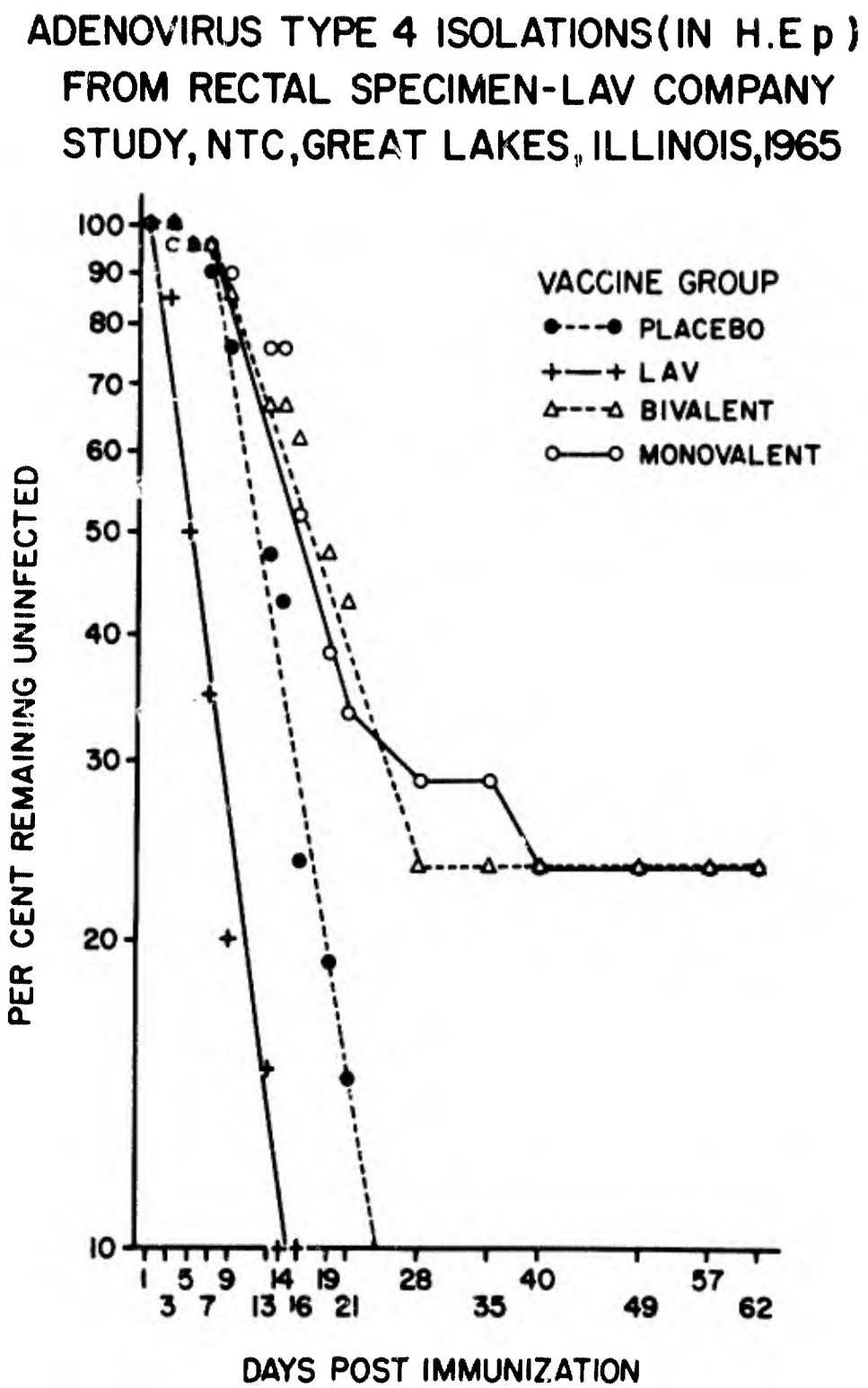

ADENOVIRUS TYPE 4 ISOLATIONS(IN H.E p)
FROM RECTAL SPECIMEN-LAV COMPANY
STUDY, NTC, GREAT LAKES, ILLINOIS, 1965
VACCINE GROUP
PLACEBO
LAV
BIVALENT
MONOVALENT
PER CENT REMAINING UNINFECTED
100
90
80
70
60
50
40
30
20
10
1 3 5 7 9 13 14 16 19 21 28 35 40 49 57 62
DAYS POST IMMUNIZATION

FIGURE 2

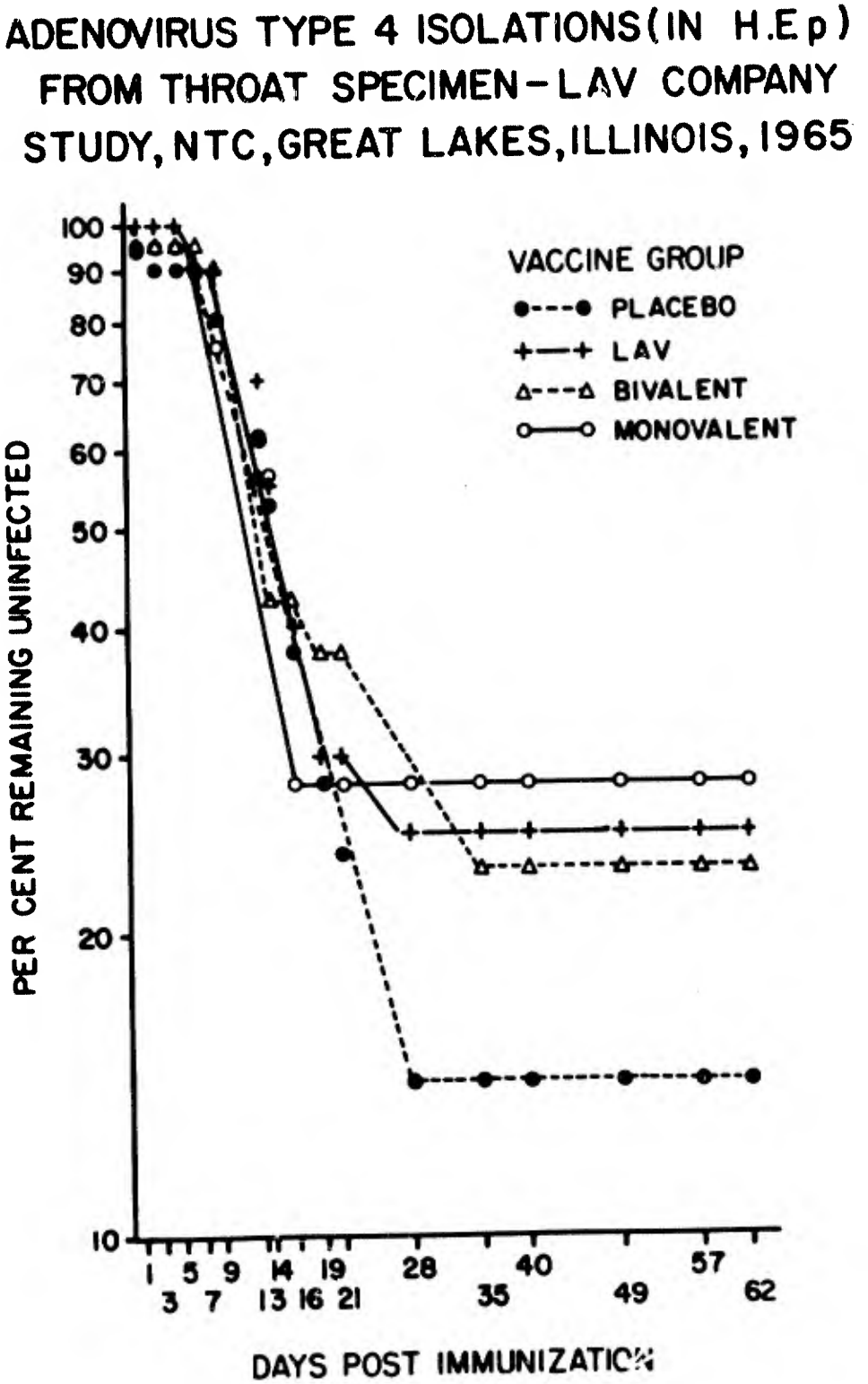

ADENOVIRUS TYPE 4 ISOLATIONS(IN H.Ep)
FROM THROAT SPECIMEN-LAV COMPANY
STUDY, NTC, GREAT LAKES, ILLINOIS, 1965
VACCINE GROUP
PLACEBO
LAV
BIVALENT
MONOVALENT
PER CENT REMAINING UNINFECTED
100
90
80
70
60
50
40
30
20
10
1
3
5
7
9
13
14
16
19
21
28
35
40
49
57
62
DAYS POST IMMUNIZATION

FIGURE 3

RESPIRATORY DISEASE ADMISSIONS, NAVY AND MARINE RECRUITS SAN DIEGO, CALIFORNIA, 1963

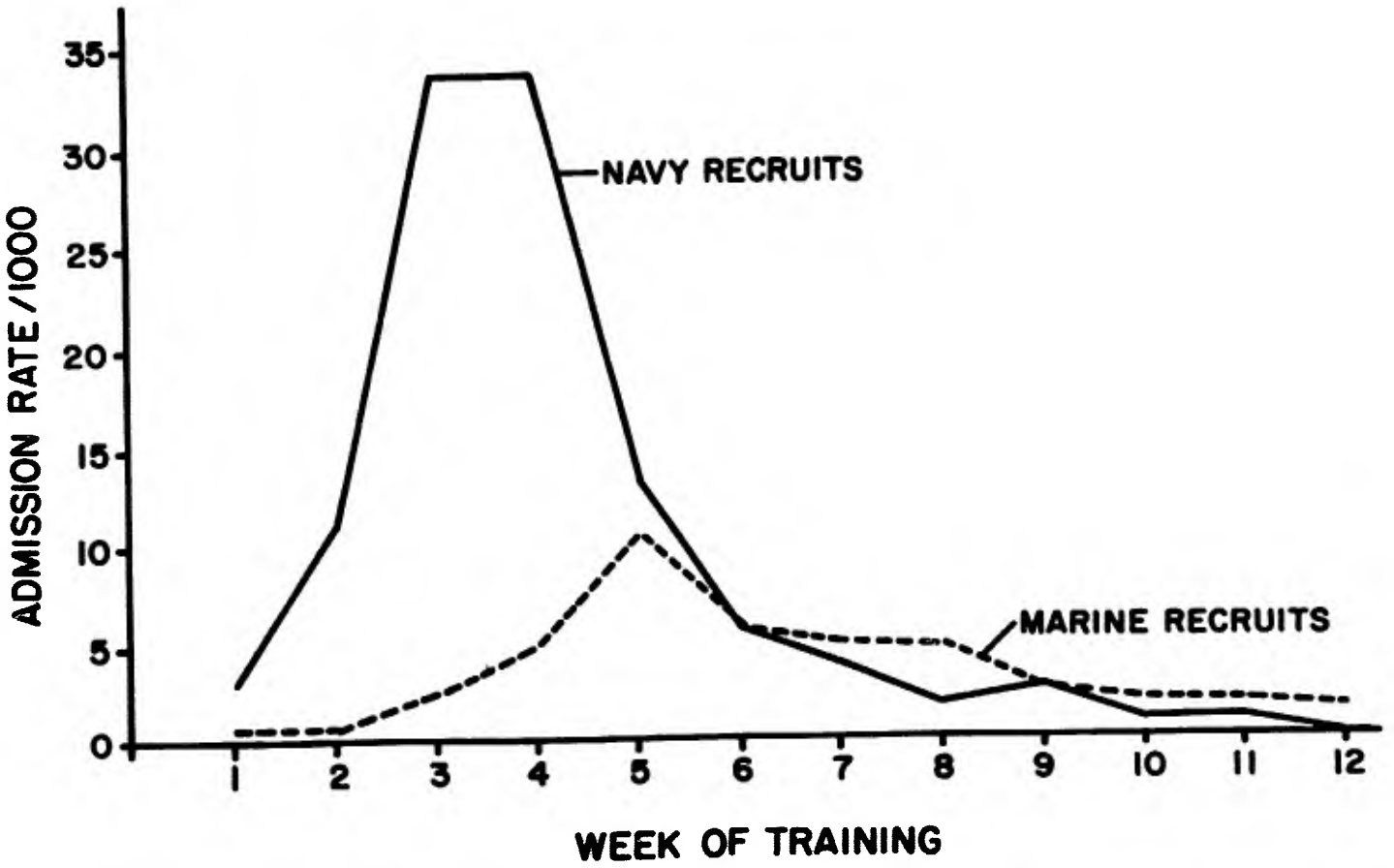


FIGURE 4

CUMULATIVE PER CENT MEN EXCRETING ADENOVIRUS TYPE 4 IN RECTAL SPECIMENS FROM VACCINATED MARINE RECRUITS MCRD, SAN DIEGO, CALIFORNIA, 1965

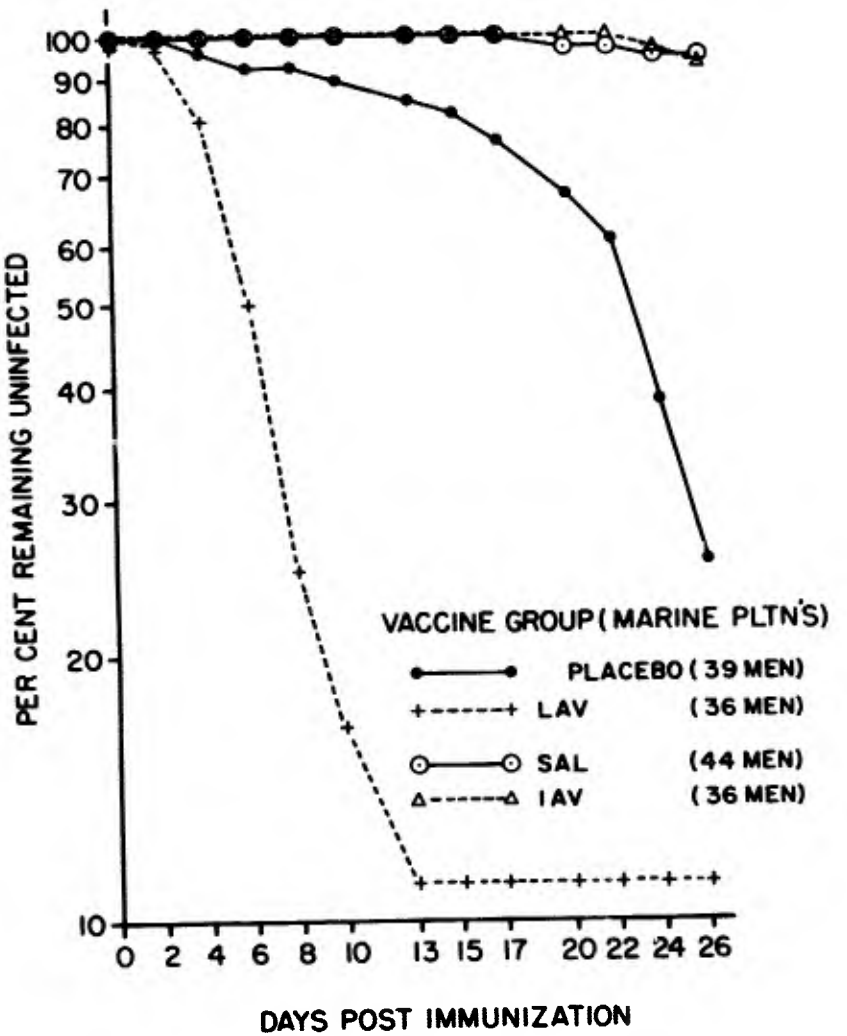


FIGURE 5

CUMULATIVE PER CENT MEN EXCRETING ADENOVIRUS TYPE 4 IN RECTAL SPECIMENS FROM VACCINATED NAVY RECRUITS NTC, SAN DIEGO, CALIFORNIA, 1965

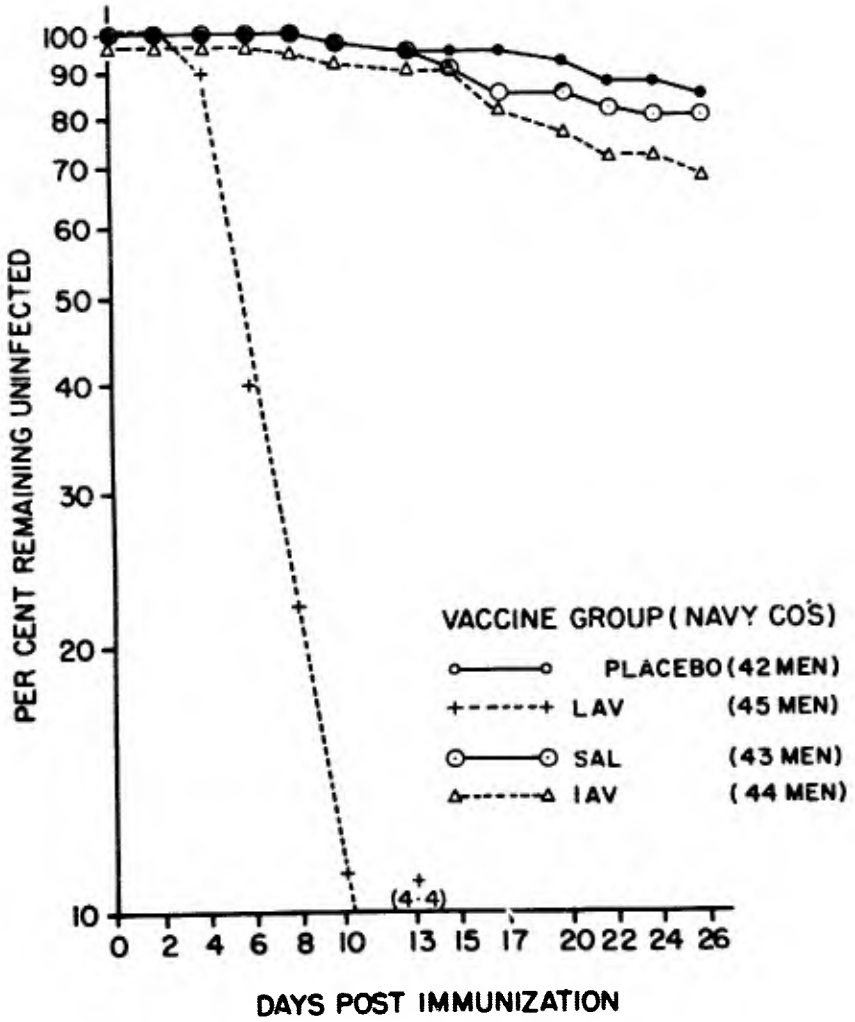


FIGURE 6

CUMULATIVE PER CENT MEN EXCRETING ADENOVIRUS TYPE 4 IN THROAT SPECIMENS FROM VACCINATED NAVY RECRUITS NTC, SAN DIEGO, CALIFORNIA, 1965

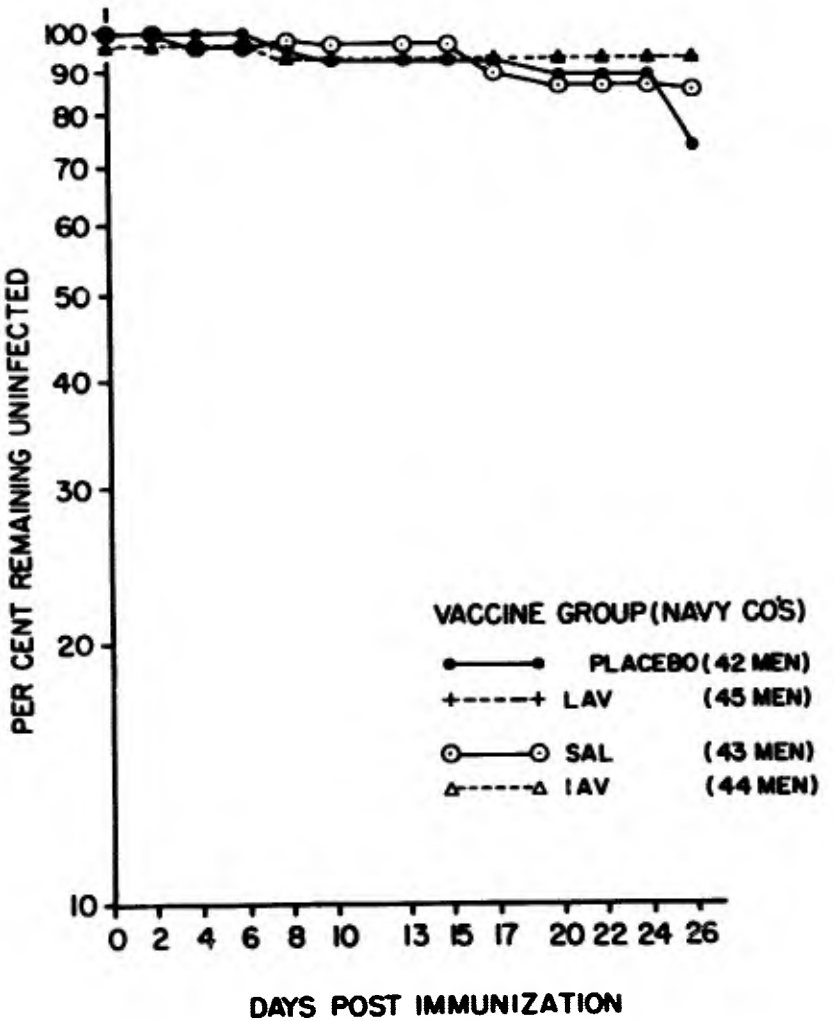
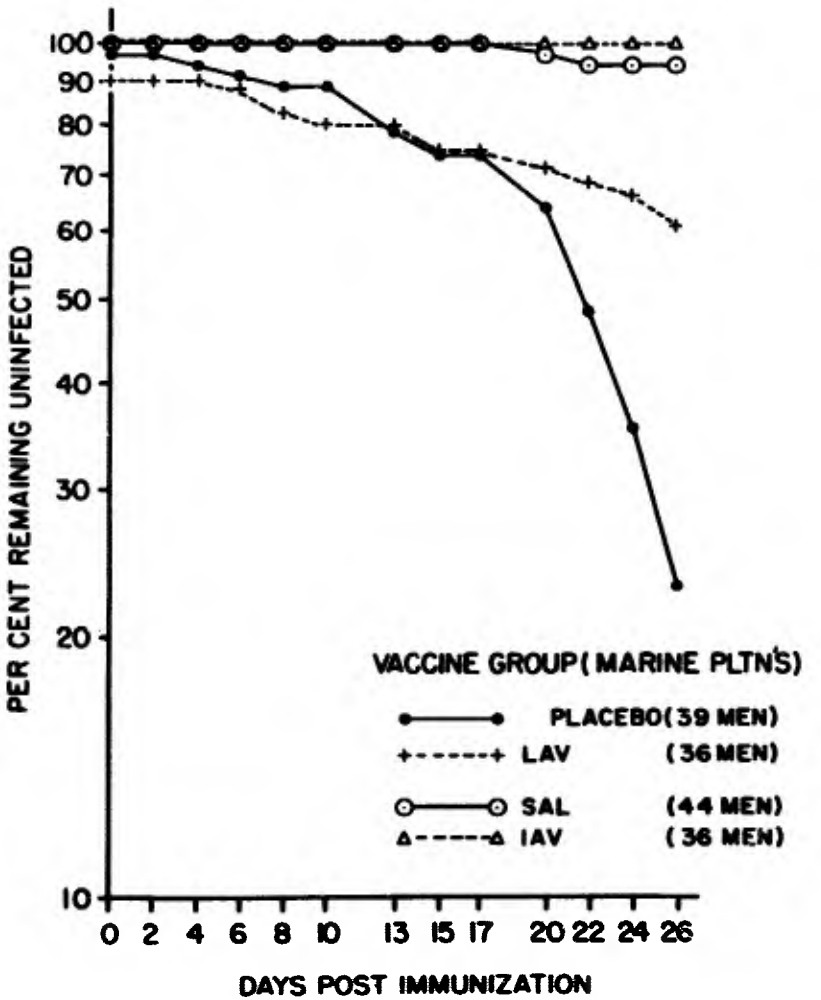


FIGURE 7

CUMULATIVE PER CENT MEN EXCRETING ADENOVIRUS TYPE 4 IN THROAT SPECIMENS FROM VACCINATED MARINE RECRUITS MCRD, SAN DIEGO, CALIFORNIA, 1965



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SEROLOGICAL RESPONSE FOLLOWING ADENOVIRUS VACCINATION

Edwards, E.A., Rosenbaum, M.J., Sullivan, E., Pierce, W.E.,

Frazier, W., Howard, D., Mueller, R.,

and

Peckinpaugh, R.O.

One of the problems in evaluating the efficacy of a vaccine is the lack of a satisfactory method of assay. The only true measure of acquired resistance is demonstration of protection against disease. In respiratory disease, this measure is complicated by a diversity of clinical manifestations, some of which could be due to latent agents temporarily removed from the initial infection (1).

In using the serologic response to determine the efficacy of a vaccine, no two people respond to the same degree to an immunizing dose and more importantly, most of the tests for antibody level measure only the secondary effects of the primary antigen-antibody interaction (2). What is actually determined is the capacity of an antiserum to produce a secondary effect such as fixing complement, inhibiting hemagglutination in some way, neutralizing the cytopathic effect of certain viruses, etc. These indicators may not reflect the total antibody content of the antiserum or its protective effect with respect to any particular infection. The implication is that an individual's antibody, resulting from a given antigen stimulus, may find it easier to bind complement (the measure of antibody in the CF test) than does antibody synthesized from the same antigen stimulus in another person. This difference between individuals depends upon a number of factors, but past immunological experience and individual genotypic differences must contribute substantially (3).

Complement fixing antibodies to rhinoviruses and live or inactivated adenovirus vaccines are relatively difficult to produce when compared with the ease of synthesis of antibodies neutralizing the cytopathogenic effects of these viruses. The data to be presented, as well as the data of other laboratories, support such a concept. The search for a test system which more accurately measures antibody is continuous. The indirect complement fixation test (4) has been helpful in certain antigen-antibody complexes

which fix complement poorly or not at all. However, no improvement in the detection of complement fixation antibody could be shown by using the indirect complement fixation test in a small sample of the live adenovirus vaccine complement fixation negative sera.

These data will give an idea as to how the recruit, coming to Great Lakes, responds to a constant adenovirus vaccine dose --- the vaccine having been given at about the same time he was experiencing an intense natural challenge.

METHODS

Two populations of recruits were used in this study. One company (Phase I) of recruits entering recruit training the first of February 1965 were randomly divided into four vaccine groups. One-fourth received the oral live adenovirus type 4 vaccine (LAV); one-fourth received an inactivated monovalent adenovirus type 4 vaccine (monovalent); one-fourth received an inactivated bivalent adenovirus types 4 and 7 vaccine (bivalent); and one-fourth received a saline placebo. A pre-vaccine and 7, 14, 21, 28, 40 and 62-day post-vaccine bloods were collected and serum stored at -20°C until tested.

The second population (Phase II) used was 35 companies of recruits, each company being made up of from 80 to 100 men. One-half of the men in each company received a vaccine and one-half received a placebo. The vaccines used in this study were the same as in Phase I. Ten companies received the oral live adenovirus type 4 or placebo (386 men receiving either vaccine for a total of 772 men in this vaccine group). Ten companies received the inactivated monovalent type 4 or placebo (375 men received the inactivated monovalent vaccine and 391 a placebo for a total of 766 men). Twenty-five companies received the inactivated bivalent adenovirus vaccine or placebo (963 received the bivalent vaccine and 986 men a placebo for a total of 1,949 men). A pre-vaccine, 4th and 9th week of training blood, was collected as was an acute and convalescent blood collected from those recruits admitted to the hospital or dispensary. All sera were stored at -20°C until tested. Sera were inactivated for 30 minutes at 56°C just prior to testing.

The micro-complement fixation method (5) was used, modified to use two exact units of complement. The micro-neutralization test (6) using from 10-32 $TCID_{50}$ doses of the virus was used in all the neutralization studies. Various tissue culture cells were used for determining sensitivity in the neutralization tests. HEp-2 cells were obtained from the American Type Culture Collection; primary rhesus monkey kidney cells were prepared by standard technics, as were second passage embryonic kidney cell cultures.

RESULTS

The rapidity of neutralizing antibody formation, as measured in HEK cells is shown in Figure 1. There is a rise of adenovirus type 4 antibody by day 7 in all vaccine groups with a titer of 1:80 attained by day 28. The lagging of the neutralizing antibody response in the saline placebo group was consistent with a somewhat later natural challenge by wild adenovirus in this population, but indicates an exposure period very early in training.

A comparison of antibody response in recruits receiving the live oral vaccine, as measured in different tissue culture systems, is shown in Figure 2. The results clearly show the greater sensitivity of the HEK cells over either the HEp-2 cells, or the monkey kidney cell system,* using the same virus strain as was used in the live oral vaccine, or a 1965 NAMRU-4 prototype isolate as the challenge virus. In making the same comparison with recruits who had received the saline placebo (in other words in individuals experiencing natural adenovirus infection) (Fig. 3), it was still evident that the HEK tissue culture was the most sensitive method for measuring neutralizing antibody to adenovirus type 4 in these men whether the antibody was due to the live vaccine or natural infection. A comparison of the neutralizing antibody response of the four vaccine groups to adenovirus type 4 is shown in Fig. 4. Regardless of treatment group, the HEK cell was the more sensitive tissue system to demonstrate neutralizing antibody to vaccine or wild strains of adenovirus type 4.

The adenovirus complement fixation antibody response of the recruits in Phase II is shown in Figure 5. It should be noted that all of the placebo groups had a higher antibody response than any of the vaccine groups. This was not observed when measuring the adenovirus type 4 neutralizing response (Fig. 6). With the exception of the live adenovirus vaccine group, the neutralizing antibody response in HEp-2 cells was the same for any vaccine group or placebo, though a less sustained increase in the live adenovirus recipient was noted. The heterotypic response is shown in Figures 7 and 8. The bivalent inactivated vaccine containing types 4 and 7 adenovirus produced, not only a homotypic response to type 7 neutralizing antibodies, but a measurable heterotypic response to type 3 adenovirus which was not observed in recipients of either the live type 4 or the inactivated type 4 monovalent vaccines. No evidence of natural infection due to either types 3 or 7 adenovirus was apparent in the measurement of neutralizing antibodies in the placebo group.

Approximately 90% of the men reporting to the Naval Training Center have an adenovirus type 4 neutralizing antibody titer of less than 1:8 (Fig. 9). Recruits reporting aboard with an initial adenovirus type 4 neutralizing titer of 1:32 or greater did not experience either serological

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*Results kindly supplied by Dr. Anthony Morris, NIH, Bethesda, Md.

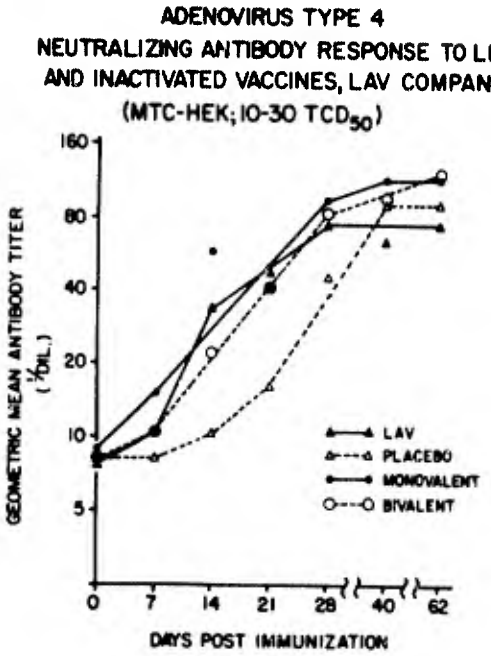
infection due to this agent, or a booster response to live or inactivated adenovirus vaccination.

Again in this phase, the percent of recruits responding with a 4-fold or greater neutralizing antibody response to adenovirus type 4 was similar regardless of whether the response was due to vaccine or natural infection (Fig. 10). However, this was not true when the complement fixation conversion rate was compared. Approximately 40% of the recruits who received the live or inactivated monovalent vaccine responded with a 4-fold or greater antibody rise compared to 70% conversion rate in the placebo groups. The percent of recruits with a heterotypic adenovirus types 3 and 7 response was about two-thirds as much in those recruits who received the live or monovalent vaccine as those who experienced natural adenovirus type 4 infection. This indicated a greater heterogenicity (or immunological recall) of antibodies in natural infection than was associated with either vaccine.

CONCLUSIONS

From serologic response of recruits entering the Naval Training Center, Great Lakes, Illinois, the neutralizing antibody titer conversion rate (4-fold or greater) is the same whether the recruits received the live adenovirus, inactivated adenovirus, or placebo. The response associated with the vaccines were measurable by day 7 post-vaccination. The antibody titer attained was about the same in either the vaccine group or placebo. The live adenovirus vaccine did not provoke the complement fixation conversion rate that natural infection did. Whether this can be attributed to a quantitative or qualitative difference is under investigation. Recruits entering training with an initial titer of 1:32 or greater did not show evidence of infection or booster effect. Natural infection provides a greater heterotypic antibody response to adenovirus types 3 and 7 than the live adenovirus enteric-coated tablet, or the parenteral injected inactivated monovalent adenovirus type 4.

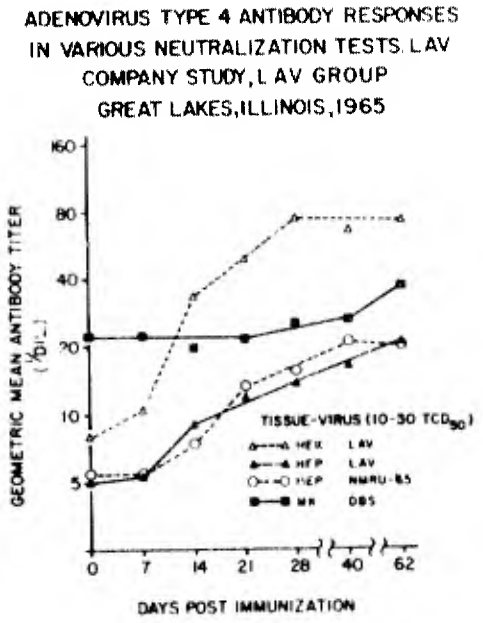
FIGURE 1



LAV = Those recruits receiving the oral live adenovirus type 4 vaccine.
PLACEBO = Those recruits receiving a saline placebo.
MONOVALENT = Those recruits receiving an inactivated monovalent adenovirus type 4 vaccine.
BIVALENT = Those recruits receiving an inactivated adenovirus type 4 & 7 vaccine.

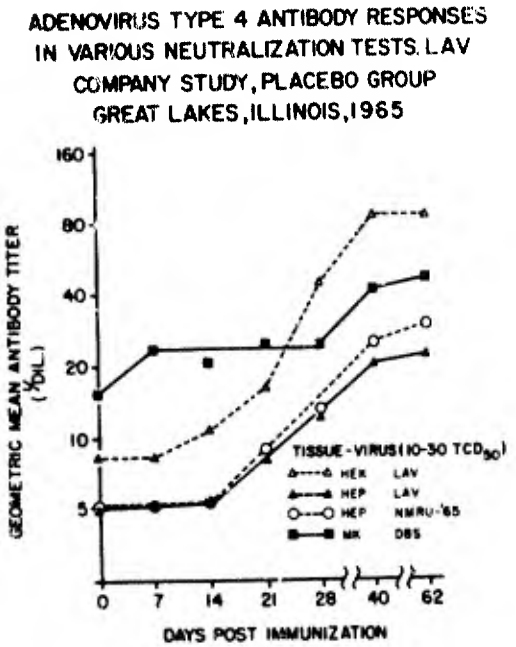
Challenge virus was the same adenovirus type 4 as was used in the vaccine.

FIGURE 2



HEK = Human embryonic kidney tissue culture.
HEP = Human epithelial tissue culture.
MK = Monkey kidney tissue culture
LAV = Challenge virus was the same adenovirus type 4 as was used in the vaccine.
DBS = Results kindly supplied by Dr. Anthony Morris, NIH, Bethesda, Md.
NMRU-65 = Challenge virus was a 1965 Great Lakes isolate.

FIGURE 3



HEK = Human embryonic kidney tissue culture.
HEP = Human epithelial tissue culture.
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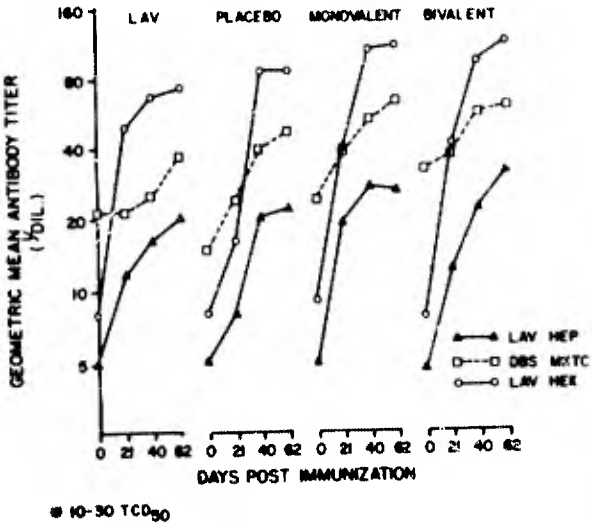
LAV = Challenge virus was the same adenovirus type 4 as was used in the vaccine.
DBS = Results kindly supplied by Dr. Anthony Morris, NIH, Bethesda, Md.
NMRU-65 = Challenge virus was a 1965 Great Lakes isolate.

FIGURE 4

COMPARISON OF ADENOVIRUS TYPE 4 NEUTRALIZING ANTIBODY TITERS AFTER VACCINATION DETERMINED BY VARIOUS VIRUS-TISSUE SYSTEMS*



* 10-30 TCD_{50}

LAV HEP = Challenge virus was the same strain that was used in the vaccine, using HEP tissue culture cells.

LAV HEK = Challenge virus was the same strain that was used in the vaccine, using HEK tissue culture cells.

DBS = Results kindly supplied by Dr. Anthony Morris, NIH, Bethesda, Md.

FIGURE 5

COMPLEMENT FIXATION ANTIBODY RESPONSE IN NAVY RECRUITS RECEIVING THE LIVE ADENOVIRUS VACCINE, INACTIVATED ADENOVIRUS VACCINE, OR PLACEBO, GREAT LAKES 1965

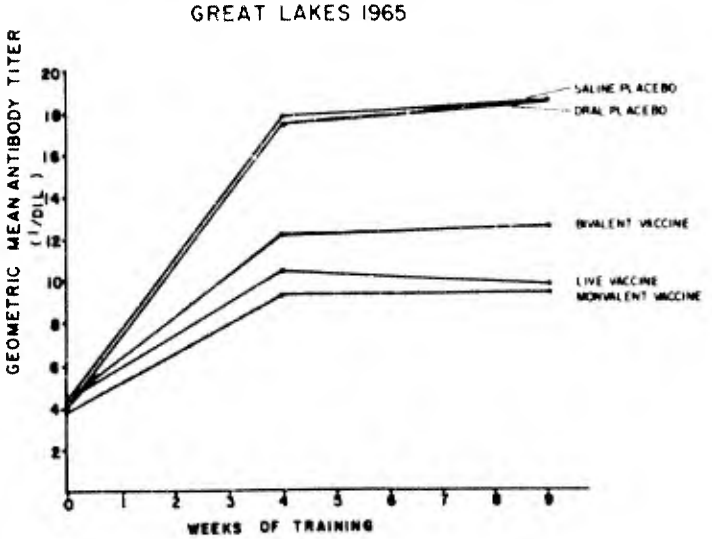


FIGURE 6

ADENOVIRUS TYPE 4 ANTIBODY RESPONSE IN NAVY RECRUITS RECEIVING THE LIVE ADENOVIRUS VACCINE, INACTIVATED ADENOVIRUS VACCINE, OR PLACEBO GREAT LAKES 1965

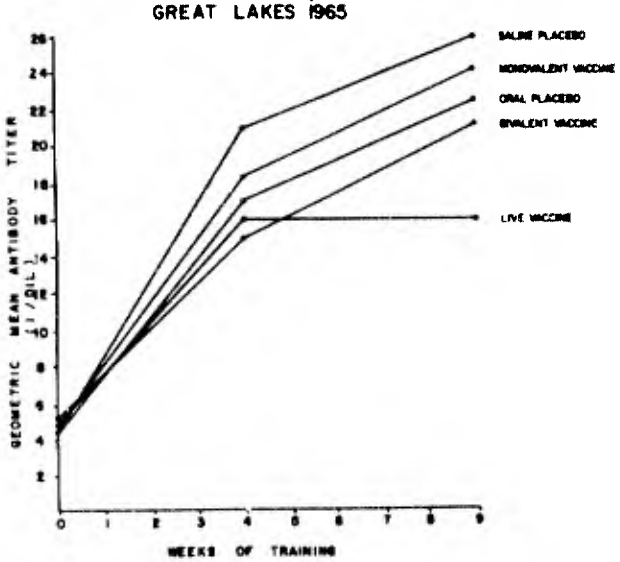


FIGURE 7

ADENOVIRUS TYPE 3 ANTIBODY RESPONSE IN NAVY RECRUITS RECEIVING THE LIVE ADENOVIRUS VACCINE, INACTIVATED ADENOVIRUS VACCINE, OR PLACEBO GREAT LAKES 1965

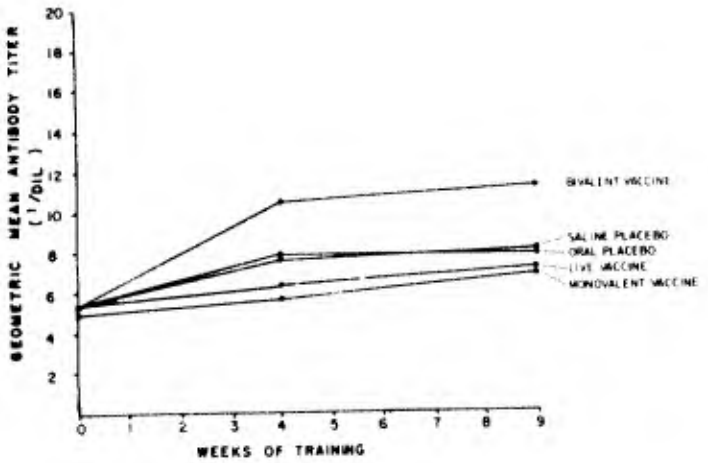


FIGURE 8

ADENOVIRUS TYPE 7 ANTIBODY RESPONSE IN NAVY RECRUITS RECEIVING THE LIVE ADENOVIRUS VACCINE, INACTIVATED ADENOVIRUS VACCINE, OR PLACEBO GREAT LAKES 1965

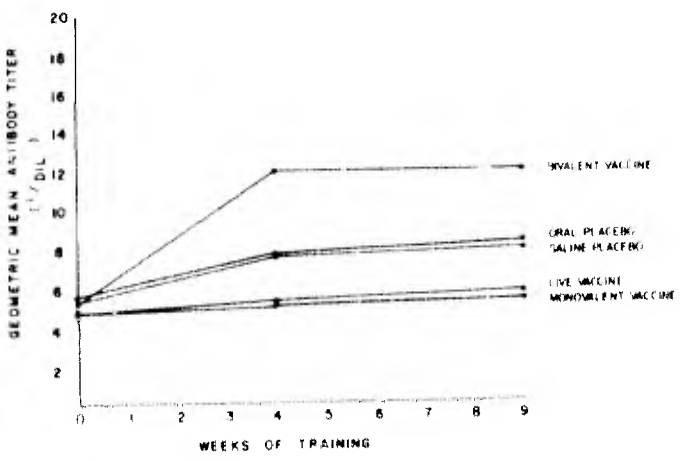
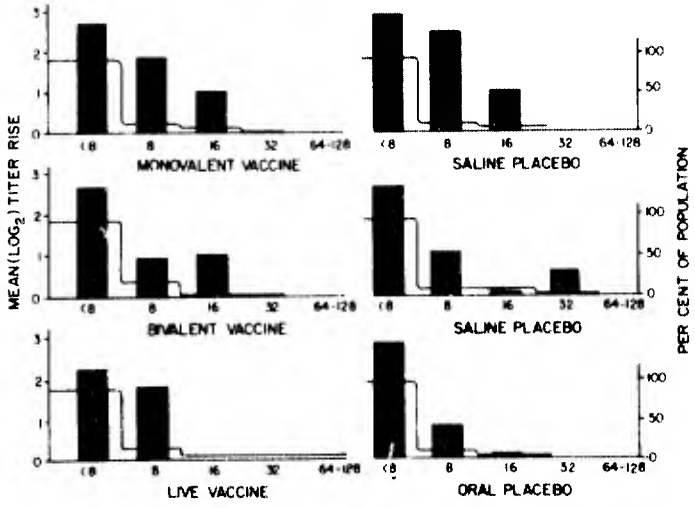


FIGURE 9

ADENOVIRUS TYPE 4 ANTIBODY RESPONSE IN NAVY RECRUITS RECEIVING THE LIVE ADENOVIRUS VACCINE INACTIVATED ADENOVIRUS VACCINE OR PLACEBO WHEN COMPARED TO THE INITIAL ANTIBODY TITER GREAT LAKES, ILLINOIS, 1965

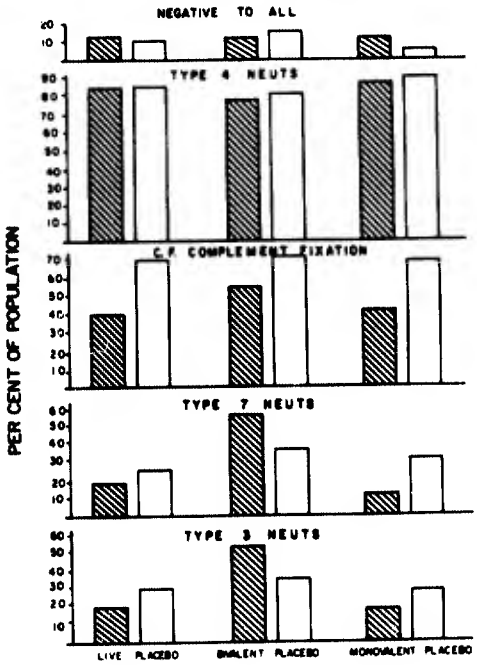


Bars represent mean titer.

Solid lines represent percent of population.

FIGURE 10

PER CENT OF RECRUITS RESPONDING WITH A
4 FOLD OR GREATER ANTIBODY RESPONSE TO
VARIOUS ADENOVIRUS ANTIGENS OR PLACEBO
GREAT LAKES, ILLINOIS, 1965



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CLINICAL EVALUATION OF THE PROTECTIVE EFFECT OF VACCINES IN A NAVAL RECRUIT POPULATION

Griffin, J.P. and Greenberg, B.H.

Adenovirus infection is a persistent problem in the recruit population at Great Lakes, Illinois. Serologic surveillance programs over the past several years have demonstrated that between 60 and 90% of recruits experience adenovirus infections during their 9-week training period (1). Inactivated adenovirus vaccines have effected an average reduction in total respiratory admissions of approximately 50% in this population (2). Previous studies have utilized data collected indirectly from outpatient visit records, inpatient admission rosters, and barracks interviews. Because of the multiplicity of infectious agents associated with recruit respiratory disease, the specific protective effect of a given respirovirus vaccine has been difficult to quantitate. In an attempt to clarify specific vaccine effect in field trials, Stille (3) developed a method of determining the intrinsic vaccine effect (IVE) from "specific infection" rates. His calculations have been based on the results of specific virus isolation and seroconversion as indices of infection in admitted respiratory illnesses in placebo groups.

Clinical evaluation has been infrequently used in previous vaccine field trials at NAMRU-4. Since the adenovirus has been etiologically implicated in a specific respiratory syndrome, namely that of acute undifferentiated respiratory disease (ARD) (4), it was considered desirable to measure the effectiveness of adenovirus vaccines in reducing admissions due to this specific syndrome. This study also provided the opportunity of observing a large population of recruit recipients of live oral adenovirus type 4 vaccine for any signs of toxicity.

METHODS

The study population consisted of young men, between the ages of 17 and 21, undergoing recruit training at the Naval Training Center, Great Lakes, Illinois, between February and June, 1965. Several days after their arrival here, all participating recruits received, in a double-blind manner, one of three adenovirus vaccines, or placebo. The characteristics of the vaccines used and the epidemiologic setting in which they were employed have been previously described (5).

All recruits requiring admission to a medical facility were examined within 24 hours by one of the authors. Pertinent findings were recorded on a standard medical form. A posteroanterior chest teleoroentgenogram was obtained on each patient.

At the completion of the study, and with knowledge of the chest film reading, a specific medical diagnosis was assigned to each case. The various respiratory illnesses were grouped by syndrome according to their primary manifestation; the terminology used, closely coinciding with that of the Commission on Acute Respiratory Diseases (6).

The respiratory disease syndrome observed in this study were: 1) acute undifferentiated respiratory disease (ARD) --- in acute inflammation of the respiratory tract with predominantly constitutional symptoms, and an oral temperature of greater than 100°F; 2) common cold syndrome --- an acute inflammation of the upper respiratory tract with coryza as the prominent feature and an oral temperature of less than 100°F; 3) exudative pharyngitis --- an acute respiratory illness in which exudate was present on the tonsils or pharynx; 4) "viral" exanthem --- an acute illness characterized by fever, lymphadenopathy, and a morbilliform rash; 5) "primary atypical pneumonia" --- an acute respiratory illness associated with pulmonary infiltration on roentgenogram (classical lobar pneumonia was not observed in this study). All participating recruits had received the standard military formula inactivated influenza vaccine at the onset of recruit training. Benzathine penicillin G was administered to all non-penicillin hypersensitive recruits during their second week of training.

RESULTS

The incidence of admissions by treatment group is presented in Table I. The number of non-respiratory illnesses was small in all treatment groups. The incidence of respiratory admissions of 19.4 per one hundred men at risk in the live oral vaccine group compared favorably to 32.6 in the placebo group resulting in a relative reduction of 40.5%. Similarly, the incidence of 17.6 in the monovalent killed vaccine group and 20.1 in those receiving the bivalent killed vaccine resulted in relative reductions of 46.0 and 38.3%, respectively. The difference observed between each vaccine group and the placebo were statistically significant ($P = <0.01$), whereas the differences observed among the vaccine groups only were not. The relative reductions compared favorably with the IVE values of the vaccine treatment groups as determined from specific infection rates of the placebo groups.

A clearer delineation of vaccine efficacy is attempted in Table II. The

incidence of "common cold" syndromes was similar in all groups. Most of these illnesses were seen in recruits admitted for a non-respiratory illness. Since recruits with common cold syndromes are rarely admitted to a medical facility, the effect of the vaccines on this syndrome cannot be adequately evaluated from this study. Similarly, the incidence of exudative pharyngitis, "primary atypical pneumonia", and "viral exanthem" were similar in all groups. Although there was a trend toward more frequent admissions for "viral exanthem" in the vaccine groups, this was not statistically significant. The most striking differences were observed in the syndrome of ARD. The admission incidence of 12.5 in the live oral vaccine group compared to 24.9 in the placebo resulted in a relative reduction of 49.7%. Similarly, the incidence of 9.9 in the monovalent killed vaccine group, and 11.7 in those receiving the bivalent killed vaccine resulted in relative reductions of 60.2% and 53.0%, respectively. While a trend was observed toward the monovalent killed vaccine as being the most effective in the prevention of the ARD syndrome, the differences observed among vaccines were not significant.

There was no excess number of admissions, nor clinically unusual illnesses to suggest toxicity related to the live enteric adenoviral vaccine.

The incidence of admission symptoms by treatment group is summarized in Table III. Symptoms listed are those in which a statistically significant reduction was observed in vaccine recipients as compared to placebo. Interestingly, a perusal of these specific symptoms demonstrates that they are identical with those comprising the definition of the ARD syndrome.

SUMMARY

The determination of specific respiratory syndrome admission rates assisted in more precisely defining adenovirus vaccine efficacy. Also, aggregation of specifically reduced symptoms in vaccine recipients confirmed previous definition of the ARD syndrome and etiologically associated it with adenovirus infection.

There was no evidence of unusual or excess illness to suggest toxicity in those recruits who received the live adenovirus oral vaccine.

Clinical evaluations were used to advantage in the present study. Addition of this methodology to future vaccine trials would appear justified.

TABLE I

EFFICACY OF ADENOVIRUS VACCINES IN NAVY RECRUITS
(GREAT LAKES, ILL., SPRING 1965)

Incidence of Admissions by Treatment Group

	Type 4 live oral vaccine (386)		Type 4 killed parenteral vaccine (375)		Types 4 & 7 killed parenteral vaccine (378)		Parenteral saline or oral placebo (1168)	
	No.	%	No.	%	No.	%	No.	%
Total admissions	87	22.6	71	18.9	80	21.2	406	34.8
Non-respiratory admissions	12	3.1	5	1.3	4	1.1	25	2.1
Respiratory admissions	75	19.4	66	17.6	76	20.1	381	32.6
Relative reduction, respiratory admissions	40.5		46.0		38.3			
I.V.E.	75		78		74			

TABLE II

EFFICACY OF ADENOVIRUS VACCINES IN NAVY RECRUITS
(GREAT LAKES, ILL., SPRING 1965)

Incidence (Persons) of Admission Syndrome by Treatment Group

	Type 4 live oral vaccine (386)		Type 4 killed parenteral vaccine (375)		Types 4 & 7 killed parenteral vaccine (378)		Parenteral saline or oral placebo (1168)	
	No.	%	No.	%	No.	%	No.	%
Total persons admitted	83	21.5	69	18.4	72	19.0	386	33.1
"Acute undifferentiated respiratory disease"	48	12.5	37	9.9	44	11.7	291	24.9
"Common cold"	3	0.8	4	1.1	6	1.6	14	1.2
Exudative pharyngitis	4	1.0	4	1.1	2	0.5	13	1.1
"Primary atypical pneumonia"	7	1.8	8	2.7	11	3.4	42	3.6
"Viral exanthem"	11	2.9	11	2.9	7	1.9	17	1.5
Relative reduction, persons admitted with ARD	49.7		60.2		53.0			

TABLE III

EFFICACY OF ADENOVIRUS VACCINES IN NAVY RECRUITS
(GREAT LAKES, ILL., SPRING 1965)

Incidence of Admission Symptoms by Treatment Group

	Type 4 live oral vaccine (386) %	Type 4 killed parenteral vaccine (375) %	Types 4 & 7 killed parenteral vaccine (378) %	Parenteral saline or oral placebo (1168) %
Chills	12.7	12.5	13.7	24.5
Headache	12.4	11.5	11.1	20.3
Nasal congestion	16.8	15.5	15.3	27.6
Sore throat	16.3	14.9	15.9	29.6
Dysphagia	12.2	11.7	12.4	23.4
Hoarseness	11.7	11.7	12.2	25.4
Cough	19.4	17.1	19.6	31.7
Anorexia	9.3	10.7	11.9	20.4

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IMPLICATIONS OF STUDIES ON ENTERIC LIVE ADENOVIRUS TYPE 4 VACCINE

George Gee Jackson

Studies in volunteers conducted by the Commission on Acute Respiratory Diseases during World War II differentiated the cause of acute respiratory disease of recruits (ARD) from other causes of viral respiratory infection (1). When adenoviruses were recovered in tissue cultures about ten years later (2,3), it was possible to prove that ARD was caused by adenovirus type 4 (4). After a larger number of adenoviruses were classified, types 3 and 7 were also identified as causes of ARD in recruits, and these three types, 3, 4, and 7, accounted for nearly all of the ARD in American troops; in Holland, types 14 and 21 caused some outbreaks (5).

The Epidemiology of ARD

The epidemiology of adenovirus ARD shows a peculiar distribution between military and civilian populations. Adenovirus type 3 infects civilians and military personnel alike. The clinical manifestations may be "pharyngoconjunctival fever", "swimming pool conjunctivitis", or ARD (6); but, such infections cause less than 5% of respiratory disease among civilians (7). Adenovirus type 4 occurs almost exclusively amont military recruits. Serologic studies show that only about 15% of college students and incoming recruits have antibody against adenovirus type 4; antibody against types 3 and 7 is more common. The symptomatic expression of infection with type 7, as with type 4, is predominantly a disease of recruits. Among college students, adenovirus ARD does not become epidemic, whereas among military recruits, 70 to 80% of the subjects, or virtually all of those without prior antibody become infected in the first few weeks of recruit training (8). This is true for Army, Navy, Marine Corps, and Air Force recruits, but in the latter two services, the time of infection is later and less epidemic probably because of differences in the training schedules. Within each service, certain centers, such as Great Lakes, have much more rapid and acute infections with adenoviruses than other centers with the same training schedules, but different locations, such as San Diego. This suggests a geopathologic effect in the clinical expression of infection. The symptoms of infection also vary according to season --- recruits infected in summer usually have mild illness or no symptoms; in the winter, approximately one-quarter of those infected require hospitalization.

Killed Adenovirus Vaccines

Following the discovery and propagation of adenoviruses in tissue culture, a killed vaccine was developed from virus grown in rhesus monkey kidney. This was shown to be both immunogenic and protective in recruits (9). With further refinement and large lot production, much variation was found in the potency of the vaccine. The antibody response in test guinea pigs was related with some difficulty to the protective effect of the vaccine in man. Nevertheless, killed vaccine was used quite extensively about 1960-1963 with appreciable, but spotty effect on ARD. As some of the problems of producing high titer killed vaccine appeared near resolution, the role of simian virus 40 (SV-40) as a helper virus in the propagation of adenovirus in monkey kidney became recognized (11). Because of the oncogenicity of this virus in baby hamsters, it was not considered safe to use any cultures containing such virus even as a killed vaccine. Soon thereafter, all adenovirus vaccine grown in monkey kidney was removed from availability, even lots apparently free from SV-40 virus, because of possibility (and demonstration) of transcapsidization or hybridization of the oncogenic genome from SV-40 within the adenovirus capsid, and, thus it was undetectable as SV-40 virus (12).

Live Enteric Adenovirus Vaccine

In 1963, it was reported that an asymptomatic infection of the gastrointestinal tract could be produced with an "unattenuated" strain of adenovirus type 4 grown in human diploid cell culture, WI-38 (13). The pedigree and non-oncogenicity of the strain were developed preparatory to investigation of its use as an enteric live virus vaccine (14). The administration of a million infectious particles of lyophilized virus with enteric coating induced a serologic response in a high proportion of antibody-free men and did not produce symptoms of infection or untoward reactions when given to increasing numbers of recruits. The results of additional well-designed and carefully controlled vaccine trials indicate that we now have a means of safe and effective prevention of adenovirus type 4 ARD in recruits (15, 16).

Time of Immunization and Infection

The effect of the time of vaccination in relation to contact with infectious virus can be interpreted from the different studies. If immunization precedes the challenge by 3 to 6 weeks, as was the case in the studies at Parris Island and Camp Lejeune, protection against illness from naturally acquired infection was complete, although approximately one-third of the subjects became reinfected in the environment of an epidemic of ARD (15). When the vaccine was given 4 days after the recruit arrivea at a station with endemic disease, as in the first study at Great Lakes, no vaccine effect was found until the 10th or 11th day (16). If vaccine was given upon arrival of the recruit in the epidemic area, as in the second study, the epidemiologic effect was immediate.

One implication of these observations is that serum antibody, per se, can protect against the specific respiratory illness. It reduced, but did not eliminate the pharyngeal acquisition of adenovirus type 4 when the vaccinee contacted epidemic disease; virus acquisition, however, was without illness. Another implication is that enteric live virus, given at the time of or shortly after infection, causes no alteration of illness in infected individuals or in the epidemiologic pattern of disease until serum antibody can be detected. Killed vaccine given parenterally had the same effect (16). A third implication relating to the time of vaccination is that live virus vaccine given upon entry into an epidemic area produced a non-antibody antiviral effect with prevention of early illness and an immediate epidemiologic effect. This could be competition between vaccine and natural virus with the mass action greatly favoring the vaccine strain, or it could have been an effect on the host, such as interferon production, which inhibited new virus acquisition, or effected through another unknown mechanism.

Enteric Route of Immunization

Some potential advantages of attenuated live virus vaccines have been presented in the investigation of other viral vaccines. One that has not been discussed, but may be particularly applicable to ARD, is related to the class of immunoglobulin that is elicited by the vaccine. It is known that the major component of gamma globulin in nasal and respiratory secretions is IgA with a sedimentation value of 7S. Some recent observations have suggested that the IgA response is greater when the site of antibody formation is the intestinal tract (17). If this applies, the enteric route of infection by adenovirus vaccine may be particularly advantageous for the production of antibody against respiratory pathogens, and, thus have an extra benefit in the prevention of ARD.

Safety of the Vaccine

Approximately 50,000 doses of vaccine have not been given without any recognized adverse effects. On that basis, one can be quite reassured about its safety. The studies among military personnel also document that spread of the live vaccine virus to the pharynx of the vaccinee or to cohorts was not detectable at Parris Island, occurred in less than 5% of company contacts at San Diego, and was of unknown frequency at Great Lakes.

In studies we have done among childless married couples of civilian status in Chicago, the virus was not found to remain limited to the intestinal tract of vaccinees. It was recovered from the pharyngeal washings of about one-third of persons given enteric vaccine, and also from specimens from about one-third of the spouses of vaccinees, or a rise in serum antibody documented the transfer of infectious virus. The implication is that the activities of young married couples, which includes food preparation and physical intimacy, offers a much better opportunity for spread of enteric adenovirus infections than merely geographic contact of the type characteristic of recruits. A significant observation was that acquisition of the vaccine virus

in the throat or by the spouse was not associated with illness. The implication is that the vaccine strain is attenuated. This is further suggested by some other unreported studies.

Natural Adenovirus Infections

One can use the results obtained from vaccine studies to interpret the experience with naturally acquired adenovirus type 4 infection among recruits. Data suggest that with very rare exception, an isolated enteric infection in an adult is asymptomatic. This may account for the seasonal difference in the clinical expression of ARD. Summer infections could be predominantly intestinal, whereas infection during the winter months is usually both respiratory and intestinal. It has been shown that enteric infection, with fecal excretion of adenovirus type 4, does not spread well among recruits, possibly because of centralized food handling. Among married couples, spread was more efficient. Whether the initial spread is to the pharynx of the infected person and then to his intimate contacts or direct entero-oral spread is not known. One can conclude, however, on the basis of no self infection in recruits that the route is not a result of viremia or that the vaccine strain does not cause viremia.

Infection of the respiratory tract also can be asymptomatic. This was readily demonstrated when previously vaccinated persons were exposed to virus in a natural epidemic, but asymptomatic throat infections with adenovirus type 4 occurred following vaccination of some antibody-free civilian volunteers and in their spouses. In the former, the effect may have been from serum antibody, in the latter from attenuation of vaccine virus, but both mechanisms may impinge on the course of natural infection. If the vaccine strain is attenuated, reversion to virulence by one passage in a human was not found in the few cases of transmission to spouses observed.

The Future

Have we accomplished a means for the elimination of ARD of recruits? Time will answer that question. The most we can expect from a monotypic vaccine is the effective prevention of disease caused by that specific type. Epidemic ARD is known to have been caused by adenoviruses types 3, 7, 14, and 21, in addition to type 4. It is possible that successful suppression of adenovirus type 4, which has been predominant in U.S. recruits, may permit the emergence of one of the other types to epidemic proportions. Study of cases of ARD admitted to the hospital at Great Lakes during the period of the mass immunization study showed both a relative and absolute increase in the number of infections with adenovirus type 7. It would be wise, therefore, to turn attention now to the development of successful vaccines for some of these other types. Because adenovirus type 7 can be oncogenic in newborn hamsters (18), its use as a live vaccine is not readily recommended. In monkey kidney cultures, hybridization of adenovirus type 7 with SV-40 is a deterrent to the production of a killed vaccine of the usual type. Information is at hand, however, to suggest the feasibility of developing a sub-particle

vaccine comprised entirely of the specific hexon antigen (19) which gives rise to type-specific neutralizing antibody.

SUMMARY

Adenovirus type 4 has been the major cause of ARD of recruits in the United States during the past 20 years. Morbidity from the infection is of great importance, more in some training centers than others and usually greater in winter than summer. Trials with enteric-coated live adenovirus type 4 vaccine have produced asymptomatic intestinal infections. Antibody induced by live virus immunization was effective in preventing clinical ARD after 10 to 11 days. It reduced, but did not prevent viral acquisition and shedding. Vaccination given simultaneously with possible infection in an endemic area was about 50% effective. Immunization of all recruits immediately before exposure to endemic natural virus had an immediate protective effect and altered the epidemiologic pattern of ARD.

Little or no spread of vaccine-induced infections occurred in military personnel. Fifty thousand doses of the vaccine caused no recognized adverse effects. Some evidence suggests that the vaccine strain is attenuated. The enteric route may have an advantage in immunizing against respiratory infections. Some interpretations of natural infection with adenovirus type 4 can be made from the vaccine trials.

The immediate vaccination of all incoming recruits with enteric-coated live adenovirus type 4 appears to offer a practical means for the elimination of epidemic ARD in recruits. Alteration of ecology of respiratory viruses consequent to the control of adenovirus type 4 could produce epidemic ARD of another etiology.

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