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PROPHYLACTIC METHODS IN PREVENTION OF
DISEASE AMONG ARMY PERSONNEL

Creed D. Smith, et al

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PROPHYLACTIC METHODS IN PREVENTION OF DISEASE AMONG ARMY PERSONNEL**

ANNUAL REPORT

by

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AUGUST 1972

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<p>Surveillance studies to determine the etiologic agents of acute respiratory disease (ARD) in basic combat trainees (BCTs) were conducted during fy '72. A total of 7,296 ARD hospitalized trainees at 8 training forts, were included in these studies. Viral isolations and serological studies indicated 42.2% of ARD hospitalizations were caused by Adenoviruses, 6.2% by influenza A and B, Mycoplasma and polio, and 51.6% by agents that were not determined. Efficacy evaluations of a 1971-72 lot of type 7 adenovirus vaccine which was administered at the 8 BCT forts during the upper-respiratory-disease season indicated this vaccine was not effective in preventing adenovirus 7 disease. Laboratory titrations of virus in the vaccine pills indicated a level below that recommended for an effective pill. A newly standardized bivalent influenza vaccine (A₂/Aichi/68; B/mass/66) was administered during the fiscal year. However, most of the 8 BCT forts experienced considerable influenza, 111 flu A and 47 flu B total infections. It is envisioned that with continued use of adenovirus 4 and 7 vaccines, and decline of these viruses as a cause of ARD, the unknown agents will occupy a larger area of ARD cause. Rhinoviruses probably occupy a portion of this undetermined group.</p> <p>Studies to determine if asymptomatic re-infection with wild-type Adenovirus 4 and 7 occurs in BCTs who have been successfully immunized with live enteric type 4 or 7 vaccines were performed. One-hundred and thirty (130) BCTs of Company A, 5th Battalion, 1st Brigade at Fort Ord were subjects for this study. Neutralizing antibody titrations on nasal washes and sera, both collected at 0, 3 and 6 weeks</p>		

indicated that 64% of the group had no detectible antibody (SN titer <1:4) to adenovirus type 4 and 52% had no detectible antibody to type 7 virus at the 0 week bleeding. 34% of the group were susceptible to both viruses by this criterion. 82% of the type 4 virus susceptible individuals and 52% of type 7 virus susceptibles underwent enteric infections with the vaccine virus. 33% of individuals susceptible to both viruses sero-converted to both adenoviruses, 4 and 7 at the 3 week bleeding. 24% of the group were infected with wild-type 4 and 66% were infected with wild-type 7, 3 weeks after receiving vaccines. The infection rates by each virus were the same in sero-converters and non-sero-converters at 3 weeks post-vaccine. However, infection rates by wild-type 7 were twice as high in recruits who had no type 7 SN antibody at 0 week than in those with SN titers \geq 1:4 regardless of subsequent sero-conversion to vaccine virus. Furthermore, virus isolated from throat swabs was detected earlier in recruits who did not have detectible antibody in the 0 week serum.

A modified formulation (SPGA) for live adenovirus 4 and 7 vaccines was field tested in BCTs at Fort Ord, California. Three vaccine groups composed of 115 to 130 men in separate companies were subjects for this study. The design was to determine the serological response of BCTs to administration of (1) type 4 and 7 monovalent tablets with modified formula (2) type 4 and type 7 bivalent tablets with modified formula, and (3) the standard issue type 4 and 7 monovalent preparations. Sero-conversion rates of recruits with pre-vaccine antibody titers of less than 1:4 were similar for all three modified formula vaccines tested. Four-fold sero-conversion rates varied from a low of 73% to a high of 79% when measured against wild virus prototype strains in the microtiter serum neutralization procedure. There was no evidence of viral interference with either sero-type of virus in recruits that were defined as susceptible to both viruses. A comparison of the conventional serum neutralization procedure performed in human embryo cell culture tubes to the microtiter procedure performed in 96 well microtiter plates did not reveal significant differences in sensitivity or specificity. However, vaccine virus strains were shown to be more sensitive in detecting response to immunization than were the prototype wild type virus strains.

Continuing efforts to develop more rapid methods for the recognition of infectious respiratory virus disease agents in nasopharyngeal specimens have made only limited progress. One of the approaches

under evaluation is the indirect fluorescent antibody technique, using rubella virus infected cell culture monolayers as a model. The level of background fluorescence on final slide preparations is high enough to preclude rapid identification of an agent under circumstances where any one of many agents may be involved. The alternative to a successful indirect procedure is to produce labeled antisera for each of the possible agents involved in ARD.

These meningococcal vaccine studies were performed in conjunction with and under the consultation of the Department of Bacterial Diseases, WRAIR. As new lots of meningococcal polysaccharide vaccines were made available to the Army, they were tested in human volunteers at Fort Ord California. Meningococcal carrier surveys were also performed monthly on at least 3 different BCT companies. These combined studies were designed to (1) test the antigenic quality of each vaccine lot, (2) assess the effect of meningococcal polysaccharide vaccine on the meningococcal disease rate, (3) monitor the emergence of serogroups other than "C", and (4) determine the effects of the vaccine on the meningococcal carrier rate. Thirteen lots of meningococcal "C" polysaccharide vaccine (including controls) were tested during the period covered by this report. Each lot was administered to 40-50 BCfs, for a total of approximately 530 men receiving vaccine. All of the lots incited a favorable antibody response. Seroconversion rates of 90% or greater were obtained by all individuals who received a 50 mcg dose. A trial dose of 2 mcg caused seroconversions in 82% of the vaccinated individuals. Following the routine continuous vaccination of all receptionees at Fort Ord, there were only 3 disease cases; these were caused by "Y" (Boshard) organisms. There were no deaths. Serogroup "Y" appears to be the emerging prevailing meningococcal type, followed by serogroup "B".

Continued efforts are in progress to test modifications of charcoal viral transport media so as to formulate a media that will transport viral pathogens of the upper respiratory tract other than the adenoviruses. This study concerns comparison of standard Liebovitz Charcoal transport medium with a modified formulation containing, gentamicin, agarose and bovine albumin. Isolation rates were 48% and 49% respectively for modified CVTM. The total study consisted of 1843 tubes. Modifications of CVTM are also being tested for use in transporting specimens containing the more fastidious bacterial agents. The best survival rate of meningococci from specimens has been obtained with a 0.5% liquid additive. This concentration supported an 85% survival rate in CVTM after 5 days, and 58% of

meningococcal organisms were recovered after 11 days. Concentrations of mucin in CVTM gave less encouraging results.

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FOREWORD

During these studies emphasis, for the most part, has been placed on those diseases that (1) cause loss of basic combat training days by recruits, and (2) those that cost the government the largest for hospitalizations; they are adenovirus, influenza and meningococcal meningitidis. Attention was oriented towards antigenicity and efficacy evaluation of vaccines and other prophylactic agents to prevent these diseases before they gained in-roads to training units. The findings reported herein are results of the combined efforts of the laboratory scientist and the field epidemiologist. It is desirous that these studies will assist in the control of the more prevalent recruit diseases and suggest pathways for further study.

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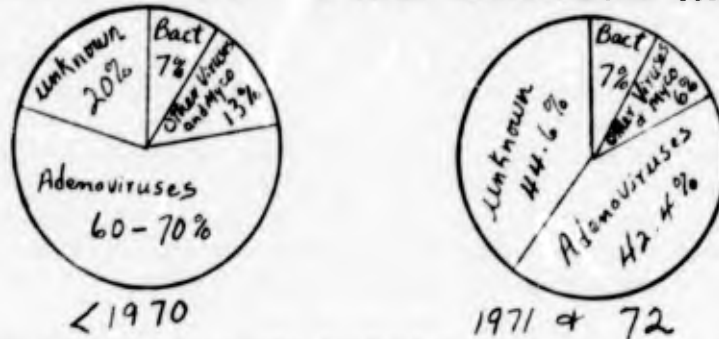
This project is concerned with methods of preventing acute respiratory disease (ARD) and infectious disease in military personnel, especially recruits. Antigenicity and efficacy studies were accomplished on the use of adenovirus 4 and 7, and meningococcal polysaccharide "C" vaccines. Some efforts were spent towards evaluating new identification techniques for the adenoviruses, and developing a more efficient transport media for respiratory viruses and fastidious bacteria.

Progress: Ensuing pages.

ACUTE RESPIRATORY DISEASE SURVEILLANCE

INTRODUCTION

Acute respiratory disease (ARD) continues to constitute the principal cause of morbidity in military recruits. During the fall and winter months up to 85 % develop acute symptoms while in the 4th to 7th week of basic combat training (BCT); and approximately 20% of these are hospitalized with acute respiratory illness. These surveillance studies were performed on BCT individuals who were hospitalized at eight BCT forts during fy'72. They indicate that following the administration of influenza and adenovirus types 4 and 7 vaccines to US Army receptionees at the reception centers of the eight BCT forts, during the 1971 and '72 respiratory disease seasons, URI etiologic agents have changed. The following two pie diagram past and present experience. With a decrease in ARD caused by adenovirus types 4 and 7, unknown etiologic agents have increased to occupy a larger portion of the pie. The serum neutralization test, which was



utilized in studies described elsewhere in this report, appeared approximately 33% more sensitive than the complement fixation procedure which was used in this surveillance study. Therefore, the area of the pie which diagrams adenovirus infections is most likely underestimated by one-third.

The Fort Ord portion of this study was performed in conjunction with, and as a part of the following studies: Infection Wild-Type Adenovirus Type 4 or Type 7 In Basic Combat Trainees Administered The Oral Enteric Phthalate-Coated Live Type 4 and Type 7 Adenovirus Vaccine; Field Trial Evaluation of Live, Oral Enteric-Coated Adenovirus Type 4 and 7 Vaccines Prepared With a Modified Formulation; Rapid Recognition Of Infectious Respiratory Virus Disease Agents In Nasopharyngeal Smears; and Field Trials Of A Modified Charcoal

Virus Transport Medium. These studies are discussed under their individual topics elsewhere in this report. However, to enhance portraying a more complete picture, results of ARD Surveillance Studies as determined by the Sixth US Army Medical Laboratory on eight BCT forts are shown.

MATERIALS AND METHODS

Approximately 7,296 Basic Combat Trainees, hospitalized with ARD, at eight BCT forts in CONUS were subjects for this surveillance. Retro-uvula swabs and acute-phase sera were obtained within 12 hours from each of these individuals, and convalescent-phase sera specimens were collected 14 to 21 days later. Paired sera were obtainable on 3,425 individuals. The swabs were placed in charcoal viral transport media and held at 5°C until examined at the Sixth US Army Medical Laboratory. Shipments were received weekly. Virus isolations were accomplished in tissue culture utilizing human embryonic kidney (HEK) and rhesus monkey embryonic kidney (MEK) monolayers. Sera were received as pairs after the convalescent collections, and they were used for detecting diagnostic rises in antibody titer, by the complement fixation test, to adenovirus, influenza A and B, and mycoplasma. Adenovirus sero-types were identified by microtiter neutralization tests utilizing Hela cell cultures. Influenza isolates were tested for by guinea pig erythrocyte hemadsorption, and sero-types were identified using specific anti-sera. Mycoplasma infections were identified by seroconversions; isolations and species determinations were accomplished only on those individuals who were also included in other studies reported elsewhere in this report.

RESULTS AND DISCUSSION

The ARD hospitalization rate per hundred per week, averaged over monthly intervals, and etiologic agents at Fort Ord during fiscal year 1972 are indicated in Figure 1. To facilitate a comparison the same information is shown for fy'71 on the same Figure. The early problem experienced in July '71 when the ARD rate climbed to 3.1/100/wk, due to adenovirus 7 infections, did not occur in 1972. However, the ARD rate began to climb in October '72, and by December it had reached the highest monthly average of 5.6 for

that year; and at that time the highest weekly rate was 6.8. The causative agent was adenovirus 7. Adenovirus 4 and 7 vaccines were administered between 1 November and 1 June. The usual holiday drop in ARD admissions occurred January, but they climbed above 3.2 in February and remained at that approximate level through June. Influenza infections began to occur in August and contributed significantly to the ARD rate throughout the year. As indicated by Figure 1, the largest number of influenza infections occurred January thru June 1972. Twenty-four (24) were caused by influenza A, and 14 by influenza B. These studies indicate that at Fort Ord, during the fiscal year, 50.3 % of ARD hospitalizations were caused by adenoviruses (93.9% type 7, and 6.1 % type 4), 9.7 % were caused by other agents, e.g., influenza A and B, and mycoplasma, and 40% were caused by agents not determined.

Figure 2 indicates the ARD hospitalization rate per hundred per week averaged over monthly intervals, and etiologic agents at Fort Lewis during fiscal year 1972. Fiscal year '71 is shown for comparison. In fy '71 adenovirus 4 vaccine was administered between October and January because of a high ARD rate in October when reached 3.7 in December, caused by adenovirus 4 agent. As is indicated, this vaccination significantly lowered the ARD rate by January. The ARD rate of 3.7 in November fy '72 was caused by adenovirus 7; however, 4 and 7 vaccines were administered. The rate fell to below 0.5 in February indicating vaccine effectiveness. Basic Combat Training was discontinued at Fort Lewis on 25 February 1972. Adenoviruses caused 31.8% of the ARD hospitalizations (98.4% type 7s, and 1.6% type 4s) and 61.9% were caused by agents not determined. There was no influenza at Fort Lewis during this study.

As indicated by Figure 3, the greatest number of ARD hospitalizations of 3.6 occurred in March at Fort Leonard Wood during fy '72. This is a shift to the right from fy '71 when the greatest number of 3.6 occurred in November. Adenovirus 4 and 7 vaccines were administered between 1 October and 15 May fy '72. Influenza, predominantly "As", appeared between August and June fy '72, with the largest number of cases occurring between January and April. Adenoviruses (93.9% 7s and 6.1% 4s) caused 29.2% of ARD hospitalizations, 9.1% were caused by other agents e.g., flu and mycoplasma, and 61.7% of the etiologic agents were not determined.

Figure 4 indicates that the largest number of ARD hospitalizations, 2.6/100/wk averaged for the month at Ft Knox in March 1972. In

1971 the peak occurred in March also. As ARD hospitalizations increased, the numbers that were caused by unknown agents increased and caused 69.4% of the ARDs for the 1972 year; Adenoviruses caused 29.9% of the ARDs, (56.8% 7s and 43.2% 4s) and 0.7% were caused by flu and mycoplasma. Adenovirus 4 and 7 vaccines were administered between 1 January and 15 May '72.

Fort Dix experienced a mild acute respiratory disease year in fy '72 when compared with '71 as indicated by Figure 5. In '72 significant ARD was not experienced until June when the rate climbed to 6.42/100/wk averaged over that month. 9% of the ARD was caused by influenza, the majority of which occurred between October and June (Flu A = 17, Flu B = 3). There were no influenza hospitalizations during the 1971 fiscal year. 22% of the '72 ARD hospitalizations were caused by adenoviruses (87.1% 7s; 12.9% 4s), and 69% of the causative agents were not determined. Adenovirus 4 and 7 vaccines were administered from September '71 to June '72.

As indicated by Figure 6, the highest ARD hospitalization rate of a 3.1 average over a monthly period occurred in March during fy '72 at Fort Jackson. The fiscal year ended with a rate of 2.5 in June. Influenza occurred between October '71 and June '72 (Flu A = 12; Flu B = 3) with the largest number of cases occurring between February and May. Adenovirus 4 and 7 vaccines were administered between January and May. 53% of the ARD hospitalizations were caused by adenoviruses (97.5% 7s and 2.5% 4s), 6.3% were caused by influenza (12 As and 3 Bs), and 40.7% of the etiologic agents were not determined. The statistics can be compared with the 1971 fiscal year.

The ARD at Fort Polk was higher during fy '72 than in fy '71, with the highest peak of 2.5 occurring in March of '72 as indicated in Figure 7. Influenza occurred between November and June (16 As and 9 Bs), with the largest number of cases in February and April. Adenovirus vaccines 4 and 7 were administered between January and May. ARD causative agents were 37.9% adenovirus (64.5% 7s and 35.5% 4s), 8.6% influenza and mycoplasma, and 53.5% of the etiologic agents were not determined.

Fort Campbell experienced rather mild ARD during the year as indicated in Figure 8. The highest monthly average of 1.5/100/wk occurred in August. There were no influenza cases. Adenovirus vaccines 4 and 7 were administered between January and March. Basic Combat Training discontinued at Fort Campbell on 15 March '72. ARD causative agents

were 35.5% adenovirus (97.7% 7s, and 2.3% 4s), and 67.5% agents not determined. There was no influenza during the year.

The average ARD hospitalization rate per hundred per week averaged over monthly intervals for all eight of the BCT Forts that were studied is indicated in Figure 9. The highest average ARD hospitalization rate occurred in March, and the lowest in July. 42% of these hospitalizations were caused by adenoviruses (85% 7s and 15% 4s). Other agents, e.g., influenza, mycoplasma and an occasional polio caused 6.2% of the influenzas, 70% were flu A and 30% Flu B. A total of 55 Mycoplasma pneumoniae cases occurred among all of the forts studied.

Table 1 indicates a break-down of adenovirus types and percentages isolated from hospitalized BCT's at each of the 8 study forts. The greatest number of isolates were from Ord, Jackson and Wood. A greater number of Adenovirus type 7s, than Adenovirus type 4s were isolated from all of the forts. The Adeno 7 vaccine most likely contained fewer logs of virus ($<10^{3.5}$) than desired for adequate immunization.

Table 2 indicates the number of Influenza cases that occurred at the 8 study forts, with a break-down by sero-type. The largest number of cases occurred at Wood (38). Sero-type A predominated at each fort (70% a; 30% B). A total of 55 Mycoplasma pneumoniae cases occurred among BCT's at these forts. Fort Leonard Wood experienced the greatest number of cases (28).

Even though a large number of these basic combat trainee ARD hospitalizations were caused by adenoviruses (42.2%) and other agents e.g., influenza and mycoplasma (6.2%), a large 51.6% of the causative agents were not determined. The more sensitive serum neutralization procedure indicated that about 1/3 of the adenoviruses might be missed by the complement fixation test which is routinely used for paired sera studies in this surveillance program. These would have been included in the undetermined group. Also making up a large portion of this group are probably the rhinoviruses. Maximum yield of rhinovirus is obtained before the recruit has severe enough URI symptoms to be hospitalized. When hospitalization eventually occurs and isolation specimens are collected, the virus is at a low level or has disappeared completely, and therefore no isolation is realized. It is envisioned that with continued use of adenovirus 4 and 7 vaccines and decline of these viruses as a cause of ARD, the unknown agents

will occupy a larger area as ARD cause. Attention should be focused towards other respiratory disease etiologic agents. Efforts in this laboratory continue towards developing a transport media that will carry viruses other than adenoviruses.

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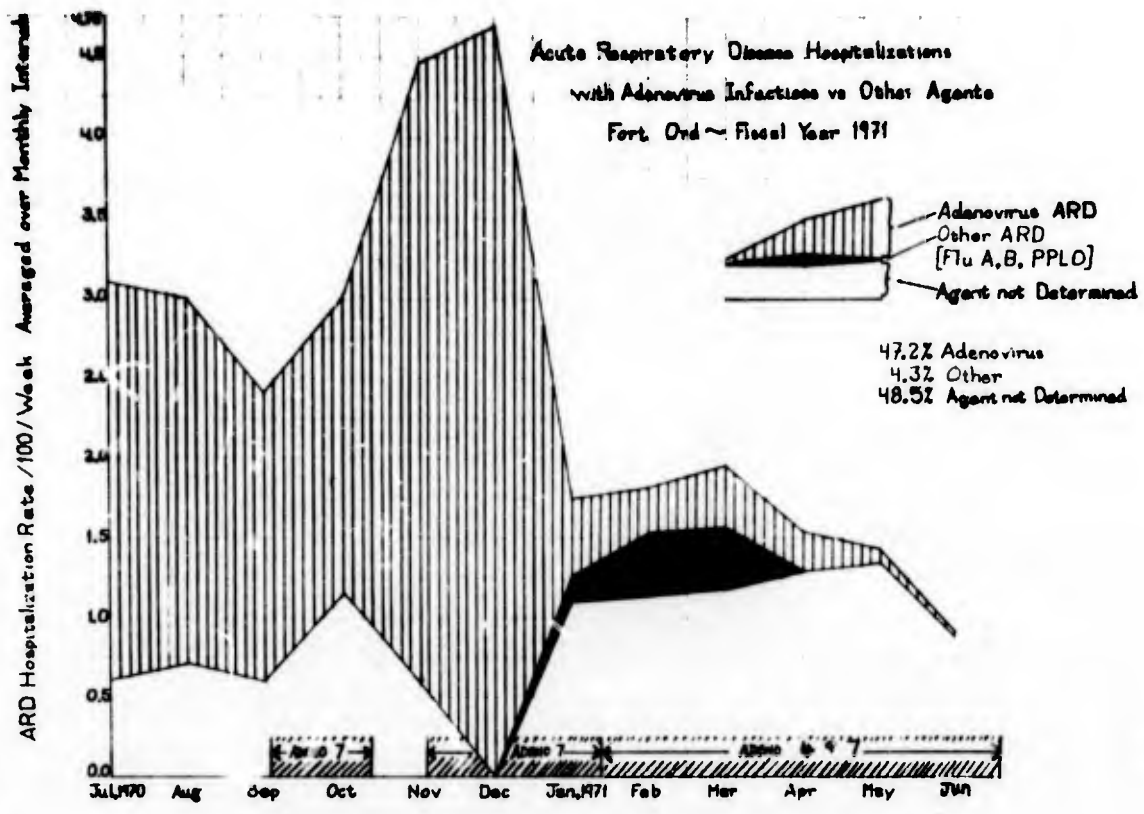


Figure 1

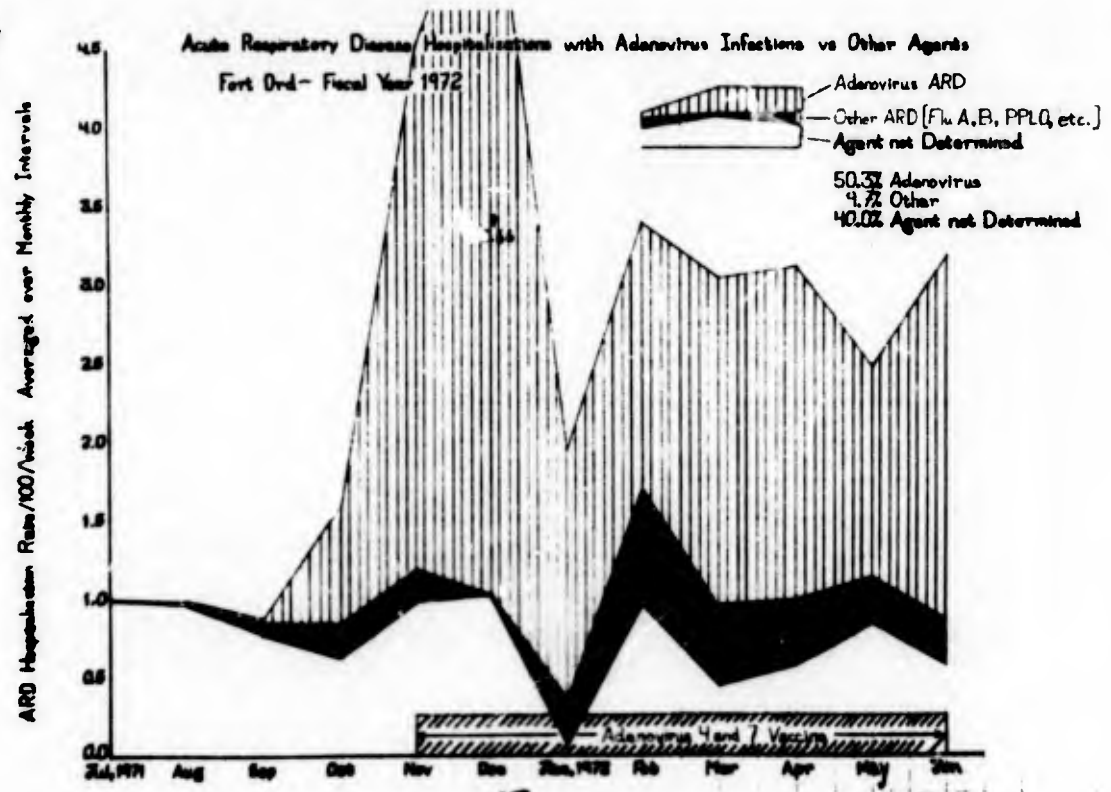
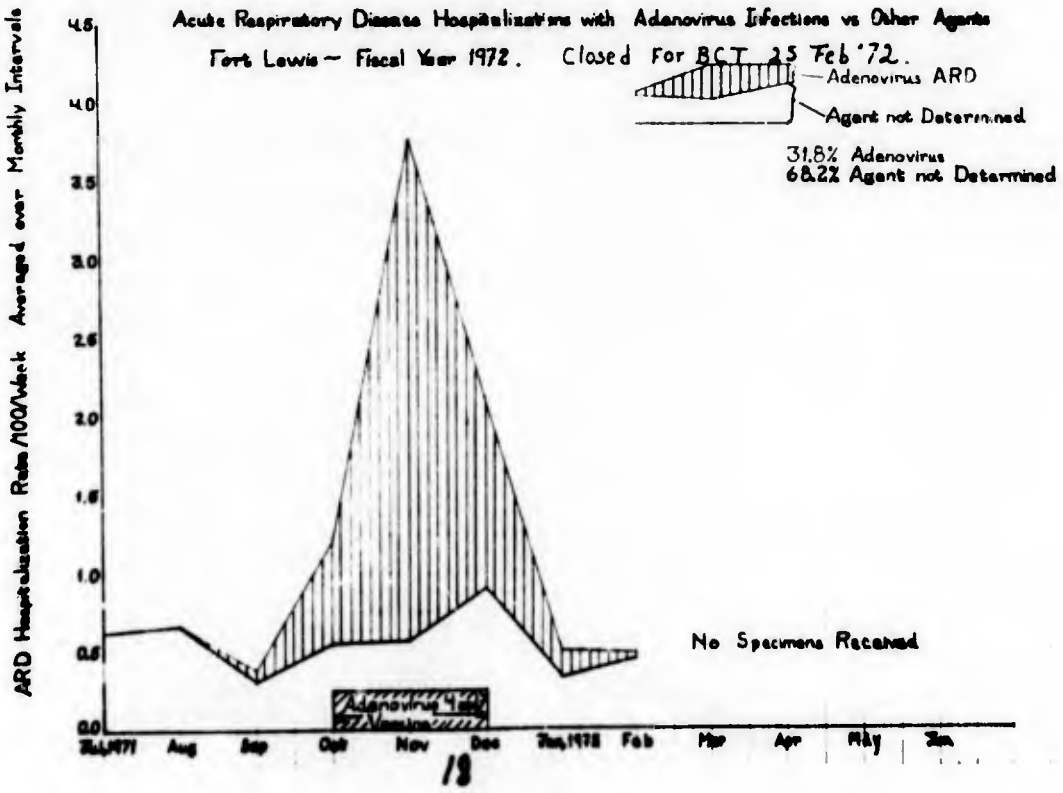
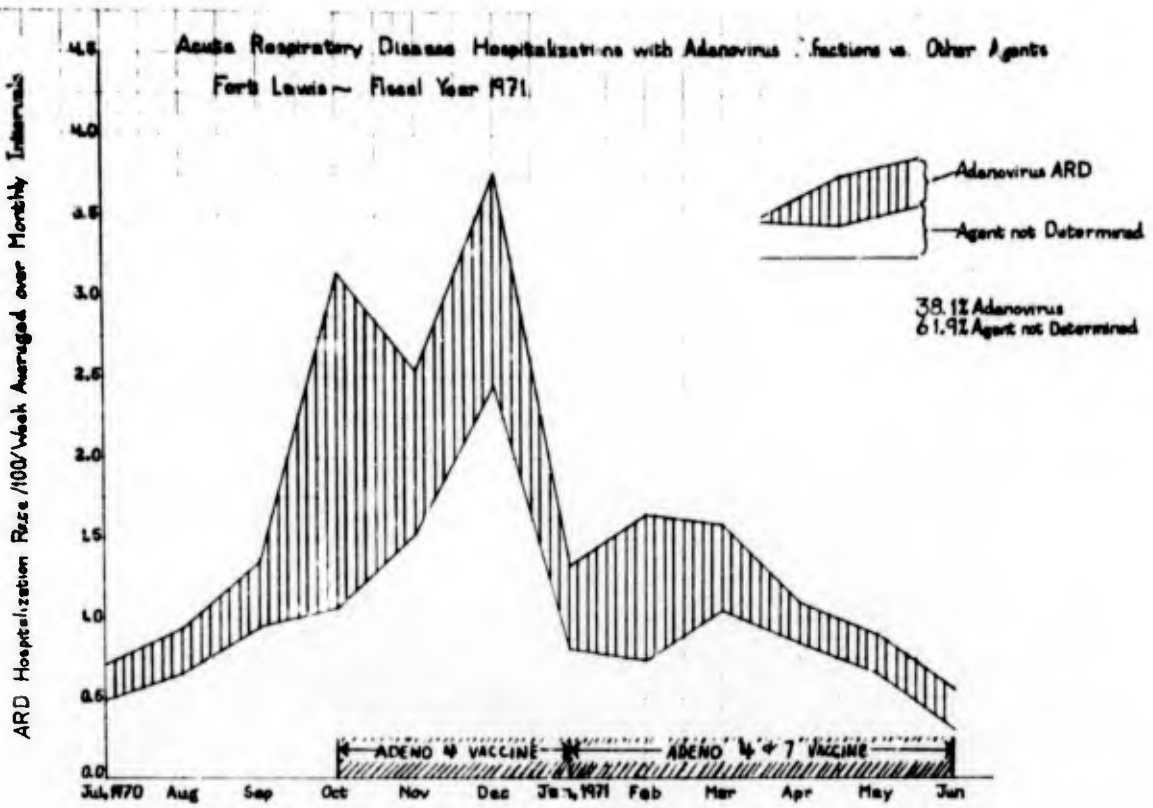


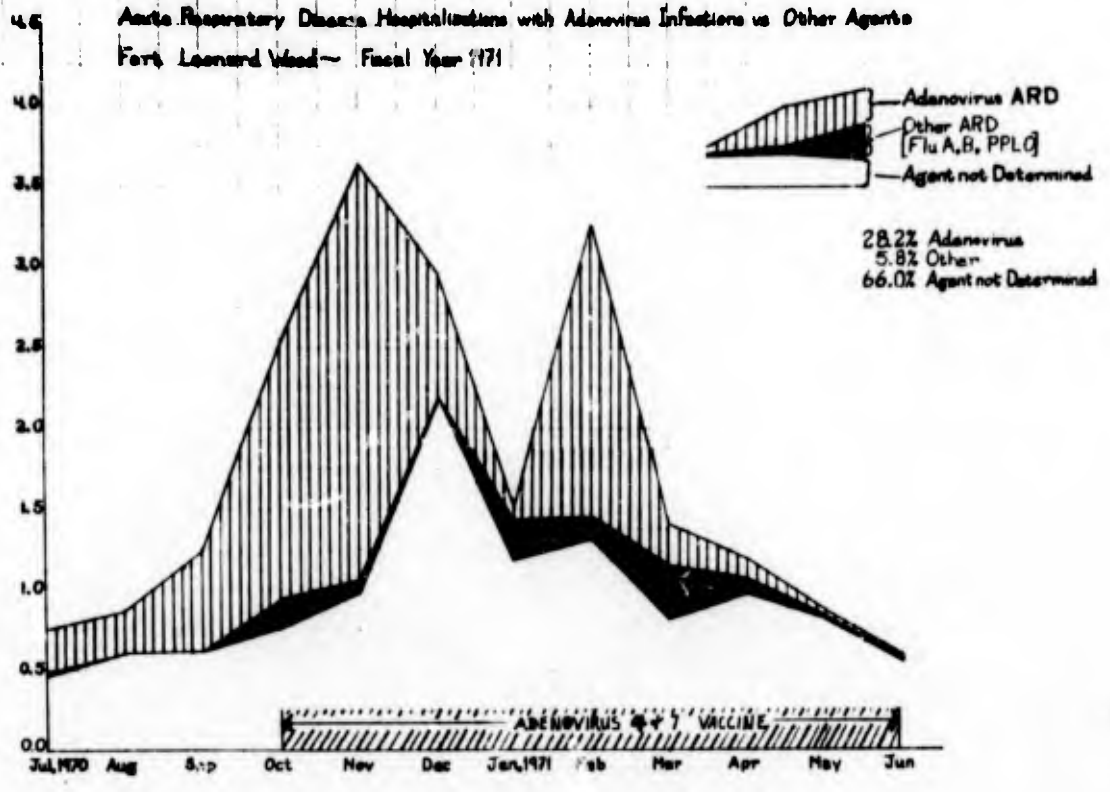
Figure 1

Figure 2



ARD Hospitalization Rate/100/Week Averaged over Monthly Intervals

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Leonard Wood - Fiscal Year 1971



ARD Hospitalization Rate/100/Week Averaged over Monthly Intervals

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Leonard Wood - Fiscal Year 1972

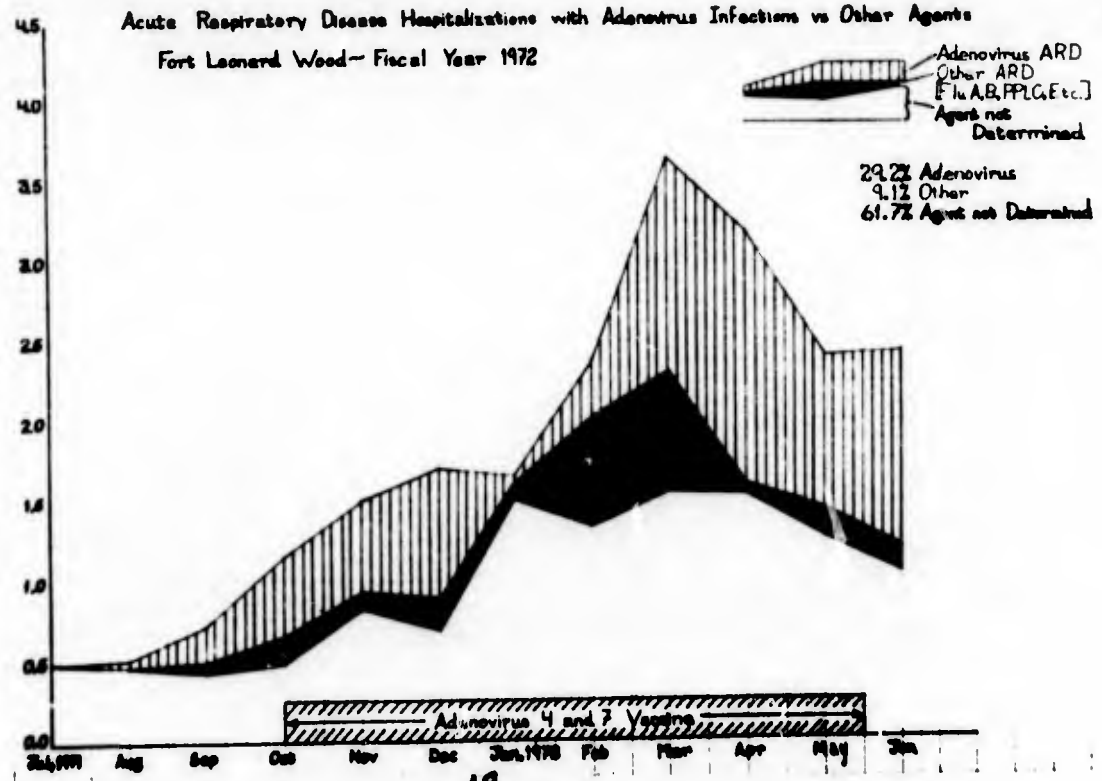
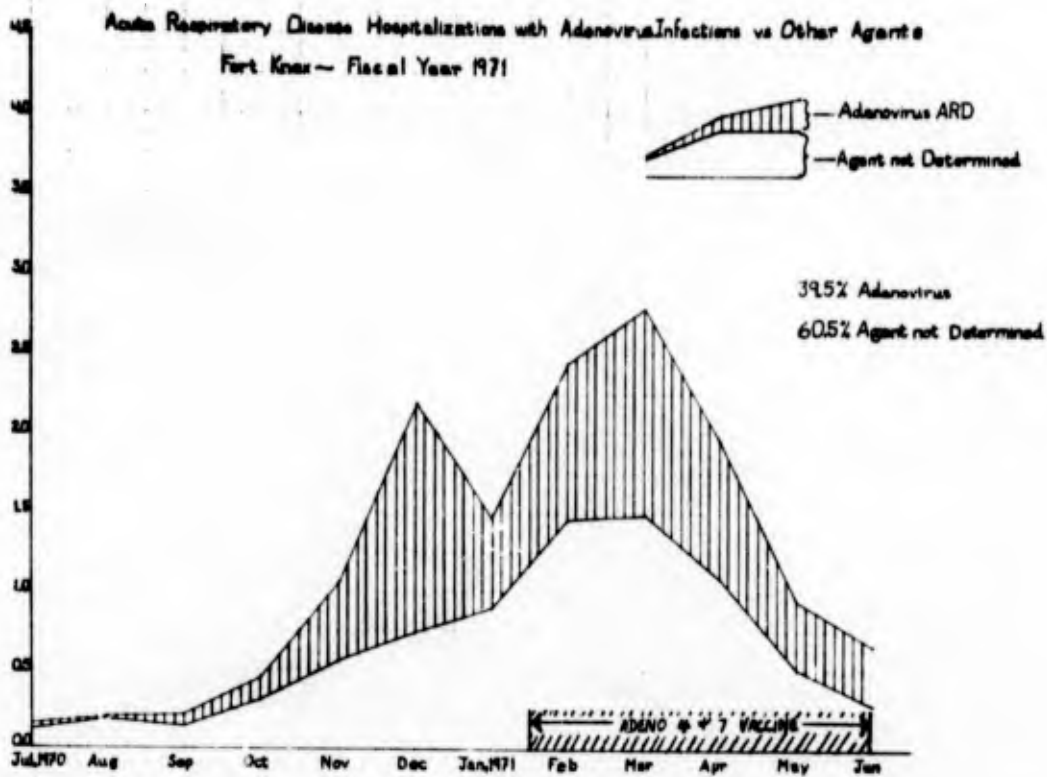


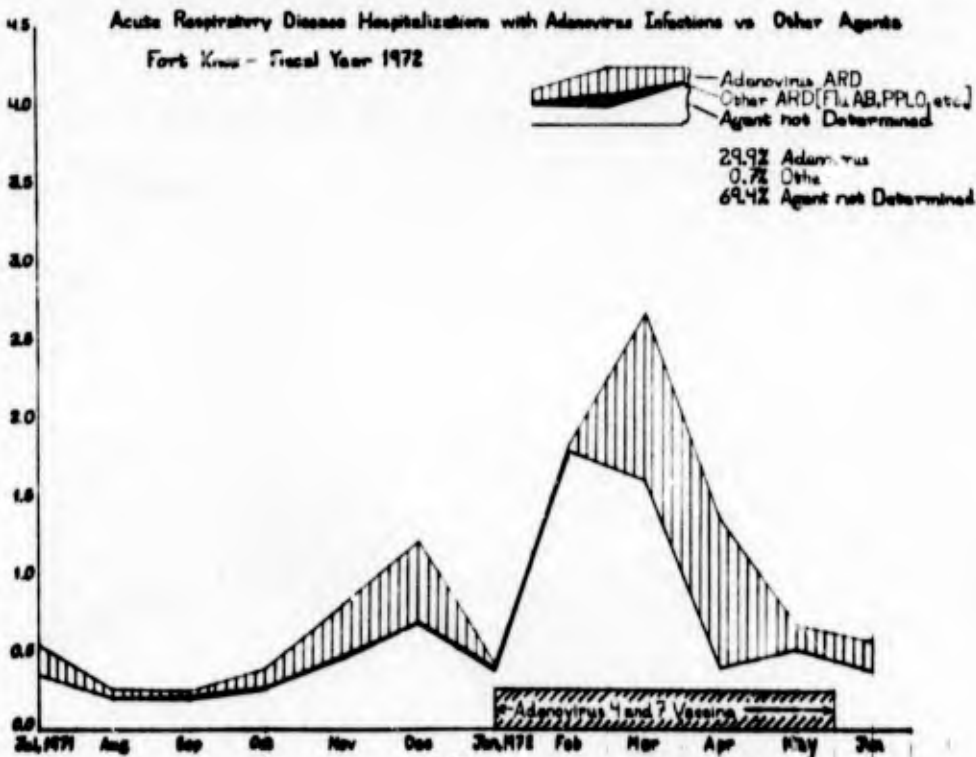
Figure 3

Figure 4

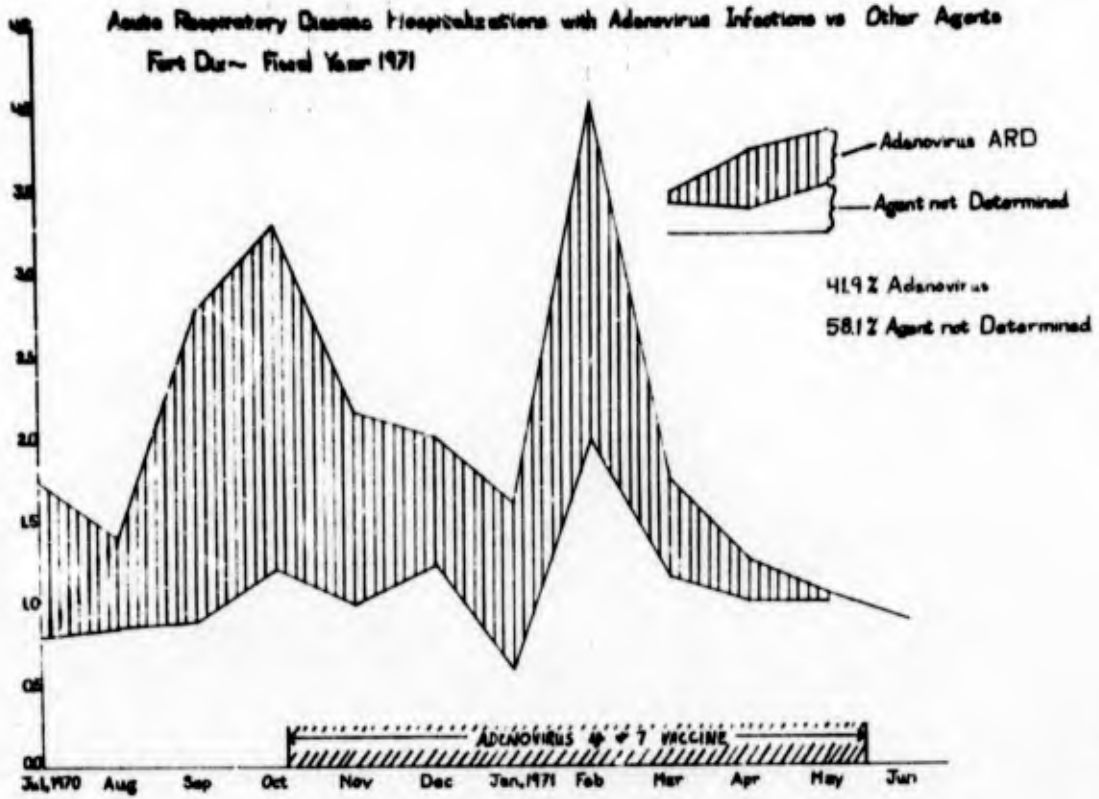
ARD Hospitalization Rate /100/Week Averaged over Monthly Intervals



ARD Hospitalization Rate /100/Week Averaged over Monthly Intervals



ARD Hospitalization Rate /100/Week Averaged over Monthly Intervals



ARD Hospitalization Rate /100/Week Averaged over Monthly Intervals

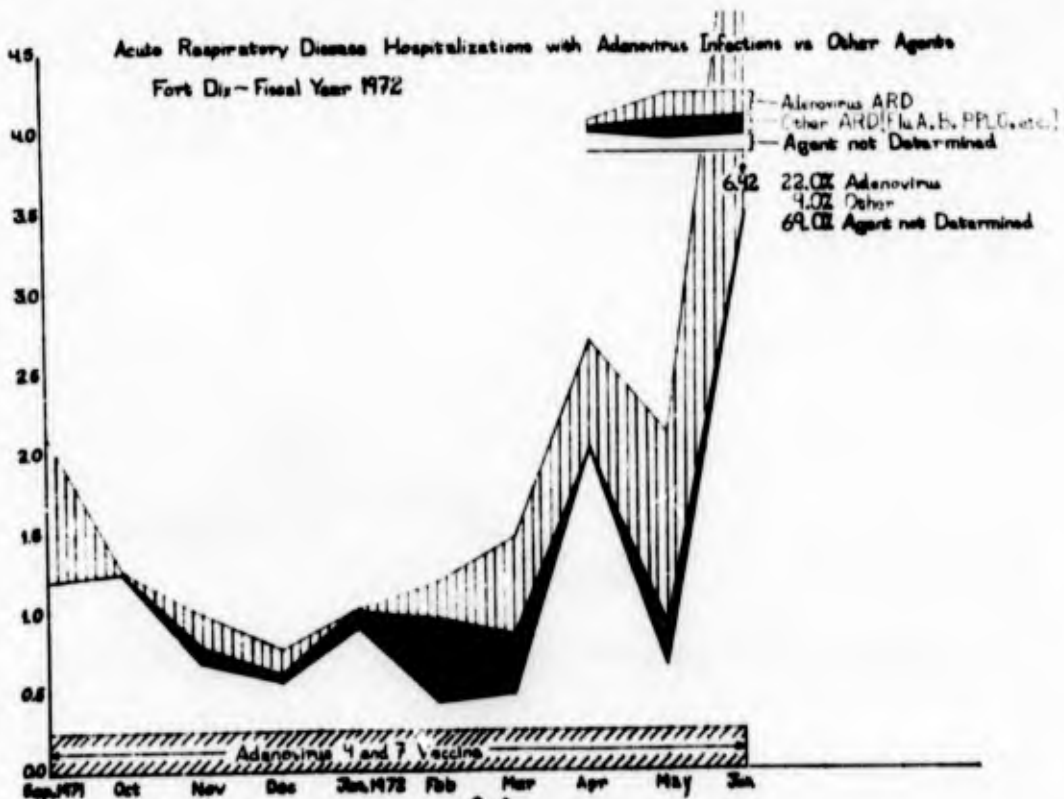
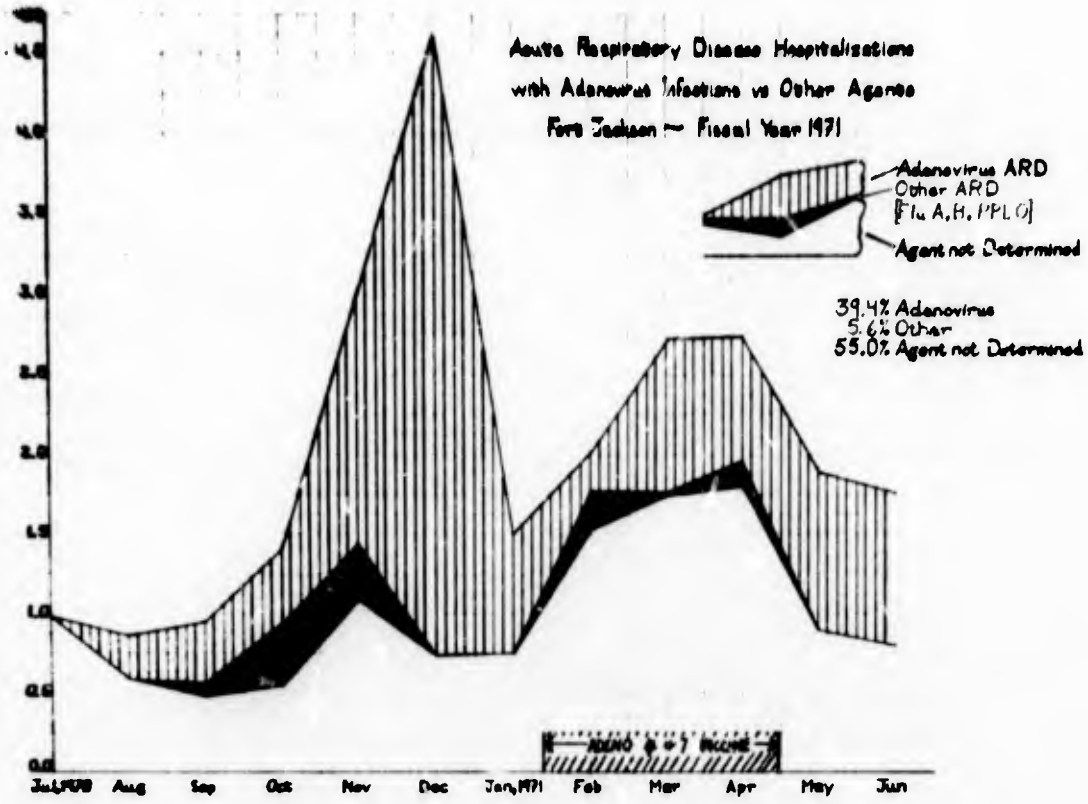


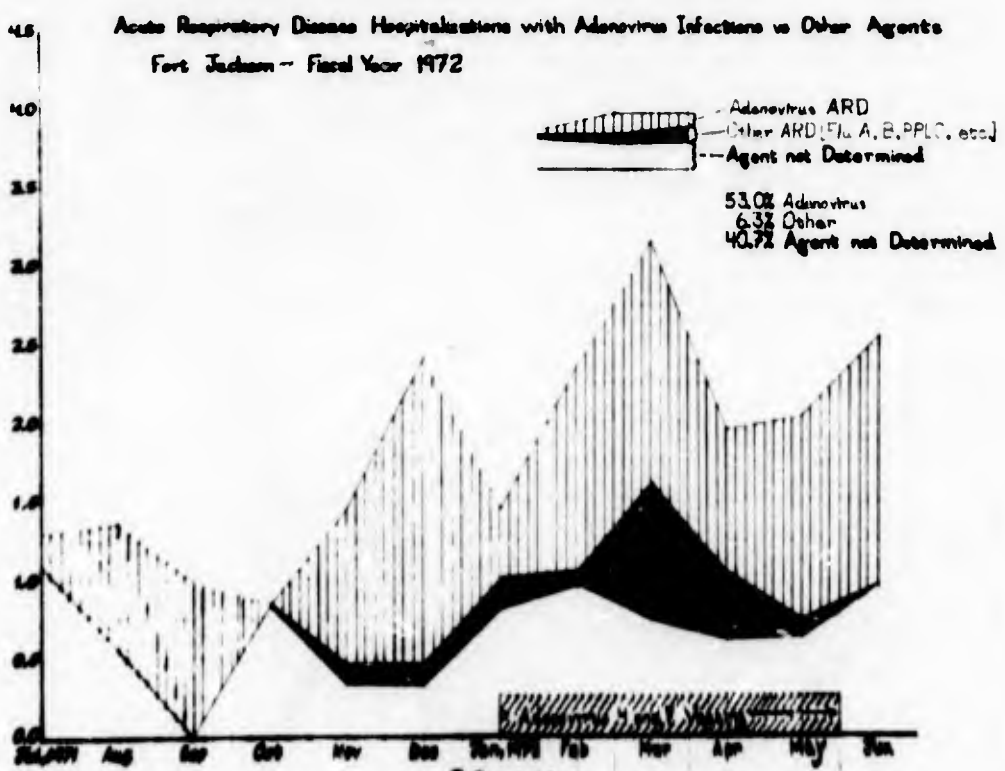
Figure 5

Figure 6

ARD Hospitalization Rate/100/Week Averaged over Monthly Intervals



ARD Hospitalization Rate/100/Week Averaged over Monthly Intervals



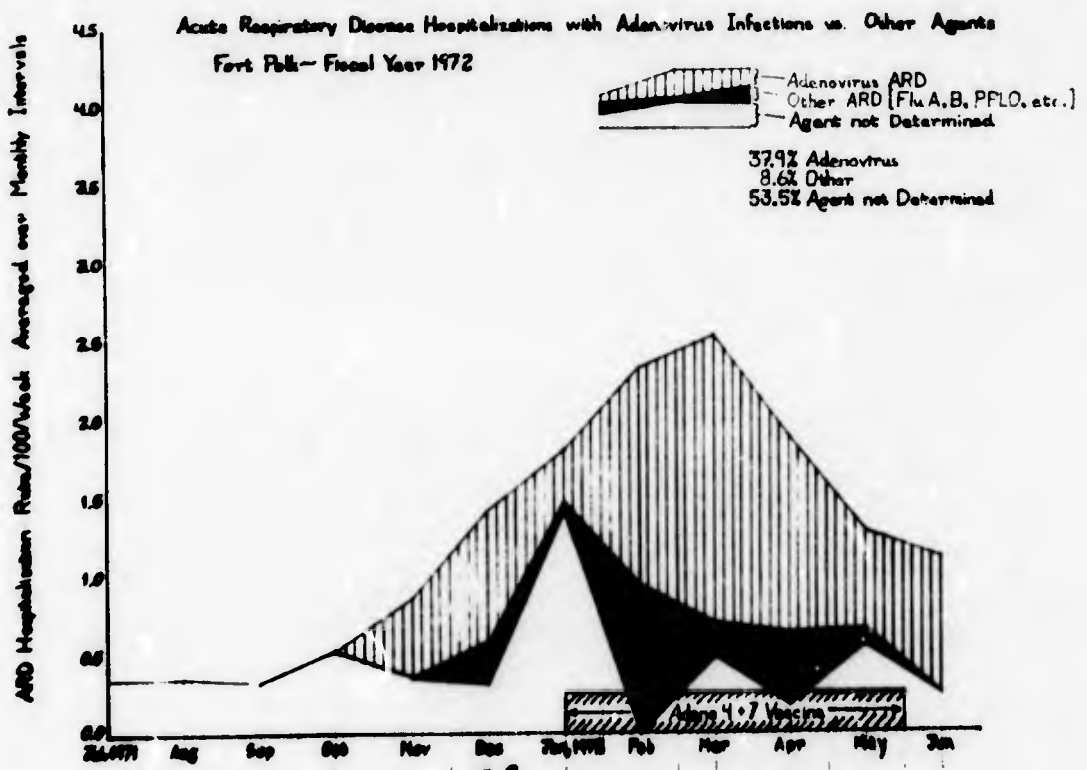
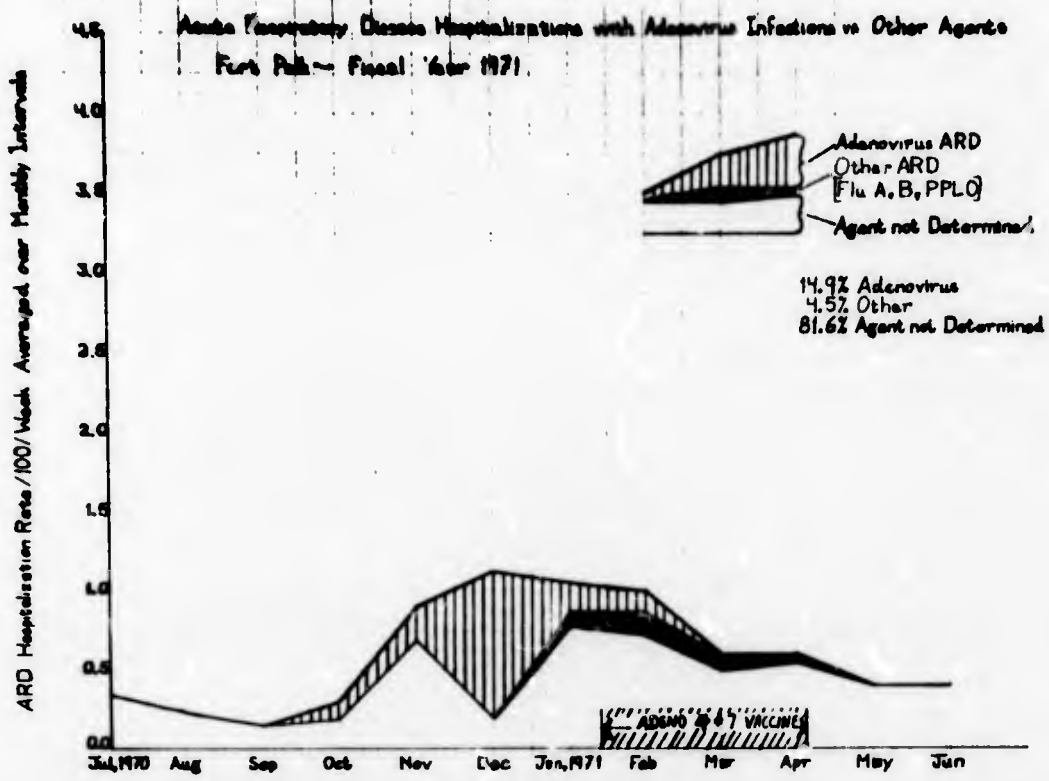
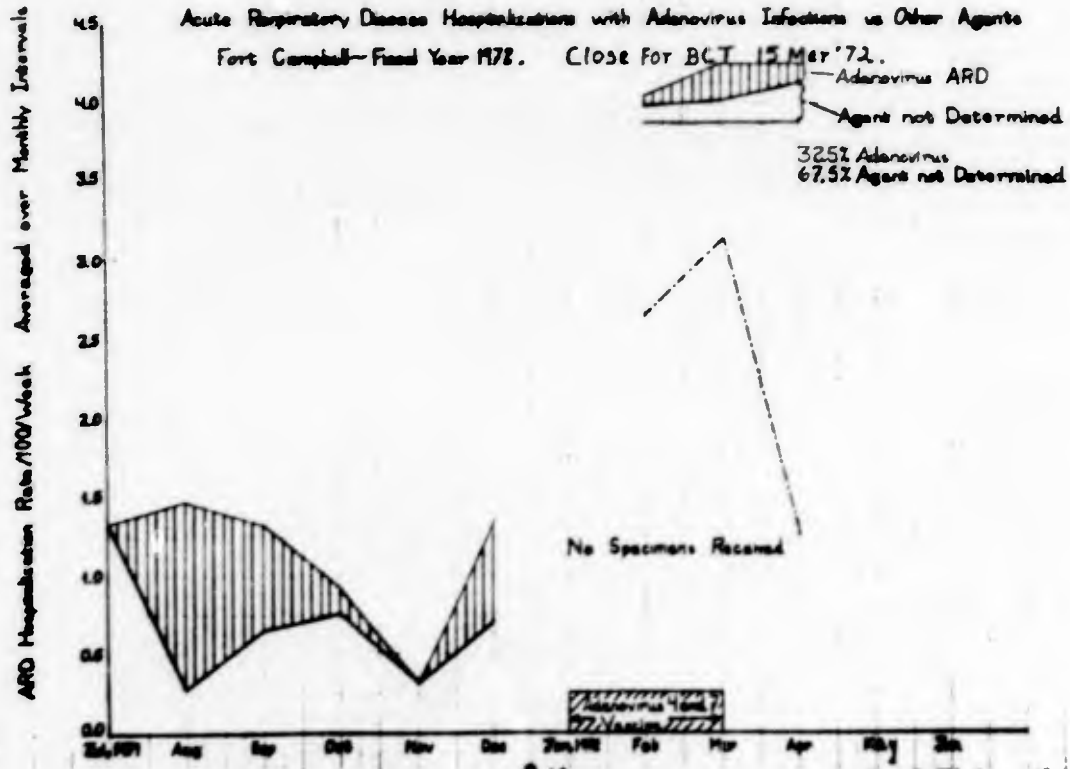
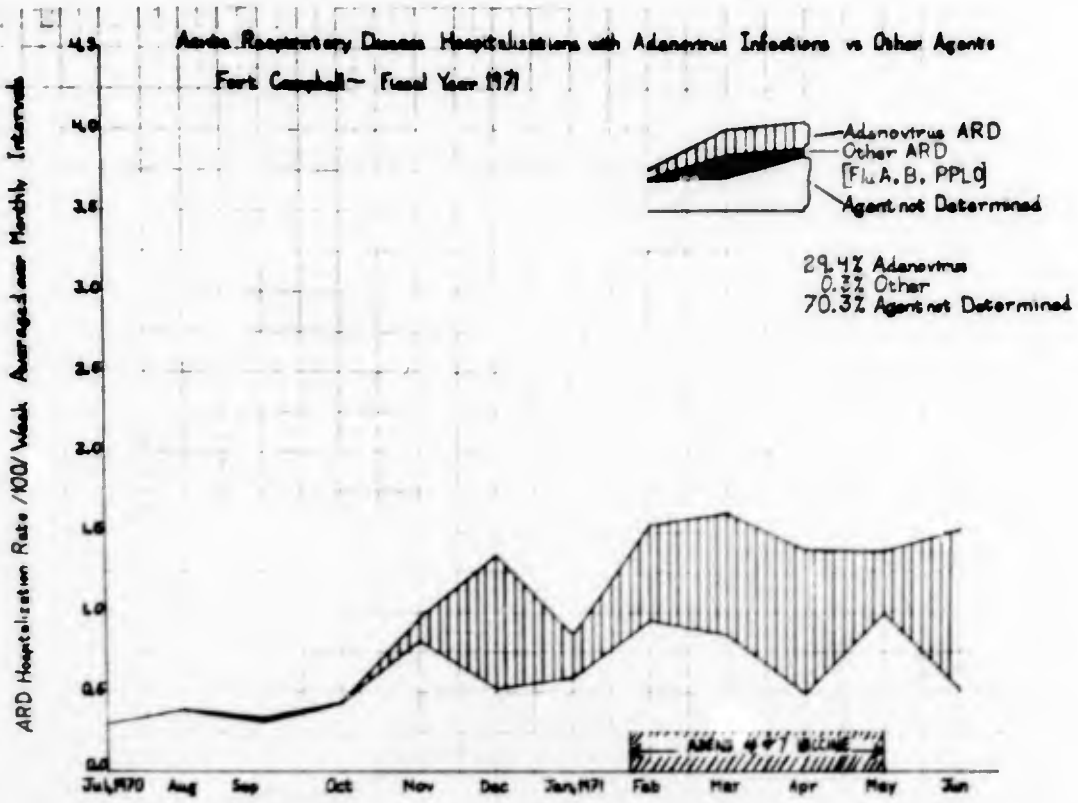


Figure 7

Figure 8



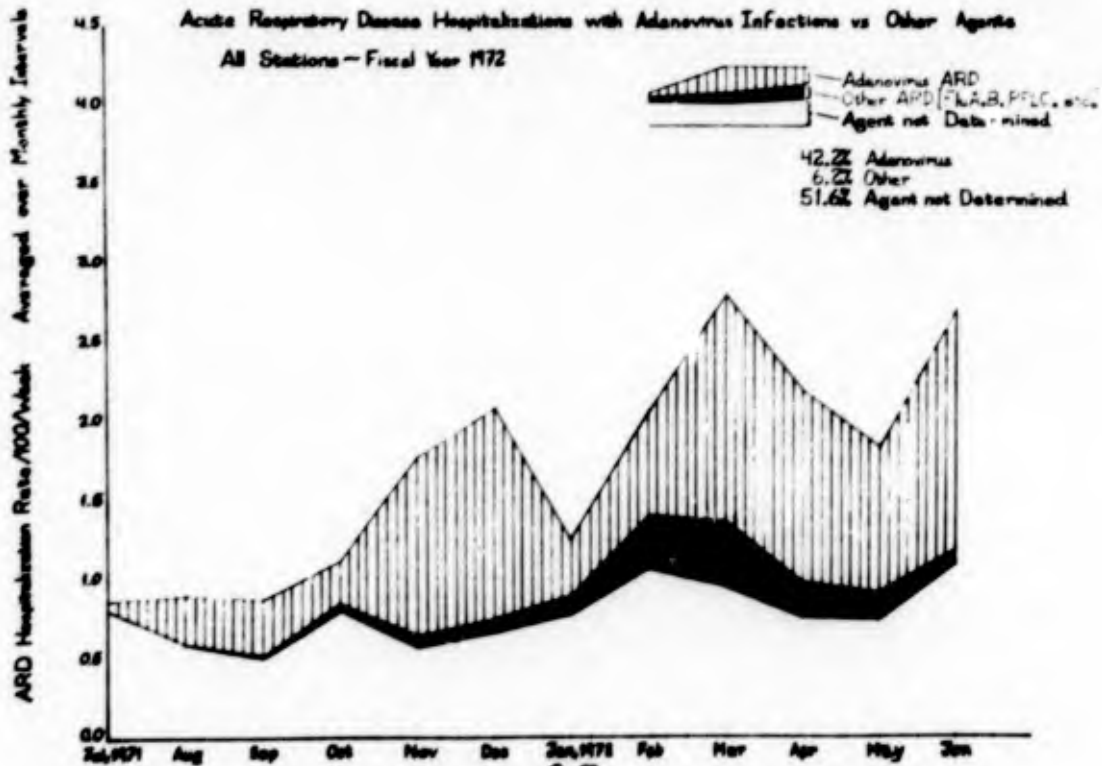
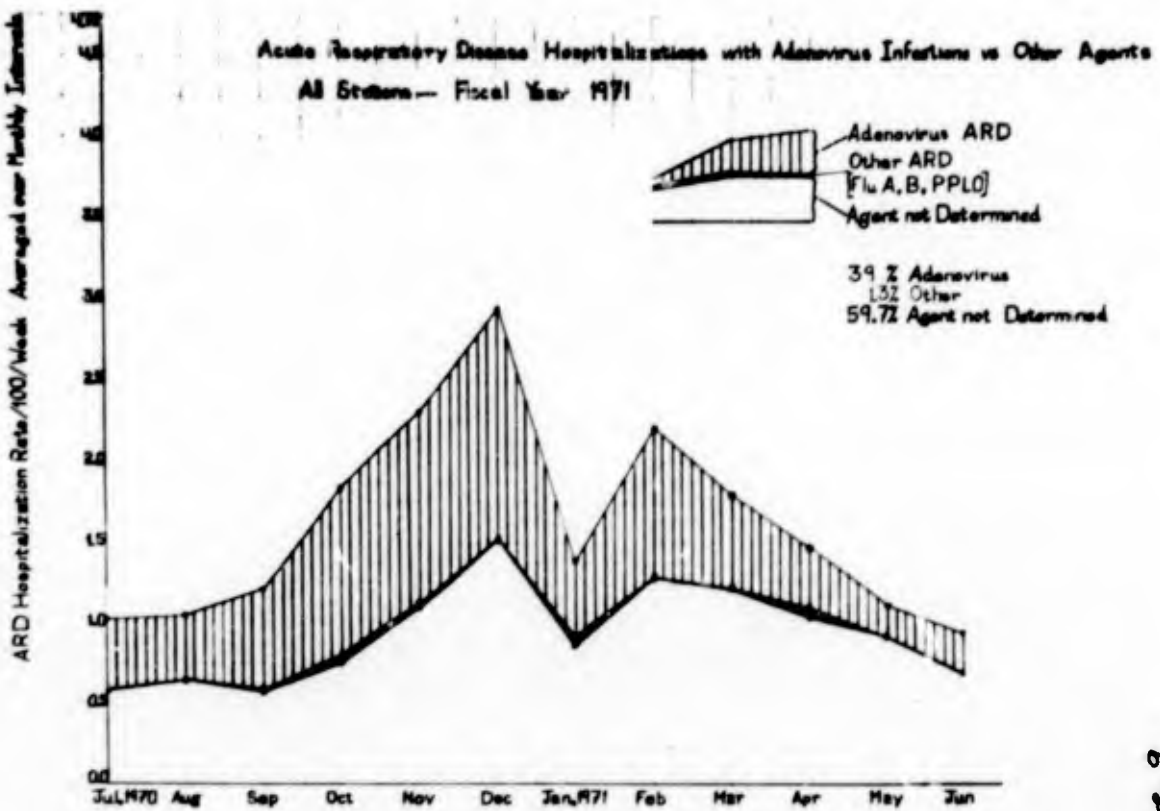


Figure 9

Table 1.

ADENOVIRUS ISOLATES

July '71 - July '72

	<u>Ad 4</u>	<u>Ad 7</u>	<u>Ad 4</u>	<u>Ad 7</u>
<u>FORT</u>				
DIX	7	32	12.9%	87.1%
KNOX	79	103	43.2%	56.8%
JACKSON	7	270	2.5%	97.5%
CAMPBELL	1	38	2.3%	97.7%
POLK	76	138	35.5%	64.5%
WOOD	14	216	6.1%	93.9%
LEWIS	2	132	1.6%	98.4%
ORD	24	325	6.1%	93.9%
TOTAL ISOLATES	<u>210</u>	<u>1254</u>	(15%)	(85%)

Table 2. - INFLUENZA CASES
July '71 - July '72

<u>FORT</u>	<u>FLU</u>	<u>A</u>	<u>B</u>	<u>PFLU</u>
DIX	17	3	3	3
KNOX	3	0	0	0
JACKSON	12	3	3	2
CAMPBELL	0	0	0	0
POLK	16	9	9	3
WOOD	38	18	18	28
LEWIS	0	0	0	2
ORD	21	14	14	16
PEARL HARBOR	4	0	0	1
	<u>111</u>	<u>47</u>	<u>47</u>	<u>55</u>
TOTAL	(70%)	(30%)		

27

Table 2

INFECTION WITH WILD TYPE ADENOVIRUS TYPE 4 OR TYPE 7
IN BASIC COMBAT TRAINEES ADMINISTERED THE ORAL ENTERIC
PHTHALATE COATED LIVE TYPE 4 AND TYPE 7 ADENOVIRUS VACCINE

INTRODUCTION

One of the expected events of successful immunization of BCTs with adenovirus types 4 and 7 vaccines was the emergence of other serologic types of adenovirus, perhaps type 14 or 21, as the principal agents of ARD at training centers. This has not occurred to-date. One possible explanation of this negative event is that recruits continue to carry and transmit adenovirus type 4 and/or type 7 in spite of successful immunization. This hypothesis was suggested by findings reported in 1970 by Smith et al from WRAIR and other studies accomplished since then: (1) Infection with enteric administered vaccine virus stimulated development of serum neutralizing antibody and prevented clinical disease, but did not stimulate homologous nasal secretory antibody, and (2) infection of the upper respiratory tract following administration of relatively high doses ($>10^5$) of type 4 vaccine virus appeared to be independent of the presence or absence of type 4 serum neutralizing antibody⁽¹⁾. The purpose of this study was to determine if asymptomatic reinfection with wild type adenovirus type 4 or type 7 does occur in basic combat trainees successfully immunized with live enteric type 4 or type 7 vaccines.

MATERIALS AND METHODS

One hundred and thirty men of Company A, 5th Battalion, 1st Basic Combat Training Brigade at Fort Ord were selected for the study. Throat swabs, nasal washes and pre-immunization bleedings were obtained during fill week prior to administration of the type 4 and type 7 tablets. Subsequent nasal washes and bleedings were obtained during week 3 and week 6 of training. Throat swabs were collected for all seven weeks of the study, and were placed in charcoal viral transport media for shipment to the Sixth U S Army Medical Laboratory. They were then processed by procedures routine for the surveillance program. Virus isolation attempts were made in tubes of Human Embryo Kidney (HEK) cell cultures which were observed 18 days for cytopathology characteristic of the group. Negative specimens were then tested by guinea pig erythrocyte hemadsorption for myxoviruses before being discarded. Adenovirus

isolates obtained from positive specimens were identified by microtiter neutralization tests in 96 well plates of Hela Cell Cultures. Serum specimens were tested for adenovirus type 4 or type 7 antibody by a constant virus-serum dilution microtiter neutralization procedure performed in Hela cells. Complement fixation titers were also determined for these sera using a CF antigen prepared at this laboratory from type 7 adenovirus infected Hela Cell cultures. A representative sample of sera that were negative or demonstrated no rise in CF titer were tested against adenovirus type 4 prepared CF antigens. Results comparable to those obtained with the type 7 antigen demonstrated the original antigen to be group specific as anticipated. Nasal washes were obtained by administration of 5cc of normal saline to each nostril of the recruits tested. Mucus was separated from the specimens with cotton tipped swabs. Particulate material was removed by centrifugation and the supernates concentrated from their original volume of 5-10 ml to 1 ml with Amicon membrane ultrafilters #CF-50. Nasal wash concentrates were tested undiluted and at a 1:4 dilution for adenovirus type 4 and type 7 neutralization antibody as described for sera above. IgA immunoglobulin concentration was determined for each nasal wash concentrate with meloy anti-IgA agar gel immunodiffusion plates. Human colostrum secretory IgA was used as standard.

RESULTS

One hundred eight of the original 130 recruits completed the study having undergone all three bleedings. Seroconversions for individuals with week 0 SN antibody titers of $<1:4$ are shown in Table 1. Sixty-four percent of the group were susceptible to adeno type 4 and 52 percent to adeno type 7 at the start of the study. One-third (34%) were susceptible to both viruses by this criterion. Eighty-three percent of the adeno type 4 susceptible group had converted by week 3 as opposed to only 52 percent of the adeno type 7 susceptible group. Seroconversion at week 3 was the only available indicator of vaccine virus infection. By week 6 virtually all of the original group of susceptibles had converted and 86 percent had seroconverted to both viruses. Two individuals of the 108 with three sera were dropped from the adenovirus type 7 analysis because of technical reasons.

Table II show the distribution of recruit SN antibody titers to adenovirus type 4 at week zero and the number of recruits that subsequently converted with four-fold rises in SN antibody titer at weeks 3 and 6. It should be noted that six of 33 individuals with SN antibody titers $\geq 1:8$ at week zero seroconverted at week 3 and five of

the remaining 27 seroconverted at week 6.

Table III presents the available evidence for wild type 4 virus infection of the week 3 sero-conversion negative recruits and re-infection of the week 3 type 4 seroconversion positive recruits. Wild type virus infection was defined as the establishment of wild type 4 virus in the oropharynx as demonstrated by virus isolation from one or more week 3 or later throat swabs and/or the development of nasal secretory antibody demonstrated in the week 3 or week 6 nasal washes. Six individuals were dropped from the analysis because they had secretory type 4 antibody in the week zero nasal wash or had a wild type 4 virus isolation from the week zero, week 1 or week 2 throat swab. It can be seen that the percentage of wild Adenovirus type 4 infections demonstrated in the week 3 seroconversion negative recruits is similar to those demonstrated in the successfully immunized (week 3 seroconversion positive) recruits. Nine of 39 recruits with less than four-fold type 4 antibody rises from week 0 to week 3 were deemed infected with wild type virus during the period week 3 to week 6. This compares to 15 infected of 63 recruits in the type 4 week seroconverters.

The data for Adenovirus type 7 seroconversions and wild type infections is presented in table IV and V. Fifty-one percent of the week zero type 7 SN antibody negative recruits were successfully immunized with the type 7 vaccine i.e. sero-converted at week 3 without evidence of an intervening wild type virus infection prior to week 3. Again, as with type 4 virus, there were a number of seroconversions in individuals with week zero SN antibody titers of $\geq 1:8$. Wild type adenovirus type 7 was quite prevalent at Ft. Ord during the period of this study. The ARD rate was over 7/100/wk at the time of the week 3 collection and type 7 isolates were running 50:1 over type 4 isolates. The study group reflected this in that 66 percent has some evidence of wild type 7 infection. Again, as with adenovirus type 4, the isolation of wild adenovirus type 7 from throat swabs or the demonstration of nasal wash antibody was independent of whether or not the recruit had seroconverted to adenovirus type 7 by week 3 of training.

There was no apparent correlation between the concentration of secretory IgA in the nasal washes and the ability to demonstrate adenovirus type 4 or type 7 antibody. Neutralization antibody was demonstrated in nasal wash concentrates with IgA levels of less than 0.25 mg/ml which was the minimum level detectible with the immuno-

diffusion technique. IgA levels varied to a high of 2.8 mg/ml with a mean of 1.1 mg/ml.

Although evidence of subsequent re-infection with adenovirus type 7 was independent of serologic evidence of response to the vaccine at week 3, it was not independent of demonstrable type 7 SN antibody at week 0. This is shown in Table VI and the data are similar for both the week 3 seroconverters and the non-responders. It can be seen that positive indicators of wild-type adenovirus type 7 infection in week zero antibody negative recruits are nearly twice that of recruits with pre-existing antibody irrespective of vaccine induced seroconversion at week 3. Further, reinfection as indicated by virus isolation occurred much earlier in those individuals that did not have week zero SN antibody. Eighteen of 31 virus isolations in this group were evident in the week 3 throat swab as opposed to only one of 7 virus isolations made in the week zero antibody positive group. The sero-conversion positive recruits which had a higher proportion of week zero antibody negative people had virus positive throat swabs at an average 3.8 weeks as opposed to the average 4.3 weeks of the seroconversion negative recruits.

Complement fixation (CF) titers were performed on sera from this study to determine how the procedure compares in sensitivity to the serum neutralization test. In Table VII it can be seen that 51 percent of the recruits that seroconverted at week 3 (vaccine responders) by SN test also converted by CF test. Conversions for the period week 0 to week 6 are shown in Table VIII. By this time the CF procedure was picking up 67% of the SN conversions. Serologic conversion by CF test versus the indicators of wild virus adenovirus type 7 infection is shown in Table IX. Fourteen percent of those recruits grouped as having undergone wild virus infection were not picked up by either serologic procedure. The nine recruits classified as infected and missed by the SN test may have been infected too late to sero-convert. The four recruits that shed virus but did not seroconvert by SN test did not shed virus until the 5th or 6th week of training.

DISCUSSION

The data resulting from this study does not negate the hypothesis that type 4 and type 7 adenovirus immunized recruits may subsequently

become infected with wild type 4 and type 7 adenovirus. They therefore may potentially contribute to the maintenance of these two strains in the recruit population. A field study such as this performed in the midst of an adenovirus epidemic is fraught with difficulties in interpretation of what is a vaccine virus infection and what is a wild type virus infection. Those counted as vaccine infections probably include some wild type virus infections, however, it is reasonable to conclude that the majority of week 3 seroconversions scored as vaccine virus infections were in fact just that. This^{is} because we know that adenovirus ARD at Fort Ord generally occurs after the second week of training (average 4.2 weeks of training)⁽²⁾ and all seroconversions scored as vaccine virus infections had negative throat swabs on week zero, one and two and a negative nasal wash SN antibody titer on week three of training. Further, there were more type 4 seroconversions at week 3 than type 7 conversions. The epidemic in the recruit population at that time was primarily adenovirus type 7 with virus isolations in the surveillance project running nearly 50 type 7 isolates for every type 4 isolate. The data for wild type virus infections in on softer ground in that it is well established that the enteric live virus vaccine does not induce nasal secretory antibody and vaccine virus administered in this manner is not subsequently shed from the oropharynx of infected recruits⁽¹⁾. Some wild type virus infections may have been missed using these criteria but there is no reason to believe they would have been missed more frequently in the week 3 non-seroconverters than those scored as vaccine infections. In view of the above it is difficult to escape the conclusion that vaccine virus infection with either type 4 or type 7 virus did not prevent subsequent infection of the Oropharynx with wild type 4 or type 7 virus and that wild type 4 and type 7 can be maintained in a population of recruits when the majority are either immune from previous type 4 and type 7 infection or have been immunized with type 4 and type 7 vaccine virus. It would appear from the data presented earlier in Table VI and the fact that wild type virus isolations were earlier and occurred more frequently in recruits without pre-existing homologous serum antibody that the timing of establishment of wild type virus infection is in large part responsible for this phenomenon. The implication is therefore that reinfection with wild type virus occurs too early for successful immunization to significantly affect virus shedding from the oropharynx.

The complement fixation test results provided some disturbing data from the standpoint of the adenovirus surveillance project. This is the only serologic procedure utilized in the project and judging from its lack of sensitivity we must conclude we underestimate the

number of ARDs attributable to adenovirus infection by at least a full one-third. Further, it had been a general assumption in the past that the CF procedure would not pick up vaccine virus infections. If we are to accept the data and supporting arguments in this study, we must conclude that the CF test demonstrates at least 50% of the vaccine virus infections. Thus, those recruit illnesses that occur before week 3 of training that are not supported by virus isolations can not be attributed to adenovirus infections on the basis of CF data if the recruits have been immunized.

SUMMARY

In summary, 130 recruits of one BCT Company at Fort Ord were pre-bled during fill week and administered the type 4 and type 7 oral enteric phthalate coated live adenovirus vaccine. One hundred eight of these recruits were followed with weekly throat swabs for virus isolation and post vaccine bleedings at week 3 and week 6 of training. Nasal washes were obtained at week 0, week 3, and week 6, and tested for adenovirus type 4 and 7 neutralization antibody. Sixty-four percent of the group had no detectible antibody (SN titer <1:4) to adenovirus type 4 and 52 percent had no detectible antibody to type 7 virus at the week zero bleeding. Thirty-four percent of the group were susceptible to both viruses by this criterion.

Fourfold increases in SN titers at week 3 indicated that 82 percent of type 4 virus susceptible individuals and 52 percent of type 7 virus susceptible recruits underwent enteric infections with the vaccine virus. Thirty-three percent of individuals susceptible to both viruses seroconverted to both adenovirus type 4 and type 7 at the week 3 bleeding.

Isolation of virus from week 3, 4, 5, or 6 throat swabs and/or development of specific neutralizing antibody in the week 3 or week 6 nasal washes were utilized as indicators of wild virus infection post immunization. These criteria indicated that 24 percent of the group were infected with wild type 4 adenovirus post immunization and that infection rates were the same for those who had or those who had not seroconverted to vaccine virus by week 3. Similar results were obtained with adenovirus type 7 though infection rates were nearly double those with type 4 virus. Sixty-six percent of the group had one or both positive indicators of wild type 7 virus infection after immunization. Again, the infection rates were the

same in those who had and those who had not seroconverted to vaccine virus by week 3. Analysis of the adenovirus type 7 data revealed that wild virus infection rates were twice as high in recruits with no week 0 type 7 antibody than in those with SN titers $\geq 1:4$ irrespective of subsequent seroconversion to vaccine virus. Further, virus isolation from throat swabs was detected earlier in recruits without detectible antibody in the week 0 serum. The complement fixation procedure as performed in this laboratory detects only 67% of those individuals that seroconvert by the microtiter serum neutralization procedure. It did, however, detect 50% of those seroconversions attributable to vaccine virus infection, a feature of this procedure that may not have been appreciated in the past. The implications of the complement fixation data in regard to the adenovirus surveillance program is discussed.

TABLE I

Recruits with negative serum antibody titers at week zero and subsequent conversion to antibody positive status.

Total		Serum Neutralizing Antibody Titer					
		<1:4 Week 0		≥1:8 Week 3		≥1:8 Week 6	
Adenovirus Recruits		#	%	#	%	#	%
Type 4	108	69	63.9	57	82.6	64	92.8
Type 7	106	55	51.9	28	50.9	52	94.5
Type 7 & 4	106	36	34.0	12	33.3	31	86.1

TABLE II

Recruit serum neutralizing antibody titers to adenovirus type 4 at week zero and subsequent four-fold conversion.

	Week 0		Week 3		Week 6		Total	
	#	%	#	%	#	%*	#	%
<1:4	69	63.9	57	82.6	7	53.8	64	92.8
1:4	6	5.7	3	50.0	1	33.3	4	66.6
1:8	17	16.0	5	29.4	2	16.7	7	41.1
1:16	10	9.4	1	10.0	3	33.3	4	40.0
≥1:32	6	5.7	0	0.0	0	0.0	0	0.0
TOTAL	108	100.0	66	61.1	13	31.0	79	73.2

* Percent of those remaining unchanged at week 3.

TABLE III

Evidence of adenovirus type 4 infection in second serum conversion recruits versus second serum no-change recruits.

Week 3 Antibody	Virus Isolation	Nasal Antibody	Both	Total	% Positive
<4 fold Rise	0/39*	9/39	0/30	9/39	23.1
≥4 fold Rise	4/63	10/63	1/63	15/63	23.8
TOTAL	4/102	19/102	1/102	24/102	23.5

* Number recruits positive over number tested

TABLE IV

Recruit serum neutralizing antibody titers to adenovirus type 7 at week zero and subsequent. Four-Fold Conversions.

	Four Fold Rises							
	Week 0		Week 3		Week 6		Total	
	#	%	#	%	#	% *	#	%
<1:4	55	51.9	28	50.9	24	88.9	52	94.6
1:4	8	7.5	5	62.5	3	100.0	8	100.0
1:8	11	10.4	6	54.5	2	40.0	8	72.6
1:16	19	17.9	4	21.1	5	33.3	9	47.3
≥1:32	13	12.3	0	0.0	0	0.0	0	0.0
TOTAL	106	100.0	43	40.6	34	54.0	77	73.6

* Percent of those remaining unchanged at week 3

TABLE V

Evidence of adenovirus type 7 infection in second serum conversion recruits versus second serum no-change recruits.

Week 3 Antibody	Virus Isolation	Nasal Antibody	Both	Total	% Positive
<4 fold Rise	10/54	14/54	12/54	36/54	66.7
≥4 fold Rise	4/40	10/40	12/40	26/40	65.0
Total	14/94	24/54	24/94	62/94	66.0

TABLE VI

The effect of pre-existing SN antibody and week 3 Serconversion on infection with adenovirus type 7.

Week 3 Antibody	Week 0 Level	Virus Isolation	Nasal Antibody	Both	Total	% Positive
<4 Fold Rise	<1:4	7/25	3/25	11/25	21/25	84.0
	≥1:4	3/29	11/29	1/29	15/29	51.7
≥4 Fold Rise	<1:4	3/25	7/25	10/25	20/25	80.0
	≥1:4	1/15	3/15	2/15	6/15	40.0
TOTAL		14/94	24/94	24/94	62/94	66.0

TABLE VII

Serologic conversion by complement fixation versus serum neutralization. A. Week 0 to week 3 conversions.

		Serologic Conversion by Serum Neutralization*			
		Adeno 4	Adeno 7	Both	Total
Serologic Conversion by CF	#	15	5	19	39
	%	46.9	31.3	67.9	51.3
# Tested		32	16	28	76

TABLE VIII

Serologic conversion by complement fixation versus neutralization. B. Week 0 to Week 6.

	Serologic Conversion by Serum Neutralization			
	Adeno 4	Adeno 7	Both	Total
Serologic Conversion by #	9	15	40	64
CF %	47.4	55.6	78.4	66.7
# Tested	19	27	51	96

TABLE IX

Serologic conversion by serum neutralization or complement fixation versus indicators of infection with adenovirus type 7.

Serologic Conversion	Indicators of Infection							
	Virus Isol.		Nasal Ab		Both		Total	
	#	%	#	%	#	%	#	%
SN	13/17*	76.5	20/25	80.0	24/24	100.0	57/66	86.4
CF	8/17	47.0	15/25	60.0	19/24	79.2	42/66	63.6
Neither	4/17	23.5	5/25	20.0	0/24	0.0	9/66	13.6

* - numbers positive over number tested

Twenty recruits converted by SN (10 by CF) that did not yield virus isolation or secretory antibody.

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- 2 Smith, C. D., G. R. French, J. Einarson, L. A. Hanson, R. L. Coultrip, W. A. Smith, and J. G. Thomas - Prophelatic Methods in Prevention of Disease Among Army Personnel. Annual Report, Sixth US Army Medical Laboratory. pp 11-25, 1971.

FIELD TRIAL EVALUATION OF LIVE, ORAL ENTERIC-COATED ADENOVIRUS
TYPE 4 AND 7 VACCINES PREPARED WITH A MODIFIED FORMULATION

INTRODUCTION

The standard formulation for adenovirus vaccines used by the military consists of live virus embedded in a solid diluent comprised of 20% skim milk, 5% gelatin and 25% human albumin. Optimal potency is reached with difficulty in the vaccine pills prepared in this formula and the shelf life has proven to be too short to be satisfactory. Wyeth Laboratories has developed a modified formula (SPGA plus additives) that they feel will greatly extend the shelf life of the vaccines. Vaccine virus has been lyophilized and tableted in the new diluent as both monovalent type 4 and type 7 tablets and a bivalent preparation of one tablet containing both viruses.

This study was designed to field trial test the modified formulations by determining the Serologic response of BCTs to administration of (1) type 4 and type 7 monovalent tablets with modified formulation (2) the type 4 and type 7 bivalent tablet with modified formulation, and (3) the standard type 4 and type 7 monovalent preparations.

MATERIAL AND METHODS

Vaccine - The modified formula monovalent type 4 tablet was lot #16CI-03801. The potency of this preparation as determined by the manufacture, Wyeth Laboratories INC. was $10^{5.7}$ to $10^{6.2}$ TCID₅₀/tablet in HEK tubes. The type 7 modified formula tablet was lot #16CV-03001 with a reported potency of $10^{5.5}$ to $10^{5.9}$ TCID₅₀/tablet. The Bivalent tablet was lot #16CXVII-00101. The type 4 component of this tablet titered $10^{4.7}$ to $10^{4.9}$ TCID₅₀/tablet and the type 7 component titered $10^{4.8}$ to $10^{5.1}$ TCID₅₀/tablet. The standard formula tablets were lot #16CI-03701 for type 4 and lot #16CV-02901 for type 7. The potency of these tablets at the time of administration for the study was not determined.

Study Design and Group - The three vaccine groups comprised of 115-130 men each were drawn from separate companies of BCTs at Ft. Ord, Ca., and designated Groups A, B, and C. All three groups were bled for pre-immunization sera on or about 21 March 1972 and administered the vaccine as follows:

(1) Group A - received one tablet each of type 4 and type 7 vaccine modified formulation (lot #s 16CI-03801 and 16CV-03001 respectively). This group is later referred to as the monovalent vaccine modified formulation group.

(2) Group B - received one tablet of the Bivalent vaccine lot #16CXVII-00101 containing both type 4 and type 7 virus. This group is later referred to as the Bivalent vaccine modified formulation group.

(3) Group C - received one tablet each of the standard type 4 and type 7 vaccines (lots #16CI-03701 and 16CV-02901 respectively). This group is later referred to as the monovalent vaccine standard formulation group

All vaccinees were bled for post vaccine sera 21 days after administration of the tablets.

Pre-immunization sera were screened at the 1:8 dilution for adenovirus type 4 and type 7 serum neutralizing antibody. Those vaccinees whose pre-serum antibody titers were less than 1:8 for either virus were the subjects of further study. The first drawn serum of these recruits were then tested again at the 1:4 and 1:8 serum dilutions, and the 21 day post vaccine sera were tested at the 1:8, 1:16, and 1:32 and 1:64 serum dilution for SN antibody to type 4 and type 7 virus.

Serum neutralization tests and challenge virus strains - SN antibody titers were performed by the microtiter plate technique unless otherwise indicated. The procedure is performed in falcon microtiter II flat bottom 96 well plates with Hela Cells and wild type adeno type 4 and adeno type 7 virus strains. The Hela Cells are prepared in suspension to 200,000 cells/ml in L-15 medium with 10% fetal calf serum. The initial serum dilution (1:4 pre-vaccine sera, 1:8 post vaccine sera) were prepared in tubes with L-15 diluent. The diluted sera were dropped in the plates (0.05 ml) and looped (Cooke Engineering 0.05 ml loops) to the final dilution. Five one hundredths ml of virus suspension was then added to each well and the reaction mixture was incubated for 30 minutes at 35°C. The virus suspensions were standardized from working stocks and preliminary titrations to contain twice that amount of virus required to produce 2⁺ CPE in 48 hours. One-tenth ml of Hela Cell suspension was then added to the reaction mixture wells, the plates were sealed with transparent tape and then returned to the incubator for an additional 48 hours. The wells were then scored for the presence or

absence of CPE. Serum titers were read as the highest dilution of serum completely inhibiting CPE at the 48 hour reading. Controls included serum wells for each test serum with L-15 diluent replacing virus to score for non-specific CPE, known negative and positive serum wells and a four well per two-fold dilution virus back titrations. Representative sera from the monovalent vaccine modified formulation group were also tested with the procedure described above using vaccine virus strains instead of wild virus strains. These sera were tested at the same time with the WRAIR Human Embryo Kidney (HEK) tube serum neutralization technique and vaccine virus strains. The HEK Tube procedure utilizes a challenge virus suspension standardized to yield 10 TCID₅₀s in a 96 hour titration.

RESULTS

Monovalent vaccine modified formulation group. The results of the microtiter plate serum neutralization tests are shown in table I. Fifty six of one hundred recruits in this group were susceptible to adenovirus type 4 (pre-vaccine titer of <1:4) and 44 of the 56 or 79% responded with post vaccine titers of \geq 1:8. Sixty four percent or 36 of the 56 susceptible responded with 8 fold or greater increases in SN antibody to type 4 virus.

Thirty eight of the 50 recruits (76%) susceptible to adenovirus type 7 had four-fold or greater increases in type 7 SN antibody and all but four of these 38 responded with 8 fold or greater increases.

Bivalent vaccine modified formulation group. Responses to the bivalent tablets were similar to those of the modified monovalent tablets and are shown in table II. The four-fold or greater response rate was 76% and 73% for type 4 and type 7 respectively. There were fewer individuals with 8 fold or greater responses to type 4 virus in this group; 48% with the bivalent tablet versus 64% with the monovalent tablet, but this may not be a significant difference due to the small numbers involved.

Monovalent vaccine standard formulation group. Responses to the standard formulation tablets are shown in table III. Conversions to either type are far fewer than expected and provide the explanation for a heavy ARD season last year at Ft. Ord. Total four-fold responses occurred in 56% of the type 4 susceptible recruits and 37% of the type 7 susceptible recruits. It is unfortunate that data are not available

on the potency of these lots at the time of the study.

Serum neutralization responses as measured by vaccine virus strains.

Paired sera from selected recruits of the modified monovalent vaccine group were tested by the microtiter SN procedure and the human embryo kidney (HEK) tube SN procedure utilizing vaccine virus strains for comparison to our standard microtiter procedure. The latter utilizes prototype wild virus strains. Seventeen pairs were tested for type 4 SN antibody and sixteen pairs were tested for type 7 SN antibody. The recruit sera tested included both conversions and non-conversions to the wild virus strains. These results are shown in table IV. In general it appears that either procedure performed with the vaccine virus strains is more sensitive than the standard microtiter procedure performed with the prototype strains. The microtiter SN test performed with the vaccine strains was particularly impressive demonstrating 8 conversions out of a possible 15 susceptibles that were not picked up with the same procedure run with the prototype wild virus strains. Seven of 30 individuals with pre-serum SN titers of $<1:4$ against the wild virus strains had $1:4$ titers against the vaccine virus strains. Not shown in the table but of interest was the finding that six of the seven individuals with pre-existing SN antibody converted post immunization with four-fold or greater rises.

Foster et al. (1964) and Edmondson et al. (1966) have demonstrated that any detectable level of SN antibody ($\geq 1:4$) to type 4 virus will almost uniformly preclude development of febrile illness in response to subsequent exposure to type 4 virus. It has since been the practice at WRAIR to consider a $1:4$ SN antibody titer post immunization in susceptible recruits (pre-immunization titer of $<1:4$) to be indicative of successful immunization. It was not possible to evaluate in retrospect the response to immunization by this criteria in the major portion of the study in that post immunization sera were not tested at the $1:4$ dilution. However, if we apply the data obtained from the SN tests performed with vaccine virus to the entire modified monovalent vaccine group we can estimate the response to immunization by the WRAIR criteria. These results would indicate that 95% of recruits susceptible to type 4 virus responded to administration of the type 4 modified vaccine tablet with post immunization titers of $\geq 1:4$. The corresponding figure for the type 7 tablet would be 86%.

Evaluation of viral interference in recruits susceptible to and simultaneously infected with both adenovirus type 4 and 7. Sero conversion

rates for the three vaccine groups as a whole were applied as expected values to sero-conversion rates of recruits susceptible to either adenovirus type 4 or type 7 but not both and to those recruits that were susceptible to both adenovirus type 4 and type 7. The purpose of this evaluation was an attempt to determine if viral interference could be demonstrated in those recruits susceptible to both viruses by comparison of their sero-conversion rates to the rates of those recruits that had demonstrable pre-immunization antibody to one or the other of the two viruses. The results of this analysis are shown in table V. The number of sero conversions demonstrated for each vaccine group appear in the rows reading across from the heading "# positive". The numbers susceptible appear across from the heading "# tested". The total conversions for the singly susceptible recruits ie those without pre-immunization antibody for one virus but not both appear under the left-half of the last column. The total conversions for the double susceptibles appear in the right hand half of the last column. Four more sero conversions were observed than expected in the singly susceptibles and one fewer occurred than was expected for the doubly susceptibles. Again, the expected values were determined from the observed sero-conversion rates of all susceptibles within groups.

When the observed versus expected values for singly and doubly susceptible recruits are tested by the Chi-square test the null hypothesis that the two groups are similar in their sero-conversion rates would be accepted.

SUMMARY

A field trial of the live, oral enteric-coated adenovirus type 4 and type 7 vaccines prepared with a modified formulation has been conducted in BCTs at Ft Ord, Ca. during March and April of 1972. The vaccines tested were either monovalent with respect to adenovirus type content or bivalent containing virus of both types. Sero-conversion rates of recruits with pre-immunization antibody titers of less than 1:4 were similar for all three modified formula vaccines tested. Four-fold sero-conversion rates varied from a low of 73% to a high of 79% when measured against wild virus prototype strains in the microtiter serum neutralization procedure. There was no evidence of viral interference with either serologic type of virus in recruits that were defined as susceptible to both viruses.

A comparison of the Conventional serum neutralization procedure

performed in human embryo cell culture tubes to the microtiter procedure performed in 96 well microtiter plates did not reveal significant differences in sensitivity or specificity. However, vaccine virus strains were shown to be more sensitive in detecting response to immunization than were the prototype wild virus strains.

TABLE I

Serologic Evaluation of the Modified Formulation for the Oral Enteric Pthalate Coated Adenovirus Type 4 and Type 7 Vaccines

1. Monovalent Vaccine Modified Formulation

	Recruits		Fold Increase in SN Titer*			
	Tested	Susceptible	<4	4	≥8	≥4
Adeno 4	#100	56	12	8	36	44
	% -	56%	21%	14%	64%	79%
Adeno 7	#100	50	12	4	34	38
	% -	50%	24%	8%	68%	76%

*SN Titer = Microtiter Serum Neutralization Titer. Susceptible Recruits had no detectable Pre-vaccine Titer (<1:4).

TABLE II

2. Bivalent Vaccine Modified Formulation

	Recruits		Fold Increase in SN Titer*			
	Tested	Susceptible	<4	4	≥8	≥4
Adeno 4	# 96	42	10	12	20	32
	%	44%	24%	29%	48%	76%
Adeno 7	# 96	37	10	2	25	27
	%	39%	27%	5%	68%	73%

*SN Titer = Microtiter Serum Neutralization Titer. Susceptible Recruits had no detectable Pre-vaccine Titer (<1:4).

TABLE III

3. Monovalent Vaccine Standard Formulation

	Recruits		Fold Increase in SN Titer*			
	Tested	Susceptible	<4	4	≥8	≥4
Adeno 4	# 98	62	27	13	22	35
	% -	63%	44%	21%	36%	56%
Adeno 7	# 98	51	32	6	13	19
	% -	52%	63%	12%	26%	37%

*SN Titer = Microtiter Serum Neutralization Titer. Susceptible Recruits had no detectable Pre-vaccine Titer (<1:4).

TABLE IV

SN RESPONSE TO THE MODIFIED MONOVALENT ADENOVIRUS TABLETS
AS MEASURED BY TWO DIFFERENT TECHNIQUES AND VACCINE VIRUS CHALLENGE

Response by Wild Virus Microtiter	Response by Vaccine Virus	Serum neutralization procedure					
		HEK Tubes			MICROTITER		
		type 4	type 7	Total	type 4	type 7	Total
<4 fold	<4 fold	5	10	15	1	7	8
	¹ / ₄ fold	3	2	5	5	2	7
	not susceptible	2	0	2	3	3	6
	not tested	0	1	1	1	1	2
	Total		10	13	23	10	13
≥4 fold	<4 fold	4	0	4	0	0	0
	¹ / ₄ fold	3	3	6	6	2	8
	not susceptible	0	0	0	0	1	1
	not tested	0	0	0	1	0	1
	Total		7	3	10	7	3

¹ Indicates those recruits with pre-serum SN titers of <1:4 against wild virus challenge but ≥ 1:4 against vaccine virus challenge.

TABLE V
ADENOVIRUS TYPE 4 AND TYPE 7 SEROCONVERSION
IN SINGLY AND DOUBLY SUSCEPTIBLE RECRUITS

Seroconversion to →	Type 4				Type 7				Type 4 or Type 7				
	Type 4		Type 4 & 7		Type 7		Type 4 & 7		Type 4 or 7		Type 4 & 7		
	Obs ¹	Exp ²	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	
Modified Monovalent	#Positive	18	16	28	29	12	11	28	26	30	27	56	55
	#Tested	20		37		15		37		35		74	
Modified Bivalent	#Positive	11	12	17	17	10	9	15	16	21	21	32	33
	#Tested	16		22		12		22		28		44	
Standard Monovalent	#Positive	13	12	20	20	5	5	14	13	18	17	34	33
	#Tested	22		36		13		36		35		72	
TOTALS	#Positive	42	40	65	66	27	25	57	57	69	65	122	123
	#Tested	58		95		40		95		98		190	

1 Obs = observed # Seroconversions
2 Exp = expected # Seroconversions - See Text for explanation

LITERATURE CITED

- 1 Forsyth, B.R., et al: Patterns of Adenovirus Infections in Marine Corps Personnel: II Longitudinal Study of Successive Advanced Recruit Training Companies, Amer. J. Hyg. 80:343-355, 1964.
- 2 Edmondson, W.P., et al: Immunization by Selective Infection with Type 4 Adenovirus Grown in Human Diploid Tissue Culture: II Specific Protective Effect Against Epidemic Disease, JAMA 195: 453-459, 1966.

RAPID RECOGNITION OF INFECTIOUS RESPIRATORY VIRUS DISEASE
AGENTS IN NASOPHARYNGEAL SMEARS

INTRODUCTION

This project was begun last year under the same title. The objective remains to develop laboratory techniques which will facilitate more rapid recognition of the viral etiologic agents of acute respiratory disease. This project was initiated in response to two observations (1) the development of effective immunization procedures for adenovirus types 4 and 7 has shifted the distribution of the etiology of ARD from principally adenovirus infections to other upper respiratory viral agents and (2) the laboratory procedures developed to monitor adenovirus infections in military recruits are not adequate to monitor the agents presently responsible for the majority of ARD.

One of the approaches still being evaluated by this lab is the indirect fluorescent antibody technique. The model utilized has been rubella virus infected cell culture monolayers.

PROGRESS

This project has seen only limited progress during the past study year due primarily to the limited personnel resources available and the unexpected requirements of projects of higher priority, principally the evaluation of the modified formula adenovirus vaccines.

The work that has been expended on this project during this year has centered around two procedural problems (1) reduction of background fluorescence associated with the indirect F Ab technique and (2) production of high titered specific antisera. Several approaches have been tried without a great deal of success. These have included adsorption of the antisera with lyophilized cells of the same species used as the test monolayer; and production of antisera in the same animal species used as the test monolayer.

The level of background fluorescence is still high enough using these procedures to preclude rapid identification of an agent under circumstances where any one of many agents may be involved. The alternative to a successful indirect procedure is to produce labeled antisera for each of the possible agents involved in ARD.

FIELD TRIALS OF A MODIFIED CHARCOAL VIRUS TRANSPORT MEDIUM

INTRODUCTION

This report is a continuation of a project started last F.Y.. The study concerns comparison of standard Liebovitz Charcoal transport medium with a modified formulation containing gentamycin, Agarose and bovine albumin. Although standard CVTM has been extremely effective in stabilizing and maintaining adenovirus at ambient temperatures for prolonged periods of shipment, its success for other viruses has been less than desirable.

MATERIALS AND METHODS

Standard CVTM - was "Difco 0834-01 Transport Medium CVTR" prepared according to the instructions on the container.

Modified CVTM - was similar to the formulation of Liebovitz except that it contained .05 g/liter of dried bovine albumin, 100 mg/liter of gentamycin and 4 g. of agarose substituted for standard agar.

The two products were color coded, and over 2000 tubes were sent to basic training camps throughout the U.S.. Isolation techniques for both types of media were identical, with routine processing of the throat swabs in human embryonic kidney cell cultures using standard techniques.

RESULTS

The results are presented in Table 1. Isolation rates were 48% and 49% respectively for CVTM and modified CVTM. Only 7 isolates other than adenovirus were found from either media for the total study of 1843 tubes. The modified media had a rate of 13% contaminated passes.

DISCUSSION

The results with the modified product indicate that it not only does not produce a significantly higher rate of isolation than the standard product, but also produces almost a 40% higher contaminated passage rate.

The agarose substitution was made to avoid the presence of inhibitory sulfated polysaccharides that are present in standard agar. This substitution did not seem to produce a significant difference in the isolation rate.

The higher contaminated pass rate in spite of the addition of 100 mg of gentamicin per liter was an unexpected and undesirable result. The increased contamination was probably due to the 0.05 g of albumin per liter, however, logic would indicate that at least the bacterial contamination would decrease. The gentamicin apparently had no effect on the fungal contaminants.

Future research on this problem will center around an entirely different media using varying types and amounts of pH buffers, antibacterial and antifungal drugs, antioxidants or oxygen receptors and substances such as activated charcoal and a solidifying substance. An appropriate selection and concentration of protective chemicals should result in an improved preservation and transportation of viruses other than adenovirus.

TABLE I

VIRUS ISOLATION FROM STANDARD AND MODIFIED CVTM

	Throat Swabs Tested	Adenovirus Isolates	Poliovirus Isolates	Other Isolates	Contaminated Passes Required
Standard CVTM	1486	713 48%	4	1	396/2972 13%
Modified CVTM	357	177 49%	2	0	145/754 18%
TOTAL	1843	890	6	1	

MENINGOCOCCAL VACCINE ANTIGENICITY AND EFFICACY STUDIES, AND CARRIER SURVEILLANCE

INTRODUCTION

Previous studies have shown that the lack of a protective level of meningococcal antibodies is the primary predisposing factor leading to meningococcal disease, and that carrier prevalence as determined by monthly surveys does not show a positive correlation with disease incidence (Smith, C.D., et al R & D Report 1971). These studies are designed to (1) test the antigenicity of various lots of meningococcal polysaccharide vaccine, (2) assess the effect of these vaccine lots on the meningococcal disease rate, (3) monitor the emergence of serogroups other than "C", and (4) determine the effects of the vaccine on the meningococcal carrier rate.

These studies represent the joint efforts of the Sixth USAML, the Preventive Medicine Division at Fort Ord, and the Department of Bacterial Diseases at WRAIR.

MATERIALS AND METHODS

Meningococcal "C" purified polysaccharide vaccine lots were administered to 530 receptionees filling various companies of Fort Ord Training Brigades during the fiscal year. Each of 13 lots was administered by needle and syringe, 0.5 ml (50 mcg in saline) subcutaneously, to 40-50 receptionees. Material swabbed from the posterior aspect of the uvula was streaked immediately onto a Columbia Chocolate Agar (CCA) plate (BBL) containing 5% laked sheep blood, 1% isovitalax (BBL), lincomycin (6mcg/ml) and polymyxin B (25 mcg/ml). The plates were incubated immediately at 37°C in a 5 to 10% CO₂ atmosphere 18-20 hours, and standard identification of meningococcal organisms and serogroupings were made. Swabs were taken in similar fashion at 2 week intervals during the 8 weeks of BCT. Blood was collected from each vaccinee at 0, and 2 weeks, and was used to determine antibody rises to "C" polysaccharide meningococcal antigen by microtiter hemagglutination technique. All sera were frozen until tested. The 0 and 2 week serum titers were performed on the same day after the 2 week bleedings. Studies on future vaccines, which will be reported later, will include a 6 week bleeding; and all sera from the 3 bleedings will be titered on the same day for reproducibility checks.

RESULTS AND DISCUSSION

Vaccine Antigenicity:

Table 1 indicates group "C" hemagglutination antibody titers in BCTs following vaccination with meningococcal "C" polysaccharide vaccine, and the meningococcal carrier rates after each bleeding. Titers are expressed as geometric mean values. The greatest mean titer change was 5.72, and all of the vaccinees seroconverted (>4-fold rise). Two of the vaccine lots incited a 100% seroconversion rate in vaccinees. All of the other lots caused seroconversion rates of above 90% indicating acceptable antigenic quality. A trial dose of 2 mcg in 34 individuals caused 28 seroconversions, 82%. There were no significant differences in the 0 and 2 week carrier rates, but as expected the 6 week carrier rate climbed as high as 94.6% in one vaccinated group; 9 groups rose above 80 and 90%.

Efficacy and Carrier Rates:

Figure 1 indicates the meningococcal carrier and disease rates among BCTs at Fort Ord comparing fy 70, 71, and 72. The actual number of total isolates and the variety of serotypes among those isolates are shown on the 1st bar for each month. The percentage of carriers among individuals surveyed is shown by the 2nd bar for each month. Under each month, on a separate line, the meningococcal disease cases are listed.

There was an even distribution of serotypes during fy '70. No vaccine was administered that year. Fifteen (15) disease cases occurred, all except 1 "B" case were caused by "C" organisms. Three of the "C" cases died. Even though the carrier rate reached 70% in May of that year, and 67% of the carriers carried "C" type organisms, there were no disease cases during the month of May.

In November of fy '71, converse to fy '70, the carrier rate was only 13%, the lowest rate of that year, with only 1 "C" carrier, however there were 4 "C" disease cases - the largest number of "C" cases during that year. These instances and others during this 3 year observation amplify the fact that prevailing carrier rates do not dictate the occurrence of meningococcal disease.

Meningococcal "C" vaccine was administered January thru May fy '71,

and there were no disease cases there after through-out the year. However, before vaccine administration, disease cases totaled 16; 14 "C" cases, 1 "B" and 1 "Y". Serotype "C" carriers declined from 40% to 0.04% after vaccine administration. However, serotype "Y" carriers increased from 0.03% to 40%.

In October of fy '72, as indicated by the 3rd diagram of Figure 1, meningococcal "C" vaccine administration commenced and was given continuously as part of the routine immunization program for receptionees. Afterwards there were no "C" disease cases, but there were 3 "Y" disease cases. During the part of the year preceding vaccine there were 7 disease cases, 4 "C"s and 3 "Y"s; there were 2 deaths due to "C" organisms.

Figure 2 indicates meningococcal disease cases at Fort Ord during fy 70, 71 and 72. This observation indicates most cases occur beyond the 4th week of Basic training, 24 above and 8 below. During these periods 4 Advanced Individual Trainees (AIT) and 1 Cadre experienced infections.

SUMMARY

Thirteen lots of meningococcal "C" polysaccharide vaccine were tested in 530 BCTs at Fort Ord, and were found to incite sero-conversion levels of antibodies in at least 90% of the individuals injected. Following continuous routine administration of the vaccine the meningococcal disease rate diminished dramatically. The cases that occurred after vaccine, were caused by meningococcus serotype "Y" organisms. Serotype "Y" carriers increased from 0.03% to 40%; and "C" carriers decreased from 40% to 0.04%. The prevailing meningococcal serotype is now "Y". Meningococcal "Y" disease appears to be less severe than "C" disease, and thus far has caused no deaths.

Individuals harboring meningococci in their throats with no evident illness have always been numerous among Basic Combat Trainees. The carrier rate among young male adults on entering the service is usually 20-30% during the winter and spring months, thus indicating the wide spread dissemination of meningococcal organisms in civilian populations. Due to the necessary close association of these men, during BCT, the carrier rate rises to 75-85% approximately six weeks after BCT begins. Even though approximately 12% of BCTs have no

detectable antibodies to meningococci, the majority of them escape overt disease. Meningococcal carrier prevalence as determined by monthly surveys has not shown a positive correlation with disease incidence. Even though the lack of a protective level of meningococcal antibodies is the primary predisposing factor leading to disease, the question of why some individuals among a group in the same "lack of antibody population" are infected and others are not remains somewhat of a mystery.

AMAML-M
SUBJECT:

Quarterly R&D Progress Report, "Prophylactic Methods in the Prevention of Diseases Among Military Personnel"

Table 1

Group "C" Hemagglutination Antibody Titers in Man Following Vaccination, and Meningococcal Carrier Rates; Titers are Expressed as Geometric Mean Titers

Vaccine Preparation	Dose	0 Week		2nd Week		6-7 Wk		Titer Change	4-fold rise/ No. tested
		Titer	Carriers	Titer	Carriers	Carriers	Carriers		
Squibb C-9 Control	50 mcg	2.03	28.6%	6.94	26.9%	82.6%	4.91	34/35	
Merck 419 (C-A258)	50 mcg	1.54	35.0%	7.16	31.5%	91.4%	5.62	36/37	
Merck 420 (C-A259)	50 mcg	1.66	20.0%	7.38	28.1%	90.4%	5.72	32/32	
Merck 422 (C-A261)	50 mcg	1.36	27.5%	6.47	11.1%	86.1%	5.11	34/36	
Squibb C-10 Pool 2	51 mcg	1.00	24.3%	6.09	13.9%	68.7%	5.09	34/35	
Squibb C-10 Pool 2	2 mcg	1.18	30.5%	4.68	14.7%	63.7%	3.50	28/34	
Squibb C-9 Control	50 mcg	1.24	28.6%	6.33	26.9%	82.6%	5.09	49/51	
Squibb C-9 Control	50 mcg	0.33	22.2%	4.22	40%	94.6%	3.99	42/45	
Merck 421 (C-A260)	50 mcg	0.30	28.2%	4.70	26.2%	92.3%	4.40	43/43	
Merck 423 (C-A262)	50 mcg	0.32	25.5%	4.30	31.8%	87.5%	3.98	38/43	
Merck 424 (C-A263)	50 mcg	0.79	47.9%	4.41	48.9%	81.4%	3.62	45/46	
Merck 426 (C-A265)	50 mcg	0.34	23.4%	4.49	36.2%	47.8%	4.15	42/47	
Merck 438 (C-AA06)	50 mcg	0.26	30.4%	4.77	40%	53.5%	4.51	42/44	

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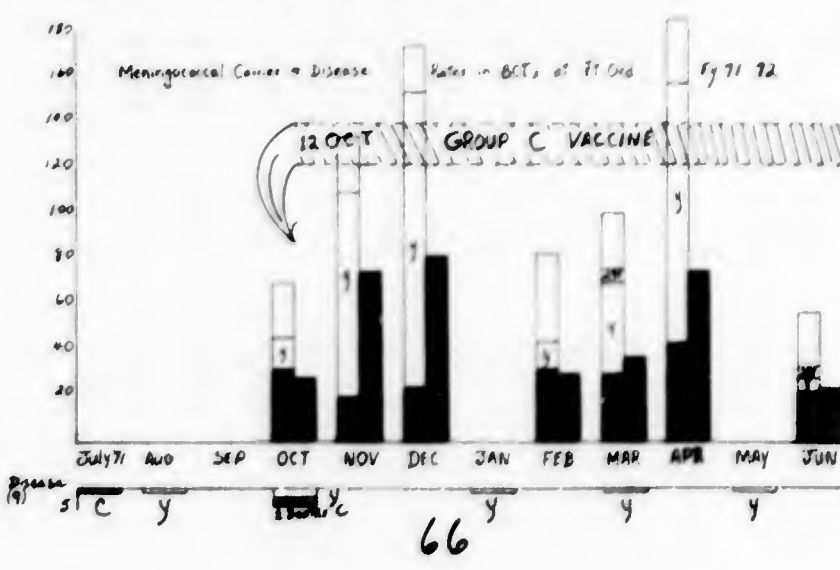
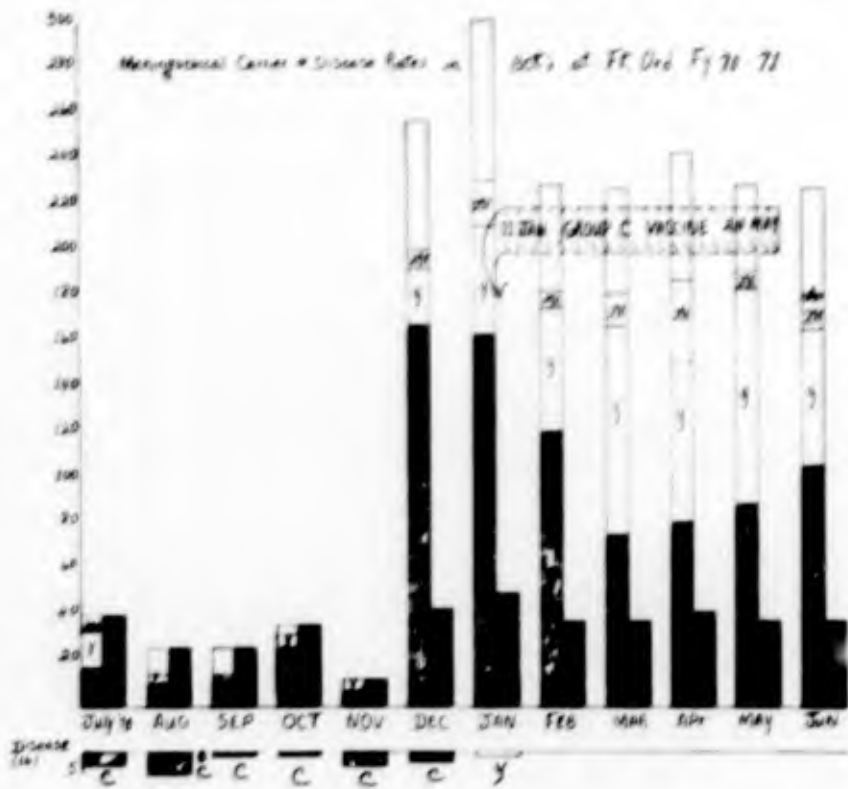
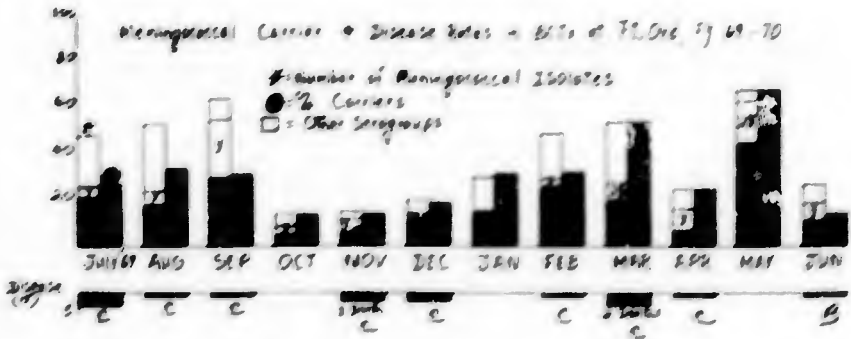


Figure 1

MENINGOCOCCAL DISEASE - FT. ORD, 1970 - 71 - 72

MONTH	#CASES	WEEK OF TRAINING										DEATHS	SEROTYPE
		0	1	2	3	4	5	6	7	8	AT/OO		
JAN 70	-												
FEB	2											2	2C
MAR	2												2C
APR	1												1C
MAY	-												
JUN	1												1B
JUL	3												3C
AUG	5												4C, 1B
SEP	1												1B
OCT	1												1C
NOV	3												3C
DEC	2												2C
	<u>21</u>												
JAN 71	1												1C
FEB	-												
MAR	-												
APR	-												
MAY	-												
JUN	-												
JUL	1												1C
AUG	1												1Y
SEP	-												
OCT	4											2	2C, 2Y
NOV	-												
DEC	-												
JAN 72	1												1Y
FEB	-												
MAR	1												1Y
APR	-												
MAY	1												1Y
JUN	-												
JUL	-												
AUG	1												1Y

Figure 2

Figure 2

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