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PROPHYLACTIC METHODS IN PREVENTION OF DISEASE AMONG ARMY PERSONNEL

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Army Medical Laboratory
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13. ABSTRACT

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An investigation into congenital anomalies, the increased occurrence of abortions, stillbirths and prematurities as results of maternal infections with influenza A virus was conducted. The total population studied comprised of 324 gravid military dependents at March AFB, Riverside County, California, and Fort Ord, Monterey County, California. Groups from both stations were similar in age and racial composition, and it was policy at both hospitals to withhold influenza virus immunization of pregnant women. A total of 107 (33%) of the 324 women experienced influenza A virus infections. There was no evidence of prematurity as indicated by mean weeks of gestation or birth weight at delivery. Two stillbirths and 9 malformations occurred, however, both stillbirths and 8 of the 9 anomalies were in the non-infected group.

A review of the records to determine the % occurrence of mycoplasma infections over the past 5 years at 7 BCT forts was conducted. Laboratory diagnoses were on the basis of >4-fold rises of complement fixation antibodies in paired sera collected at Forts Jackson, Wood, Polk, Ord, Dix, Knox, and the San Diego Navy Training Center. Of 5,842 individuals tested, 206 (3.5%) had mycoplasma infections.

Studies to determine antibody responses and clinical reactivity to 2 lots of Merck, Sharpe and Dohme lyophilized meningococcal "C" vaccine stored at 2-8°C and reconstituted with diluent containing 1:20,000 thimerosal preservative were accomplished utilizing BCTs at Fort Ord. A Squibb C-9 lot was also tested in parallel as a control. Carrier surveys were accomplished along with the pre-vaccine and 2-week post-vaccine phlebotomies. The two Merck Vaccine lots incited 95.7% (44/46) and 93.8% (44/47) seroconverters; and the Squibb lot incited 100% (43/43) seroconverters. The percent carriers at 0 week was 28.7% (19 B's, 3 C's, 2 29E's, 12 Y's, 2 X's, and 2 non-typeable organisms). The percent carriers at 2 week post-vaccine was 36.2% (27 B's, 2 C's, 3 29E's, 14 Y's, 1X, and 3 non-typeables). There were no vaccine reactions. Only two meningococcal meningitis cases occurred at Fort Ord during FY '74, 1 case in July 1973 caused by a sero-group Y organism and 1 case in January 1974 also caused by a sero-group Y. Group Y organisms contituted 29% of the carrier population, Group B's 51%, and 20% was made up of a variety of lesser occurring serotypes.

Efforts to identify new meningococcal serotypes from non-typeable isolates have produced two organisms that are agglutinated by an antiserum prepared in rabbits, and they are not agglutinated by other routinely used grouping sera. Pilot studies indicate that these strains are new serological types that have not been previously described. Non-typeable organisms are collected from meningococcal carrier surveillance studies on Basic Combat Trainees at Fort Ord, California.

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A search for rapid methods to aid in identifying yeasts and yeast-like fungi from clinical specimens continued during the fiscal year. Twenty-eight different species of yeast (A.T.C.C. and clinically isolated strains) were subjected to 190 biochemical assimilation and fermentation procedures utilizing test reagent impregnated paper disks. Insufficient differences in assimilation patterns indicate that C. parapsilosis, C. stellatoidea, and C. tropicalis may be less pathogenic mutants of C. albicans. Conversely, assimilation patterns produced by C. pseudotropicalis, C. guilliermondii and C. Krusei indicate these fungi should maintain species integrity. Inhibition patterns to sodium salts of organic acids and certain nucleic acid bases (purines and pyrimidines) indicate this technique can most likely be used to biotype C. albicans in epidemiological investigations.

14 KEY WORDS	LINK A		LINK B		LINK C	
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Mycoplasma: 1. Evaluation of Monovalent "B" vaccines.						
2. Prenatal Infections.						
Meningococcus: 1. Vaccine antigenicity, efficacy and carrier rates.						
2. Identification of new serotypes.						
Urethritis: 1. Viral & Bacterial agents in						
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PROPHYLACTIC METHODS IN PREVENTION OF DISEASE AMONG ARMY PERSONNEL

ANNUAL REPORT

by

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Henry A. Leighton, COL,MC, Clayton L. Dillavou, MAJ, MSC
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AUGUST 1974

US Army Medical Laboratory, Fort Baker, CA 94965

and

Health and Environment Activity, Office of the
MEDDAC Commander, Fort Ord, CA 93941

DOD Distribution Statement

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

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ABSTRACT

Surveillance studies to determine the etiologic agents of acute respiratory disease (ARD) in basic combat trainees (BCTs) were conducted during FY '74. These studies included 4,143 trainees hospitalized with ARD at 7 Army Basic Combat Training Centers (including Fort McClellan), and the US Navy Training Center in San Diego. Viral isolations and serological studies indicate only 9.5% of ARD hospitalizations were caused by adenoviruses, 15.5% by influenza A and B, mycoplasma, polio and occasional coxsackie, and a large 75% by agents that were not determined. Sixty-three percent (63%) of the adenovirus infections were caused by adeno 7 virus and 37% by adeno 4. Laboratory titrations of virus in the vaccine pills indicated acceptable but not high levels. Considerable numbers of influenza hospitalizations were experienced by some of the training forts (Knox, Ord, McClellan). Disease cases totaled 101; 50 flu A (49%) and 51 flu B (51%). The newly standardized bivalent influenza vaccine A/Eng/42/72/ (H_3N_2) was administered throughout the year, while monovalent B/HK/5/72 was added to this treatment in January 1974. There were 56 mycoplasma disease cases. Twenty-five (25) of these were experienced by Fort Ord and Fort Polk.

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FOREWORD

Antigenicity and efficacy evaluations of vaccines and other prophylactic agents, and other means of preventing upper respiratory and infectious diseases in military populations, have been the primary direction of these investigative efforts. Emphasis has been focused on those diseases that (1) cause loss of basic combat training days among recruits, and (2) those that cost or have the potential of costing the government the largest amount for hospitalizations, e.g., adenovirus infections, influenza, meningococcal meningitidis, and urethritis. Our greatest attention is directed towards prophylactic means of preventing diseases before they gain in-roads to combat training units. These studies represent the combined efforts of the laboratory scientist and the field epidemiologist. It is intended through these joint efforts, these investigations will assist in controlling the more prevalent recruit diseases, and suggest pathways for further studies.

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Project No. 3A061102B71Q

Title: Prophylactic Methods in Prevention of Disease Among Army Personnel

Description:

This report describes the efforts of this study group to forecast, detect and prevent acute respiratory disease (ARD) and infectious disease among military personnel, especially recruits. Surveillances at 6 male BCT Forts, 1 female BCT Fort, and a Navy Training Center were conducted to determine the need for adenovirus and influenza vaccine administration, and etiologic agents. Antigenicity and efficacy studies were accomplished on the use of adenovirus 4 and 7, influenza, and meningococcal polysaccharide A, B, and C vaccines. Some efforts were spent determining asymptomatic infections in pregnant dependents, etiologic agents in the increasing urethritis problem, and more efficient and timely techniques for identifying and classifying yeast like fungi and Herpes viruses.

Progress: Ensuing pages.

ACUTE RESPIRATORY DISEASE SURVEILLANCE

INTRODUCTION

The principal cause of morbidity in military recruit populations continues to be acute respiratory disease (ARD). During the fall and winter months most of the individuals who developed ARD (approximately 85%) were in the 4th to 7th week of basic combat training (BCT). Approximately 20% of the individuals were sick enough to be hospitalized. These surveillance studies were performed on BCT's who were hospitalized at 6 BCT Forts during FY '74. A fairly good quality of adenovirus 4 and 7 vaccine significantly reduced the number of adenovirus infections, and therefore, the overall ARD rates. As indicated by the diagrams below (FY 1973 and 1974). However, in spite of continuous influenza vaccine administration through the year, influenza cases increased. ARD caused by unknown etiologic agents increased over the last fiscal year; it is suspected that rhino viruses contributed largely to these illnesses.

Fy '73

Fy '74

The Fort Ord portion of these studies were performed in conjunction with, and part of the antigenic and efficacy evaluation of new lots of adenovirus and influenza vaccines. However, in the interest of portraying a complete picture, results of ARD surveillance as determined at all 7 BCT Forts are shown.

MATERIALS AND METHODS

Retro-uvula swabs and acute phase sera were obtained from 4,143 patients within 12 hours after they were hospitalized with acute respiratory disease (ARD) at 8 basic combat training (BCT) forts (including a Navy and WAC Training Center) in the United States. Convalescent-phase sera were collected in 14 to 21 days later. Paired sera were obtainable on 1,955 individuals. A number of individuals completed BCT and transferred before convalescent serum collection dates. Other loses were due to short discharges and AWOLs. The swab samples were placed in charcoal viral transport media and held at 5°C (usually 2-5 days) until inoculated into tissue cultures at the US Army Medical Laboratory, Fort Baker. Virus isolations were accomplished in tissue culture utilizing human embryonic (HEK) and rhesus monkey embryonic kidney (MEK) monolayers, and embryonated chicken eggs. Acute phase sera were stored at -70°C until convalescent sera collections. The paired sera were used to detect diagnostic rises in antibody titer, by complement fixation test, to adenovirus, Influenza A and B, and mycoplasma. Other viral serologies were performed when indicated by isolated outbreaks, unusual isolates or for other diagnostic aids. 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; isolation and species determinations were accomplished only on those individuals who were also included in studies reported elsewhere in this report.

RESULTS AND DISCUSSION

The total number of samples used for isolations, and the paired sera that were studied from all of the training forts surveyed during FY '74 are indicated in the Figure 1 summary sheet. Isolation and seroconversion percentages are presented in detail. From 4,143 specimens processed for virus isolations, adenoviruses were isolated from 286 (6.9%). Of 1,955 paired sera received, 234 (12%) showed seroconversions. Influenza A and B, and mycoplasma seroconversions are also indicated in Figure 1. Our adenovirus isolation rate proved to be only 57.5% as efficient as our seroconversion rate, as is indicated in Figure 1. The greatest number of samples for virus isolations (821) was received from the 5th EPMU - US Navy Training Center, San Diego. One-hundred-forty-one (17%) adenovirus isolations were obtained. Seroconversion data are also shown in this figure.

Figure 2 indicates the ARD hospitalization rates per hundred/week during FY '74 at the remaining 6 male BCT Army Forts. Figure 3, which indicates rates for the previous year FY '73, is included for comparison. It is apparent that all of the forts experienced only mild upper-respiratory-disease year during FY '74. The greatest number of ARD hospitalizations was experienced by Fort Leonard Wood. Peak ARD rates of 4/100/ week were reached in February and April. Fort Jackson reached a peak of 3/100 week in February but remained at a low level throughout other parts of the fiscal year. Except for Fort Ord's ARD rate of 2.5/100/week during the last week of January, all of the other BCT Forts remained below 2/100/week through-out the year. Figure 3 indicates rates as high as 6/100/week during FY '73 at Forts Wood, Polk, and Ord. Influenza vaccine was administered at all BCT Forts throughout the year. Bivalent vaccine (A/Eng/42/72-(H_3N_2) 700 CCA units; B/Mass/1/72 300 CCA units) was followed in 2 weeks by Monovalent B/HK/5/72 500 CCA units. Adenovirus vaccines were administered between dates as will be described under individual Forts below. Antigenicity evaluations of the adenovirus vaccines are discussed under separate heading.

The ARD hospitalization rate per hundred per month, and etiologic agents at Fort Ord during FY '74 are indicated in Figure 4. The same information is shown for FY'73 on the same figure to serve for comparison. Also, included below the ARD graph on this Figure are the number of specimens processed each month, on which these data are based. The largest numbers of specimens were received in October and January. The ARD hospitalization rate of slightly above 1/100/month in July 1973 reached a peak of 1.75/100/-month in January 1974. It dropped progressively to below 1/100/week by June 1974. Influenza infections were experienced at Fort Ord throughout the year (38% A's and 62% B's). As indicated in the top portion of Figure 4, FY '73 was a more severe ARD year at Fort Ord. Adenovirus 4 and 7 vaccines have been administered continuously at Fort Ord since July 1972. These studies indicate that at Fort Ord, during the fiscal year, 15.4% of ARD hospitalizations were caused by adenoviruses (50% 4's and 50% 7's), 18.7% were caused by other agents, e.g., influenza A and B, and mycoplasma, and a large 65.9% were caused by undetermined agents.

Figure 5 indicates the ARD hospitalization rate per hundred per month and etiologic agents at Fort Leonard Wood during FY '74. Though the ARD activity at Fort Wood during FY'74 was not as high as it was during the previous year, it ranked highest among the forts studied. The highest ARD hospitalization rate of 3/100/month was reached in January. The high of the previous year, as indicated, was reached in early December. Adenovirus 4 and 7 vaccines have been administered continuously since October 1973. Influenza Hospitalizations occurred August through June, but the bulk of these infections, 55% A's and 45% B's, occurred December 1973 through April 1974. During the year, 6.8% ARD hospitalizations were caused by adenoviruses (62% 4's and 38% 7's), 12.7% were caused by influenza A, B, and mycoplasma, and a very large 80.5% were caused by agents that were not determined.

The highest monthly ARD hospitalization rate of 1.5/100/month occurred in December at Fort Knox as indicated in Figure 6. It dropped gradually to 0.025 in June 1974. ARD hospitalizations ranged far below those of the previous year. As indicated, a small number of influenza infections (70% A's and 30% B's) occurred throughout the year, mostly between January and April. We were not successful in receiving specimens from Fort Knox in September, October or April, however, individuals hospitalized with ARD during these months remained below 1/100/week. Adenovirus 4 and 7 vaccines were administered between January and May. Adenoviruses (14% 4's and 86% 7's) caused 13.6% of ARD hospitalizations, 9.2% were caused by influenza A, B, and mycoplasma, and 77.2% could not be determined.

Peak ARD admissions <2/100/week, were experienced by Fort Dix in March as indicated by Figure 7. As with the other Forts, a relatively mild year existed. A few influenza cases occurred in July, and a larger number of cases occurred between November and May (80% A's and 20% B's). Adenovirus 4 and 7 vaccines were administered September through June. Adenovirus infections (14% 4's and 86% 7's) caused 4.8% of ARD hospitalizations, 7.7% were caused by influenza A, B, and mycoplasma, and a large 87.5% of ARD etiology was not determined.

As indicated by Figure 8, the highest ARD hospitalization rate of 1.3/100/week was reached at Fort Jackson in February. By April the rate had fallen below 1 where it remained through June. Specimens were not received for the months of August, December, January or March. As indicated on Figure 2, the rate was above 1/100/week in March, therefore, specimens should have been collected from those hospitalized individuals. Adenoviruses (100% 7's) caused 1.4% ARD hospitalizations, 1% were caused by influenza A, B, and mycoplasma, and 97.6% were not determined.

The severe ARD experience that occurred during FY '73 at Fort Polk did not occurr during the 1974 fiscal year, as is indicated by Figure 9. The largest number of ARD hospital admissions 1.1/100/month occurred in February. This is compared with a high of 5.40/100/month in FY '73. There were more influenza infections this year (100% A's) at Fort Polk than last year. No specimens were received in October or March. Adenovirus caused 5.1% ARD hospitalizations, 13.8% were caused by influenza A, B, and mycoplasma, and 81.1% were not determined.

Figure 10 is a summary and portrays the total ARD picture among BCT's, and therefore, indicates the average ARD hospitalization rate per hundred per month for 6 BCT Forts that were studied during FY '74. FY '73 as shown at the top of this Figure can be compared with FY '74 to show the drastic drop in ARD. The highest monthly average of 1.5/100/month average of 1.5/100/month for FY '74 occurred in January compared with a high of 3.5/100/month in December during FY '73. Adenoviruses (37% 4's and 63% 7's) caused only 9.5% of ARD hospitalizations among BCT's during FY '74, as opposed to 47.9% during FY '73. A fewer number of adenovirus infections were responsible for an overall lower ARD rate in FY '74. Influenza infections (49% A's and 51% B's), mycoplasma, an occasional polio and coxsackie caused 15.5% of the ARD hospitalizations. A total of 52 Mycoplasma pneumonia cases occurred among all BCT's studied. A large 75% of the ARD etiology among BCT's was not determined.

Results of an ARD surveillance study among WAC at Fort McClellan, Alabama are indicated in Figure 11. The study included 129 WAC trainees who were hospitalized or confined to quarters with acute upper respiratory disease between 16 February and 11 May 1974. Retro. uvula swabs and paired sera were collected and treated as previously described under Materials and Methods. Specimen distribution as collected throughout the 3 month period is shown across the bottom area of Figure 11. The ARD rate was 3.5/100/week in February due largely to influenza B infections. It fell below 2/100/week in March but before the end of that month had climbed to 2.75/100/week with approximately 25% of illness caused by influenza and 75% caused by undetermined agents. The next peak of 2.6/100/week was reached in April, and we received no samples that week due to personnel shortages. At the end of this survey in May, the ARD rate was at 1.7/100/week. Judging from the results of this survey there were no adenovirus ARD's at Fort McClellan during the period of this study. Influenza caused 21.8% of upper respiratory illnesses (65% B's and 35% A's), and 78.2% were caused by undetermined agents. There were 4 Mycoplasma pneumonia infections as diagnosed by paired sera complement fixations. Adenovirus vaccines are not administered to female military persons. Influenza vaccine is administered the first day of arrival on post, throughout the year.

Table 1 indicates a break-down of adenovirus types and percentages isolated from hospitalized BCT's at each Study Fort and the Navy Training Center, San Diego. The largest number of isolates were obtained out of the Navy samples (66 adenovirus 4's and 75 adenovirus 7's). Fort Wood was the only Fort experiencing more adenovirus 4 than adenovirus 7 infections. During the year, 37% of adenovirus isolates were 4's and 63% were 7's.

Table 2 indicates the number of influenza cases as determined by CF, that occurred at each study Fort and the Navy Training Center, San Diego. A breakdown by serotype is shown. Influenza vaccine is administered to receptionees during their first day on post. In Table 2, diagnostic rises that occurred within two weeks after vaccine, are considered due to vaccine. Those diagnostic rises that occurred 3 weeks and beyond after vaccine, are considered due to influenza disease. Fort Leonard Wood experienced the largest number of influenza cases (10 A's and 16 B's), followed by Fort McClellan (7 A's and 13 B's). Mycoplasma pneumonia cases totalled 52, with a significant number occurring at Fort Polk and Fort Ord. The Navy Training Center usually experiences the largest number of mycoplasma cases - only 8 were determined during FY '74. During the year, 49% of influenza cases were due to flu A and 51% to flu B.

Of the 75% unknown ARD etiology, retro-spective studies indicate approximately 7% were due to bacteria. It is felt that most of this unknown etiology was caused by rhinoviruses. Since rhinoviruses reach maximum yield before the recruit develops severe enough symptoms to warrant hospitalization, by the time he is admitted the virus population may be so low that chances of obtaining isolations are drastically decreased.

A moderately potent adenovirus vaccine decreased ARD rates during FY '74. Even though the over-all rate decreased, the number of cases caused by unknown agents increased.

FIGURE 1. ADENOVIRUS SURVEILLANCE PROGRAM

JULY 1973 - 1 JULY 1974

Forts	Reporting Laboratory	Spec Rec'd	Isolations	Percent	Paired Sera Rec'd	ADENOVIRUS ISOLATION / SEROCONVERSIONS Fourfold Rise: Adeno	Flu A	Flu B	Myco.
Knox	USAML-FT BAKER	455	43	9.5%	116	20(17.2%)	20(17.2%)	12(10.3%)	3(2.6%)
	USAML-FT MEADE	336	31	9.2%	98	6(6.1%)	14(14.3%)	8(8.2%)	0(0.0%)
Ord	USAML-FT BAKER	262	18	6.9%	226	32(14.2%)	13(5.8%)	22(9.7%)	12(5.3%)
	USAML-ST LOUS	235	10	4.3%	170	30(17.6%)	1(0.6%)	18(10.6%)	0(0.0%)
Polk	USAML-FT BAKER	192	2	1.0%	135	7(5.2%)	9(6.7%)	8(5.9%)	13(9.6%)
	USAML-FT HOUSTON	149	5	3.4%	58	7(12.1%)	1(1.7%)	4(6.9%)	6(10.3%)
Wood	USAML-FT BAKER	423	16	3.8%	248	25(10.1%)	28(11.3%)	23(9.3%)	7(2.8%)
	USAML-ST LOUIS	395	8	2.0%	236	16(6.8%)	12(5.1%)	28(11.9%)	0(0.0%)
Dix	USAML-FT BAKER	252	3	1.2%	122	14(11.5%)	11(9.0%)	4(3.3%)	5(4.1%)
	USAML-FT MEADE	249	7	2.8%	101	4(4.0%)	6(5.9%)	4(4.0%)	0(0.0%)
Jackson	USAML-FT BAKER	132	1	0.8%	25	3(12.0%)	3(12.0%)	1(4.0%)	0(0.0%)
	USAML-FT McPHERSON	113	1	0.9%	23	4(17.4%)	0(0.0%)	0(0.0%)	0(0.0%)
McClellan	USAML-FT BAKER	129	0	0.0%	110	0(0.0%)	9(8.2%)	23(20.9%)	4(3.6%)
US Navy TC	USAML-FT BAKER	821	141	17.2%	287	66(23.0%)	4(1.4%)	16(5.6%)	8(2.8%)
TOTALS BY AREA LABS	USAML-FT MEADE	585	38	6.5%	199	10(5.0%)	20(10.0%)	12(6.0%)	0(0.0%)
	USAML-FT McPHERSON	113	1	0.9%	23	4(17.4%)	0(0.0%)	0(0.0%)	0(0.0%)
	USAML-ST LOUIS	630	18	2.9%	406	46(11.3%)	13(3.2%)	46(11.3%)	0(0.0%)
	USAML-FT HOUSTON	149	5	3.4%	58	7(12.1%)	1(1.7%)	4(6.9%)	6(10.3%)
	USAML-FT BAKER	2666	224	8.4%	1269	167(13.2%)	97(7.6%)	109(8.5%)	52(4.1%)
TOTAL		4143	286	6.9%	1955	234(12.0%)	131(6.7%)	171(8.7%)	62(3.1%)

Adenovirus Isolation Efficiency = 57.5%

FIGURE 2.

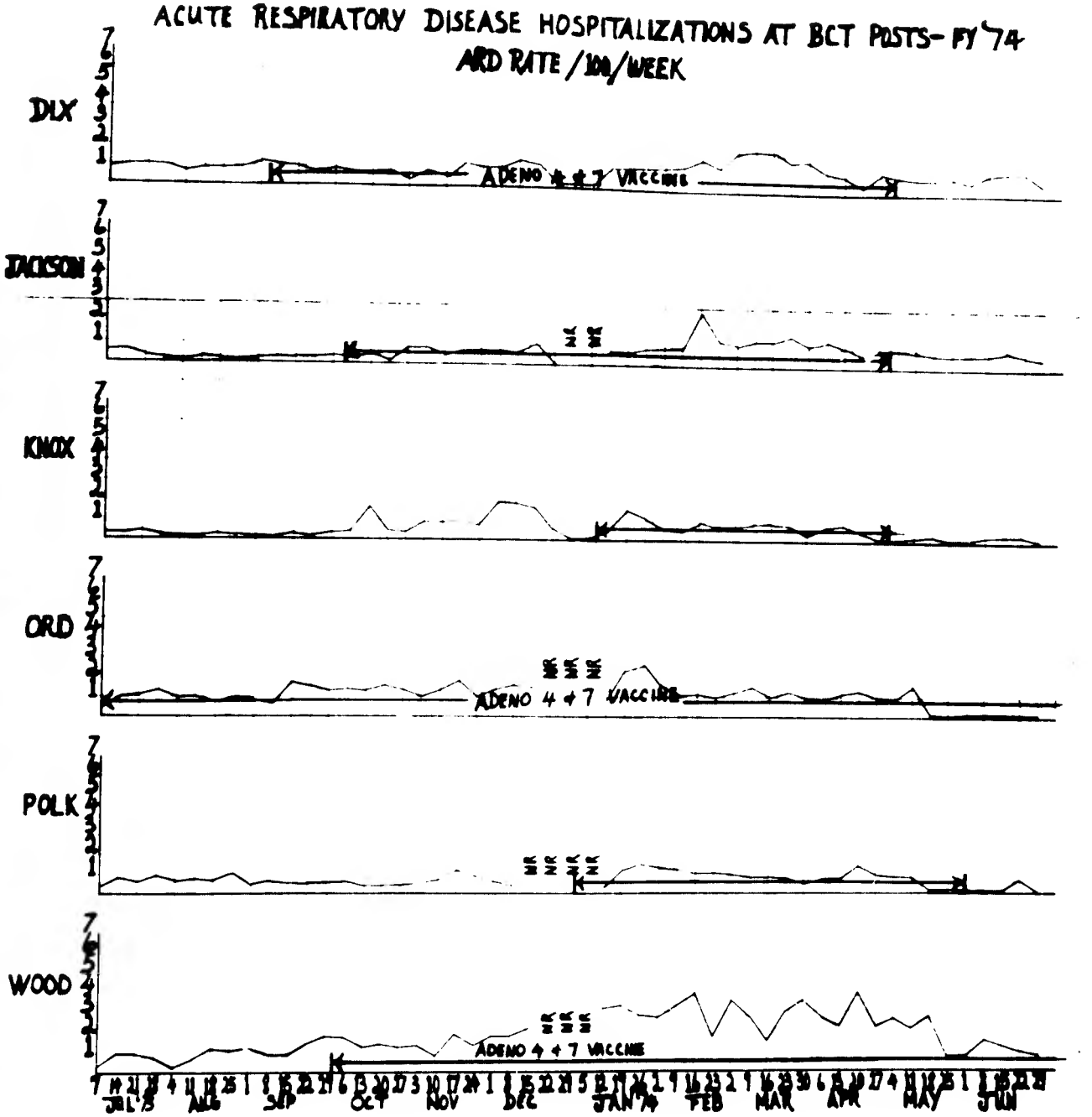

ACUTE RESPIRATORY DISEASE HOSPITALIZATIONS AT BCT POSTS- FY '74
ARD RATE / 100 / WEEK
DLX
ADENO 4 & 7 VACCINE
JACKSON
KNOX
ORD
ADENO 4 + 7 VACCINE
POLK
WOOD
ADENO 4 + 7 VACCINE
JUL '73
AUG
SEP
OCT
NOV
DEC
JAN '74
FEB
MAR
APR
MAY
JUN

FIGURE 3.

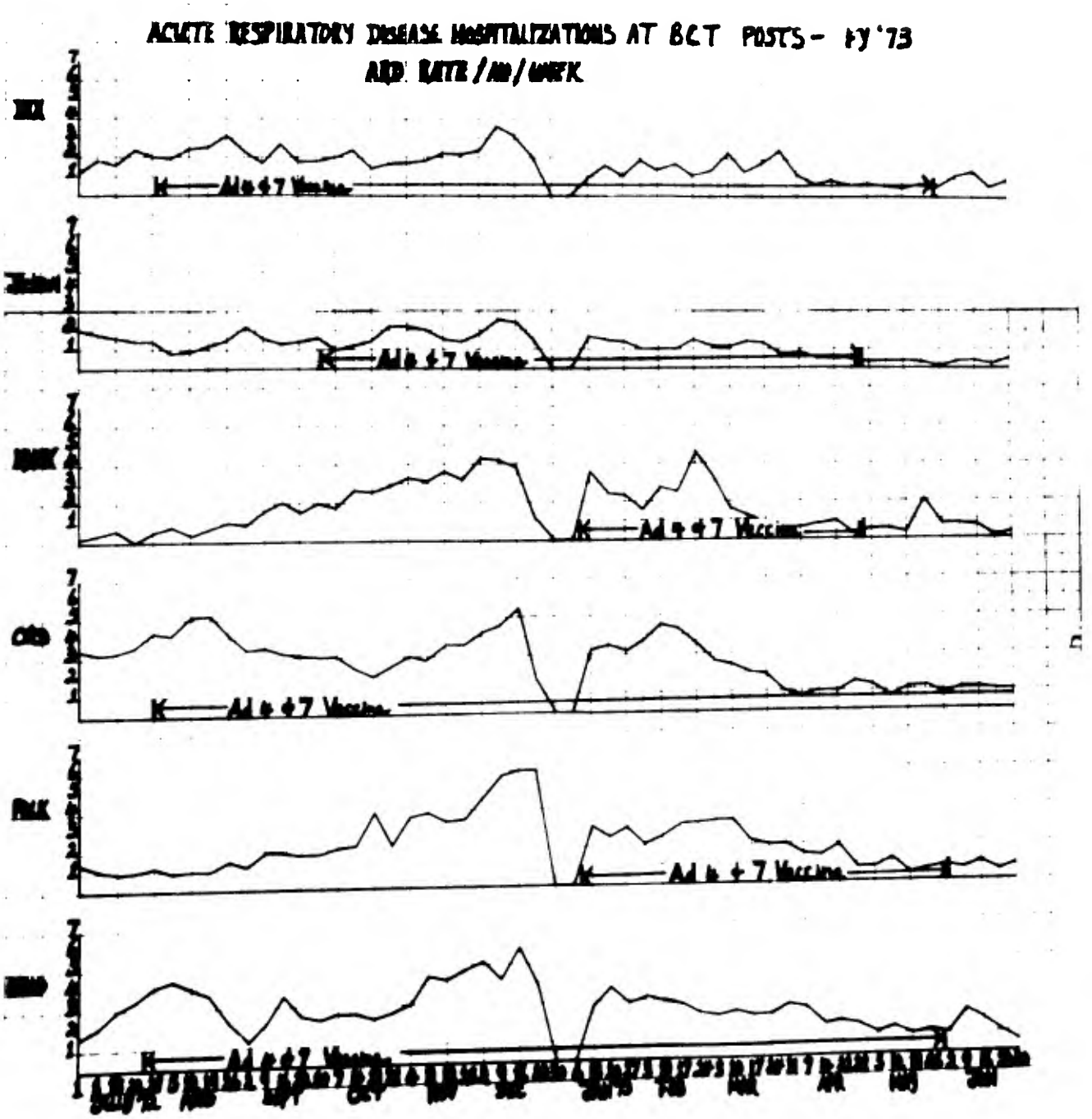
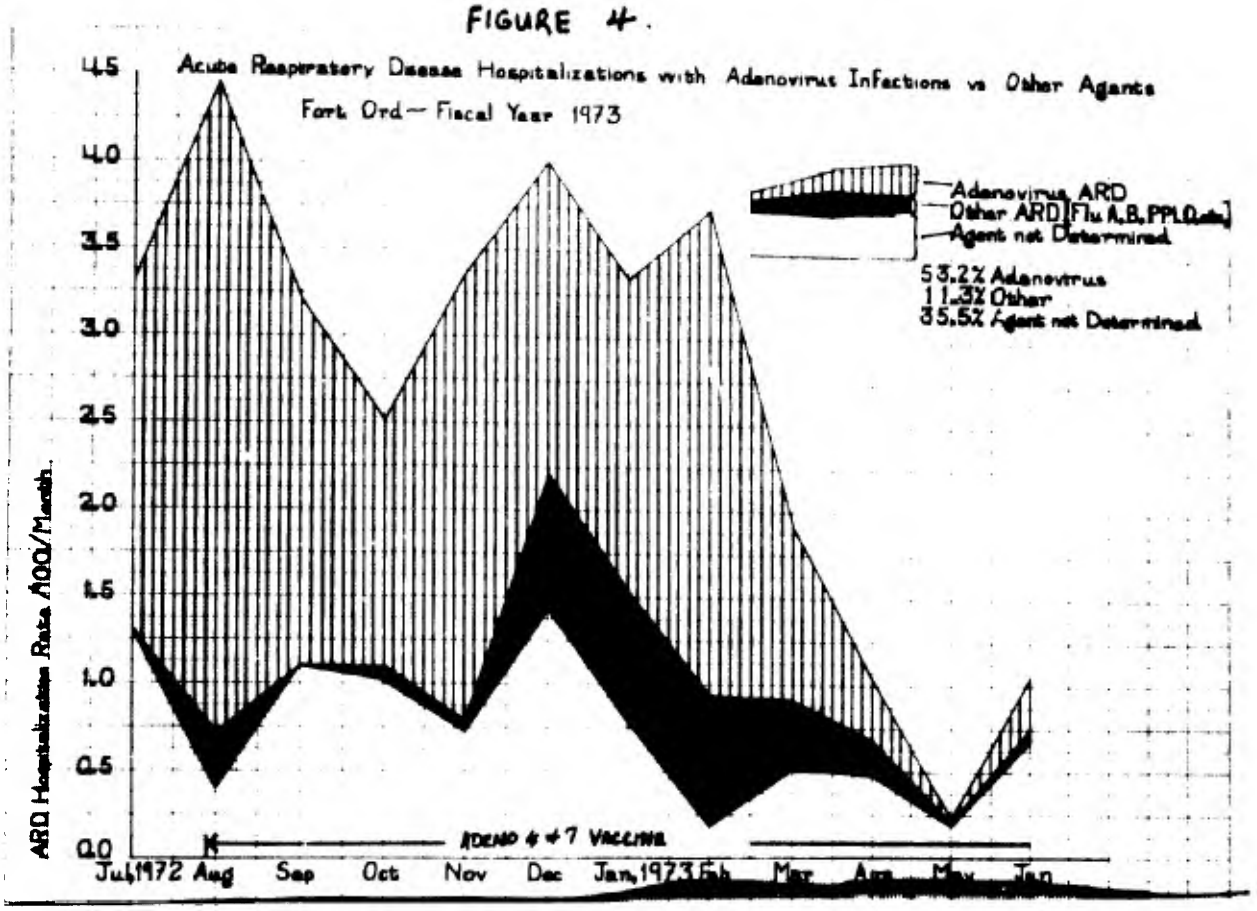

ACUTE RESPIRATORY DISEASE HOSPITALIZATIONS AT BCT POSTS - FY '73
AVG. RATE / MO / WORK

FIGURE 4.


Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Ord — Fiscal Year 1973
ARD Hospitalization Rate/100/Month
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Adenovirus ARD
Other ARD [Flu A, B, PPLO, etc.]
Agent not Determined
53.2% Adenovirus
11.3% Other
35.5% Agent not Determined
ADENO 4+7 VACCINE
Jul 1972
Aug
Sep
Oct
Nov
Dec
Jan 1973
Feb
Mar
Apr
May
Jun

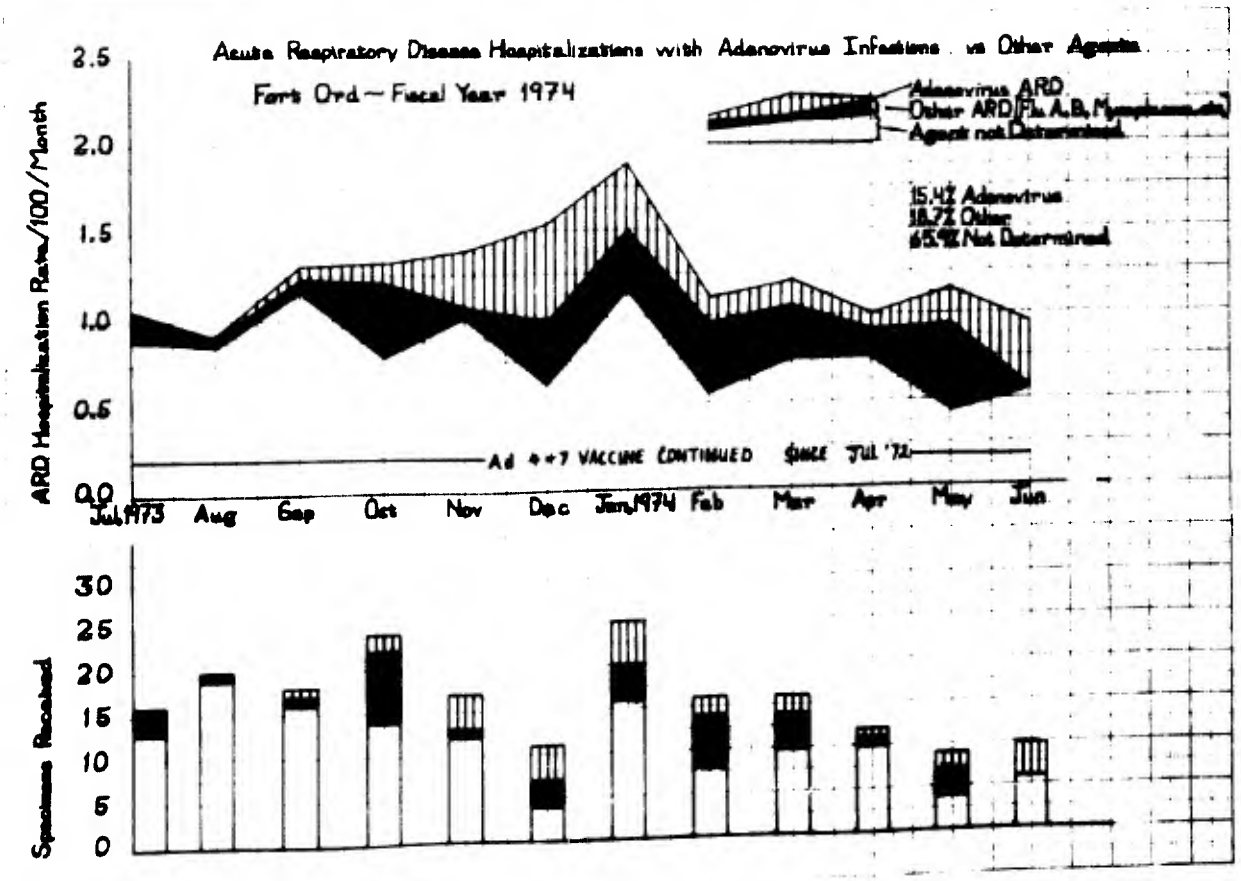
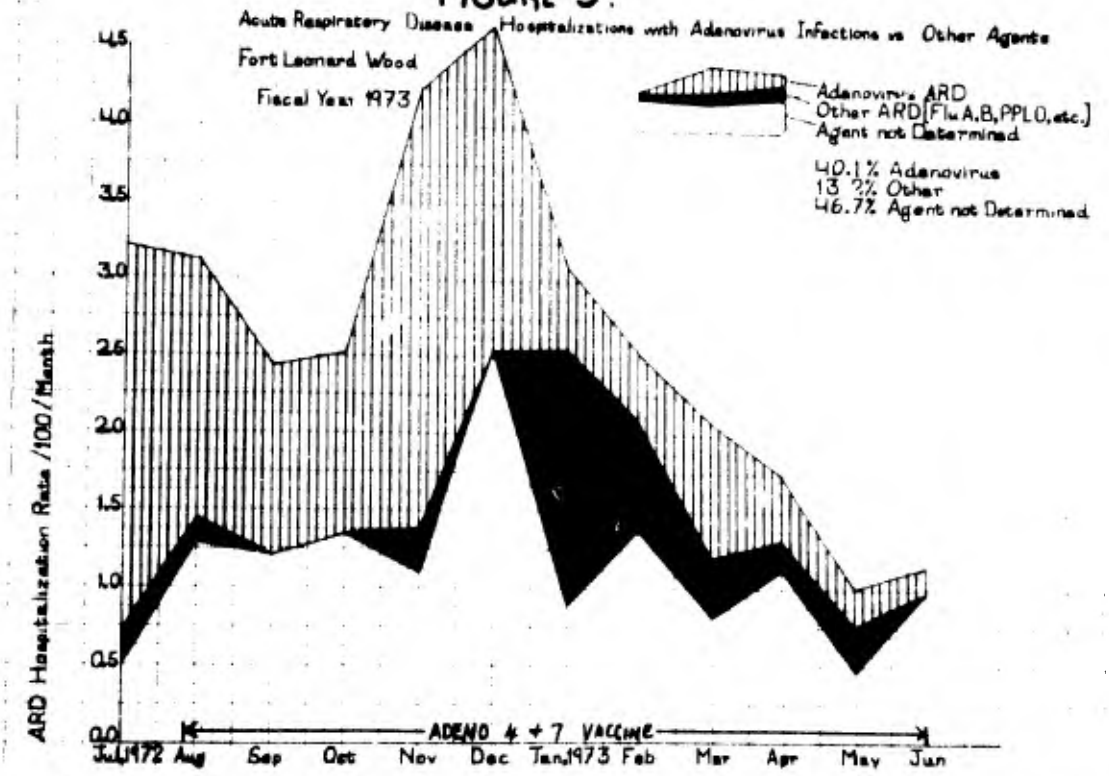

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Ord — Fiscal Year 1974
ARD Hospitalization Rate/100/Month
2.5
2.0
1.5
1.0
0.5
0.0
Adenovirus ARD
Other ARD [Flu A, B, Mycoplasma, etc.]
Agent not Determined
15.4% Adenovirus
18.7% Other
65.9% Not Determined
Ad 4+7 VACCINE CONTINUED SINCE JUL '72
Jul 1973
Aug
Sep
Oct
Nov
Dec
Jan 1974
Feb
Mar
Apr
May
Jun
Specimens Received
30
25
20
15
10
5
0

FIGURE 5.


Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Leonard Wood
Fiscal Year 1973
Adenovirus ARD
Other ARD [Flu A,B, PPLO, etc.]
Agent not Determined
40.1% Adenovirus
13.2% Other
46.7% Agent not Determined
ARD Hospitalization Rate /100/Month
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ADENO 4 + 7 VACCINE
Jul 1972
Aug
Sep
Oct
Nov
Dec
Jan 1973
Feb
Mar
Apr
May
Jun

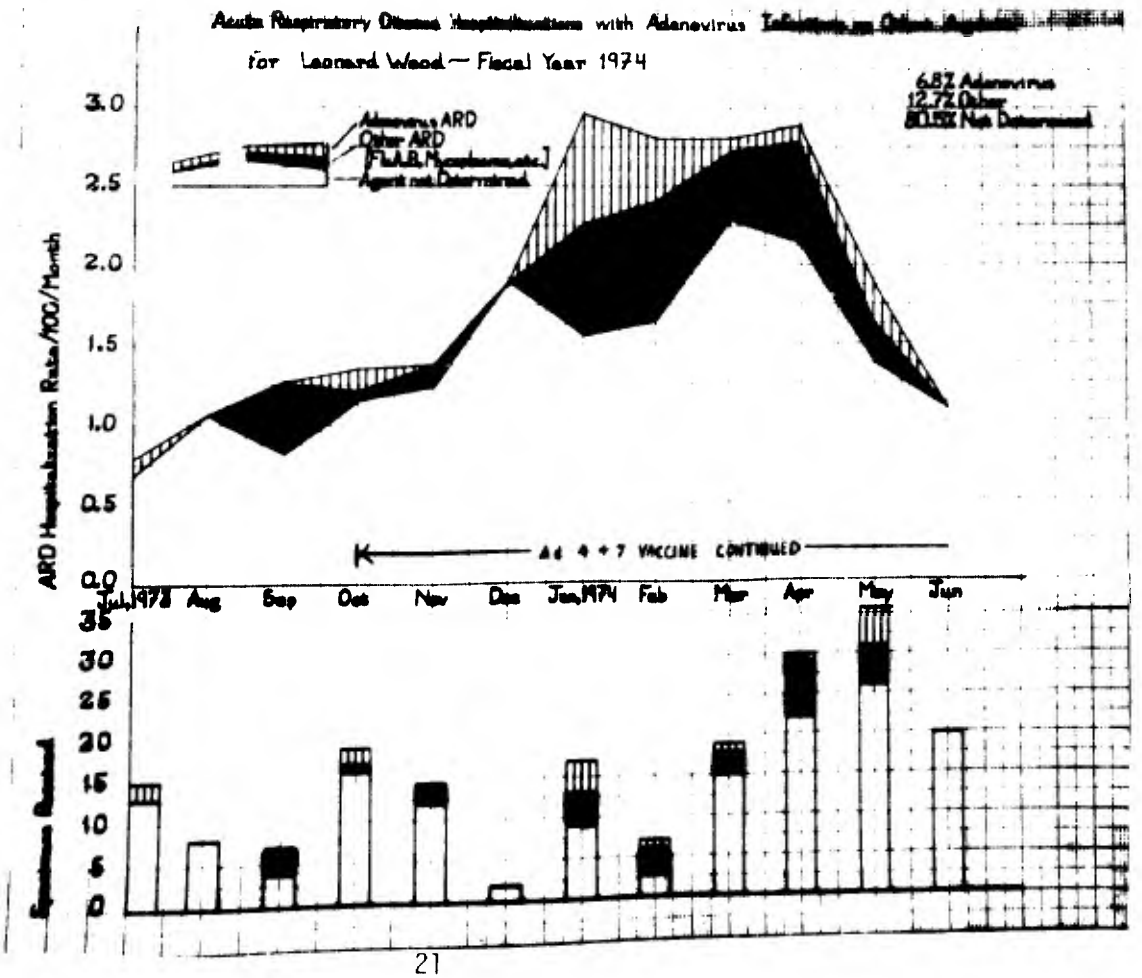
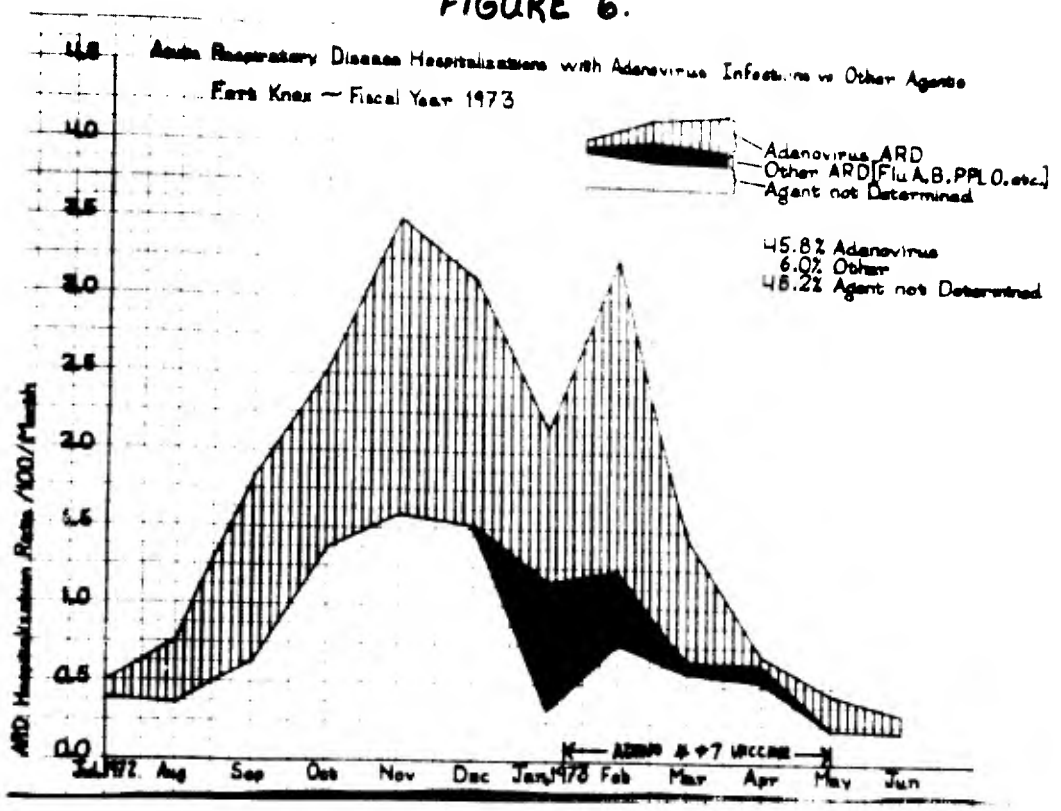
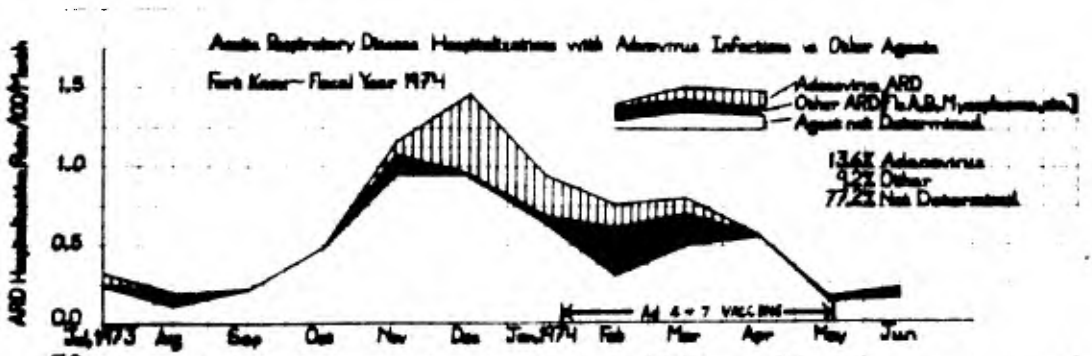

for Leonard Wood — Fiscal Year 1974
6.8% Adenovirus
12.7% Other
80.5% Not Determined
Adenovirus ARD
Other ARD
Agent not Determined
ARD Hospitalization Rate/100/Month
3.0
2.5
2.0
1.5
1.0
0.5
0.0
4 + 7 VACCINE CONTINUED
Jul 1973
Aug
Sep
Oct
Nov
Dec
Jan 1974
Feb
Mar
Apr
May
Jun
35
30
25
20
15
10
5
0

FIGURE 6.


Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Knox — Fiscal Year 1973
Adenovirus ARD
Other ARD [Flu A, B, PPI 0, etc.]
Agent not Determined
45.8% Adenovirus
6.0% Other
48.2% Agent not Determined
ARD Hospitalization Rate /100/Month
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Jul 1972
Aug
Sep
Oct
Nov
Dec
Jan 1973
Feb
Mar
Apr
May
Jun


Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Knox — Fiscal Year 1974
Adenovirus ARD
Other ARD
Agent not Determined
13.6% Adenovirus
9.2% Other
77.2% Not Determined
ARD Hospitalization Rate/100/Month
1.5
1.0
0.5
0.0
Jul 1973
Aug
Sep
Oct
Nov
Dec
Jan 1974
Feb
Mar
Apr
May
Jun

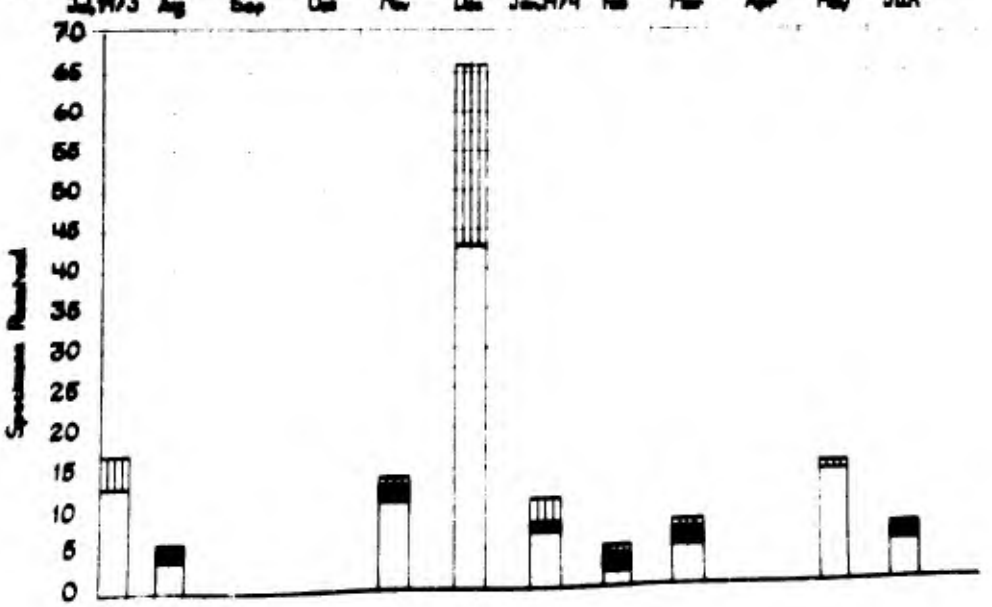

Specimens Received
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0

FIGURE 7.

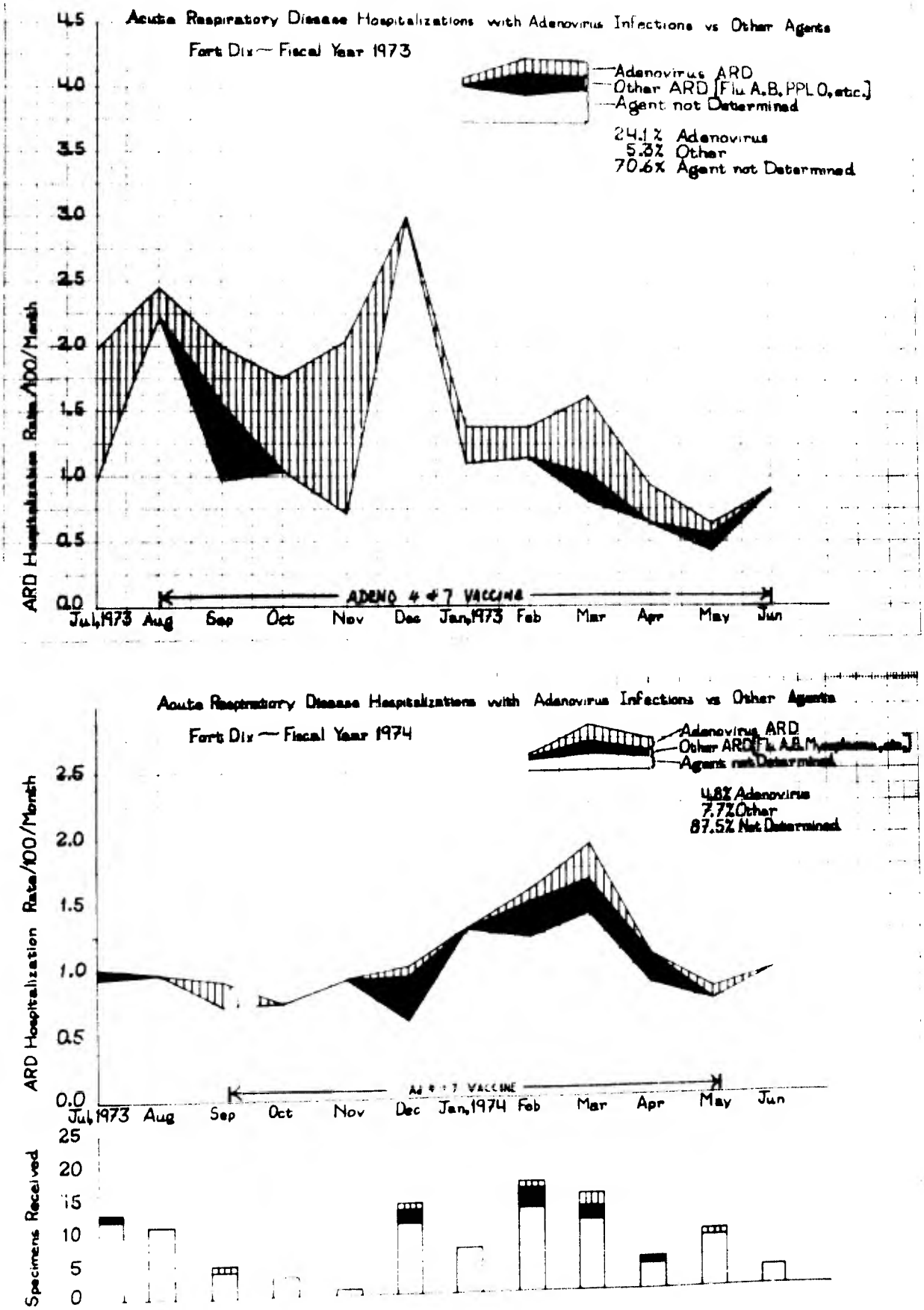

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Dix — Fiscal Year 1973
Adenovirus ARD
Other ARD [Flu A.B. PPLO, etc.]
Agent not Determined
24.1% Adenovirus
5.3% Other
70.6% Agent not Determined
ARD Hospitalization Rate/100/Month
ADENO 4 & 7 VACCINE
Jul 1973 Aug Sep Oct Nov Dec Jan 1973 Feb Mar Apr May Jun
Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Dix — Fiscal Year 1974
Adenovirus ARD
Other ARD
Agent not Determined
4.8% Adenovirus
7.7% Other
87.5% Not Determined
ARD Hospitalization Rate/100/Month
Ad 4 & 7 VACCINE
Jul 1973 Aug Sep Oct Nov Dec Jan 1974 Feb Mar Apr May Jun
Specimens Received

FIGURE 8.

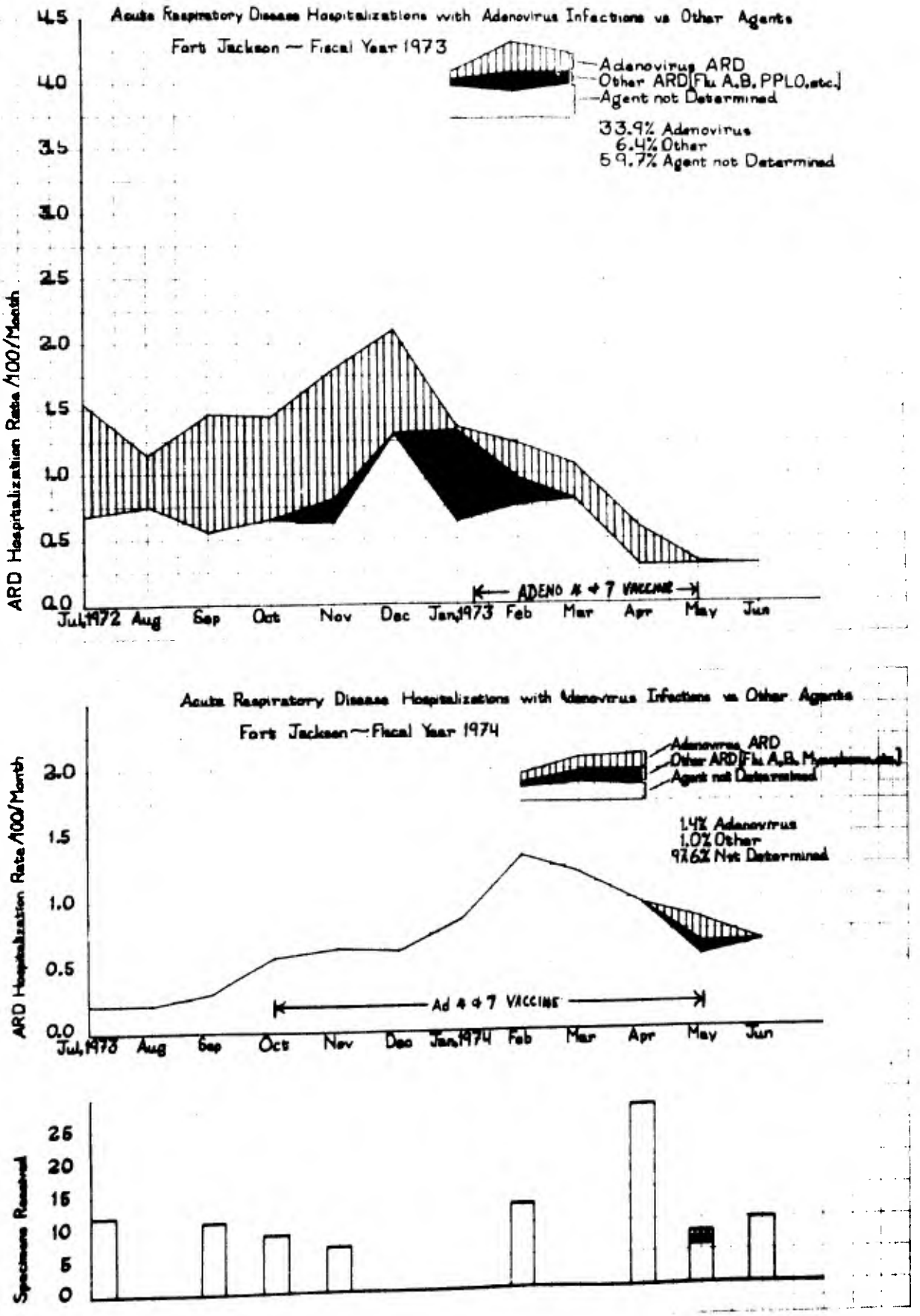
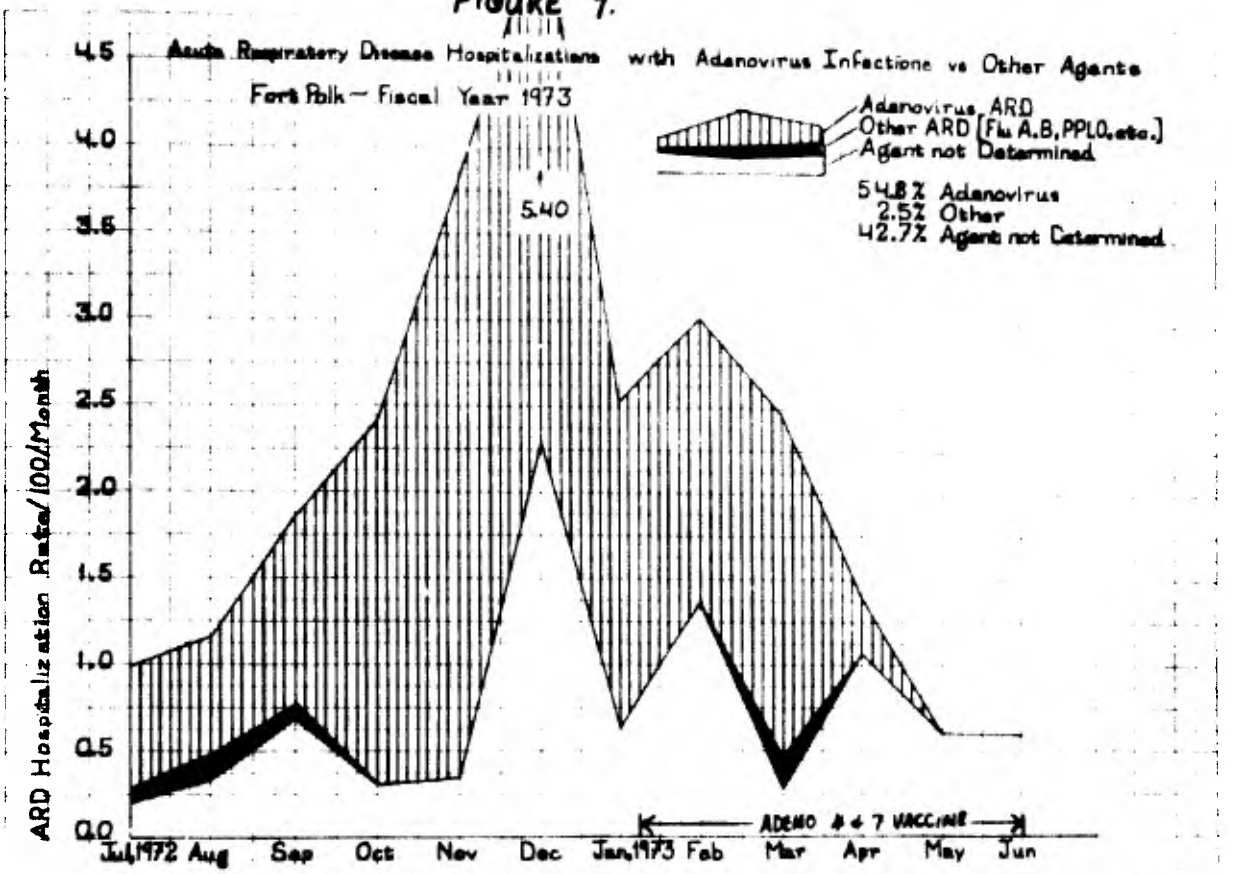

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Jackson — Fiscal Year 1973
Adenovirus ARD
Other ARD (Flu A,B, PPLO, etc.)
Agent not Determined
33.9% Adenovirus
6.4% Other
59.7% Agent not Determined
ARD Hospitalization Rate/100/Month
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ADENO 4 & 7 VACCINE
Jul 1972
Aug
Sep
Oct
Nov
Dec
Jan 1973
Feb
Mar
Apr
May
Jun
Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Jackson — Fiscal Year 1974
Adenovirus ARD
Other ARD (Flu A,B, Mycoplasma, etc.)
Agent not Determined
1.4% Adenovirus
1.0% Other
97.6% Not Determined
ARD Hospitalization Rate/100/Month
2.0
1.5
1.0
0.5
0.0
Ad 4 & 7 VACCINE
Jul 1973
Aug
Sep
Oct
Nov
Dec
Jan 1974
Feb
Mar
Apr
May
Jun
Specimens Received
25
20
15
10
5
0

FIGURE 9.


Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Polk — Fiscal Year 1973
Adenovirus ARD
Other ARD [Flu A,B, PPLO, etc.]
Agent not Determined
54.8% Adenovirus
2.5% Other
42.7% Agent not Determined
5.40
ARD Hospitalization Rate/100/Month
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ADENO # 4 & 7 VACCINE
Jul, 1972 Aug Sep Oct Nov Dec Jan, 1973 Feb Mar Apr May Jun

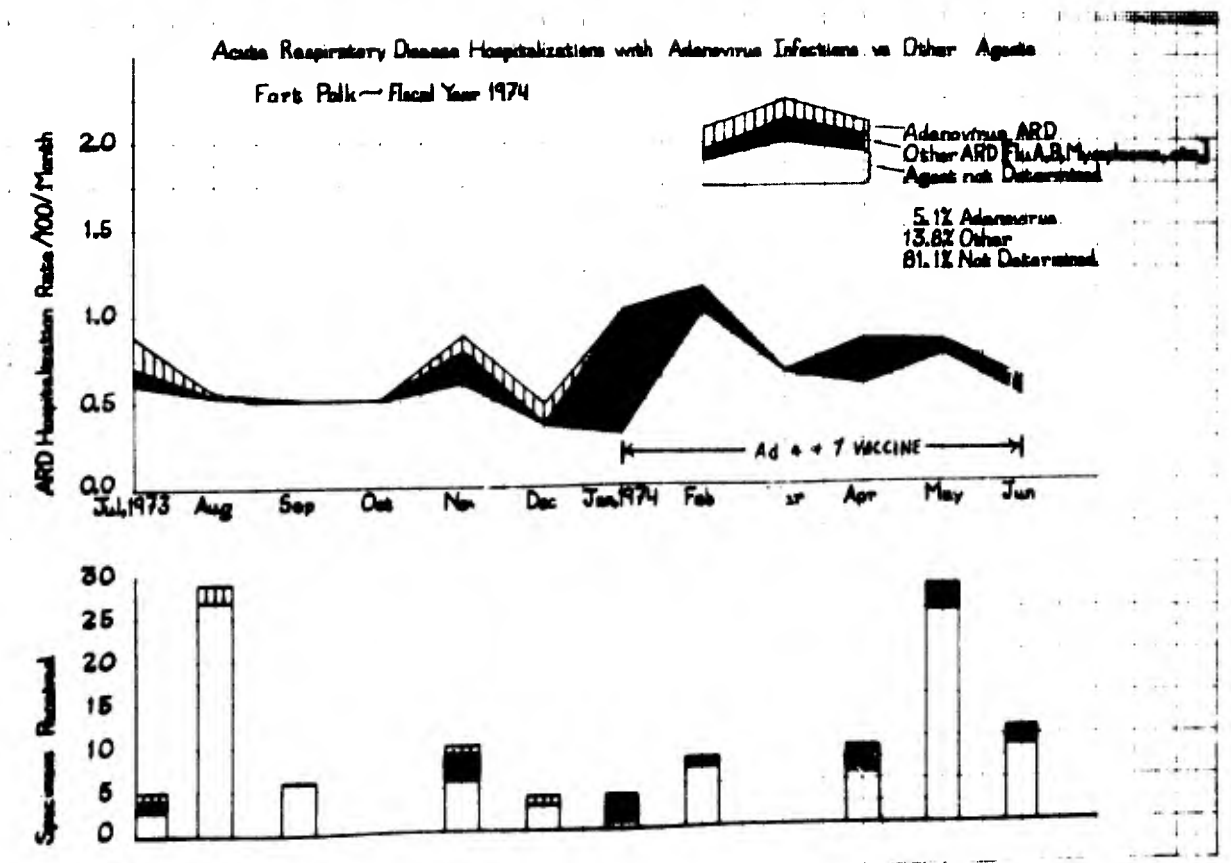

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort Polk — Fiscal Year 1974
Adenovirus ARD
Other ARD
Agent not Determined
5.1% Adenovirus
13.8% Other
81.1% Not Determined
ARD Hospitalization Rate/100/Month
2.0
1.5
1.0
0.5
0.0
Ad 4 & 7 VACCINE
Jul, 1973 Aug Sep Oct Nov Dec Jan, 1974 Feb Mar Apr May Jun
Specimens Received
30
25
20
15
10
5
0

FIGURE 10.

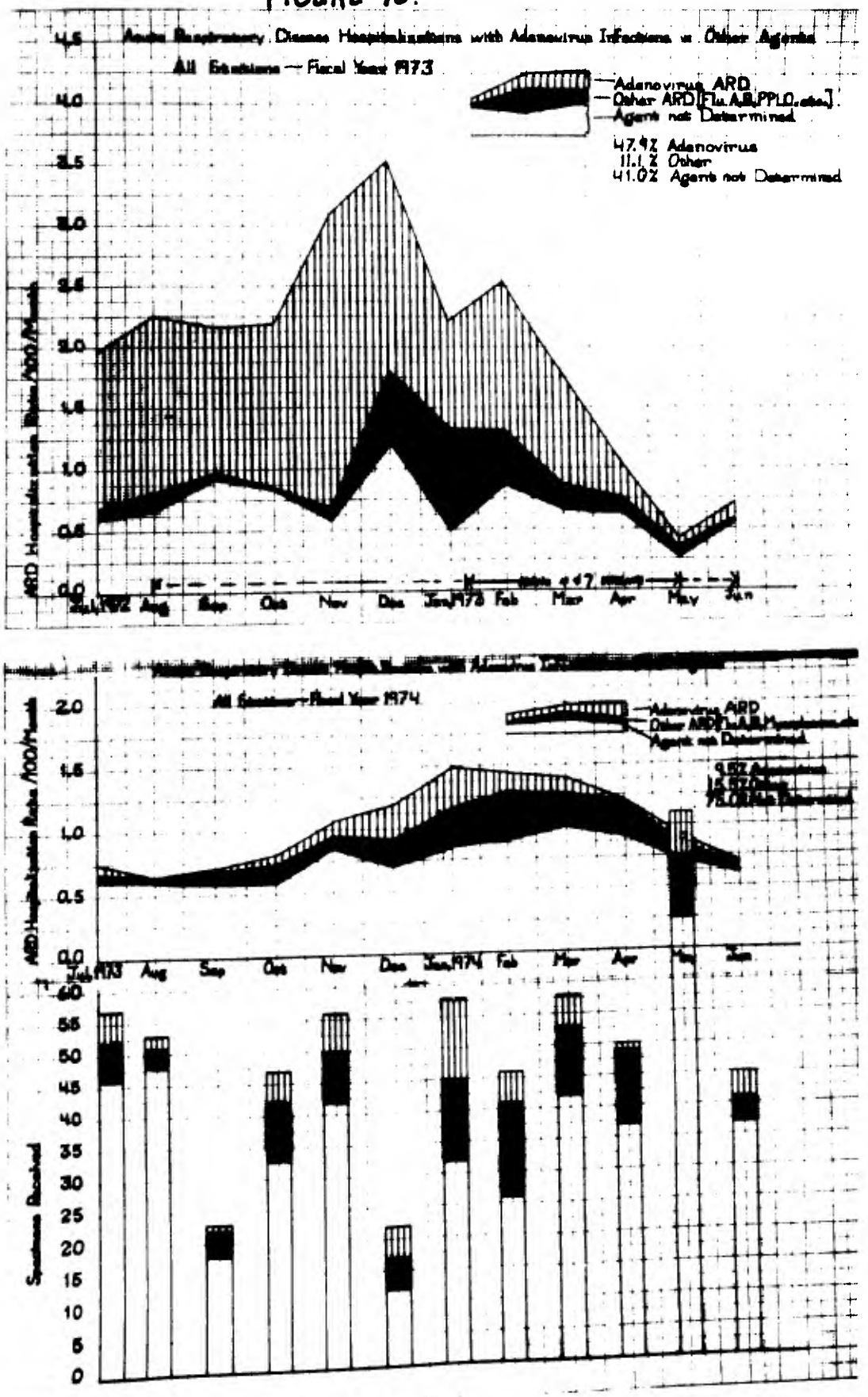


FIGURE 11.

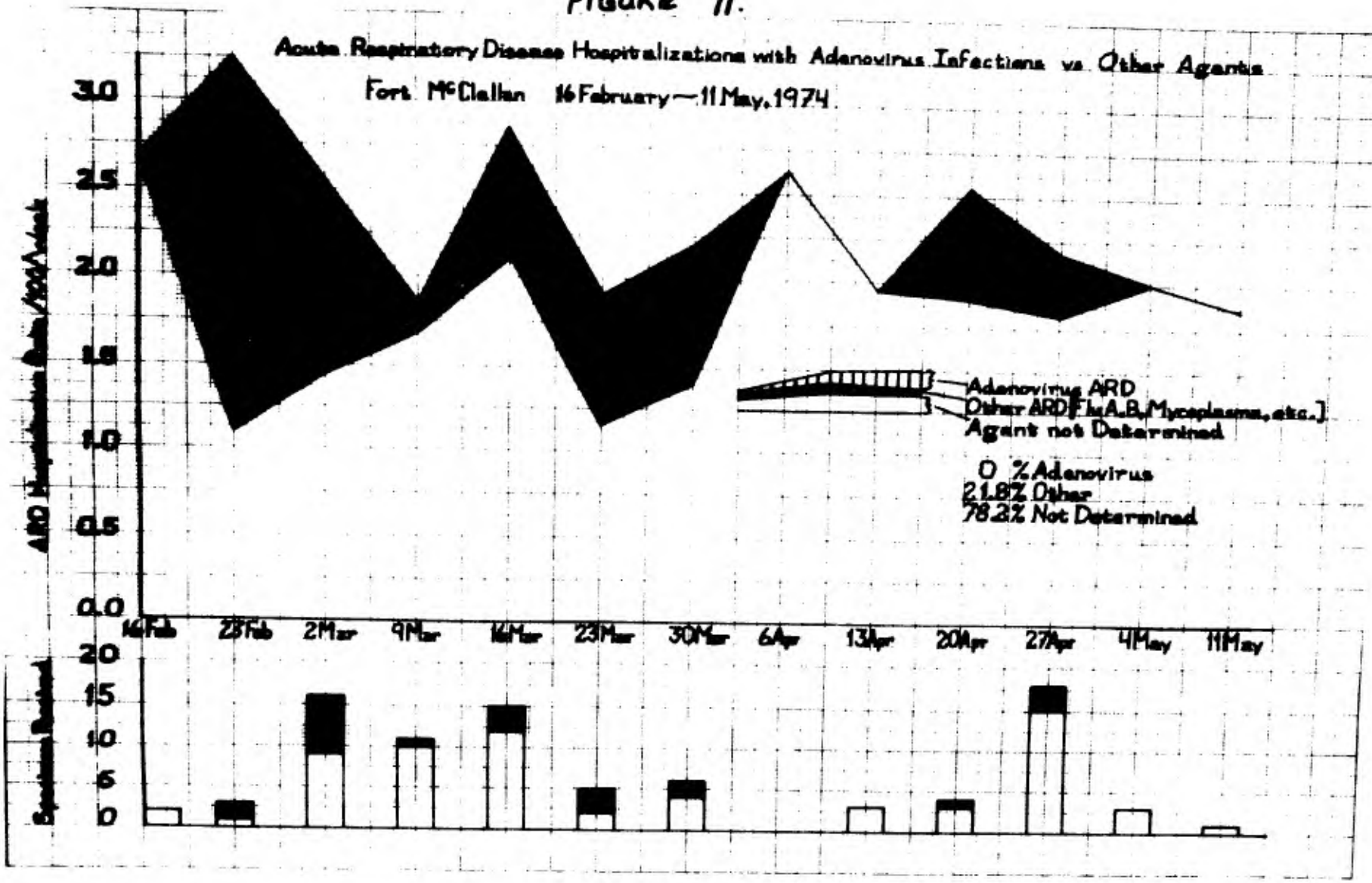

Acute Respiratory Disease Hospitalizations with Adenovirus Infections vs Other Agents
Fort McClellan 16 February — 11 May, 1974
ARD Hospitalizations Rate/1000 Admissions
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Adenovirus ARD
Other ARD [Flu A, B, Mycoplasma, etc.]
Agent not Determined
0 % Adenovirus
21.8% Other
78.2% Not Determined
16 Feb
23 Feb
2 Mar
9 Mar
16 Mar
23 Mar
30 Mar
6 Apr
13 Apr
20 Apr
27 Apr
4 May
11 May
Specimens Received
20
15
10
5
0

TABLE 1.

ADENOVIRUS ISOLATES

July '73 - July '74

FORT	Ad 4	Ad 7	Ad 4	Ad 7	Total Samples Processed
DIX	6	37	14%	86%	250
KNOX	6	37	14%	86%	455
JACKSON	0	1	0%	100%	132
POLK	0	2	0%	100%	192
WOOD	10	6	62%	38%	423
ORD	9	9	50%	50%	262
McCLELLAN	0	0	0%	0%	129
NTC (SAN DIEGO)	66	75	47%	53%	821
	97	167			2666
	(37%)	(63%)			

TABLE 2.

INFLUENZA CASES

July '73 - July '74

FORT	FLU A		FLU B		MYCO
	< 2 wks	> 3 wks	< 2 wks	> 3 wks	
DIX	7	4	3	1	5
KNOX	1	12	5	7	3
JACKSON	0	0	0	0	0
POLK	3	6	7	0	13
WOOD	18	10	15	8	7
ORD	3	10	6	16	12
McCLELLAN	2	7	10	13	4
NTC (SAN DIEGO)	3	1	10	6	8
TOTAL	37	50	56	51	52
		(49%)		(51%)	

ANTIGENICITY AND EFFICACY EVALUATION OF ADENOVIRUS VACCINES FY '74

Wyeth adenovirus vaccine lots #04902 (Type 4) and #05302 (Type 7) were tested for infectivity on HEK cells according to Standard Procedures. The results were as follows:

1. Titration of 30 October 1973

   a. Wyeth Lot #04902 type 4 $10^{4.1}$ $TCID_{50s}$/pill

   b. Wyeth Lot #05302 type 7 $10^{3.4}$ $TCID_{50s}$/pill

On site evaluation of type 4 and type 7 vaccines were conducted this year at Fort Ord, Fort Leonard Wood and Fort Jackson. The accumulative results of serum neutralization tests conducted on paired sera drawn pre and post vaccine is shown below:

	Conversion/Susceptibles	% Conversions
Adenovirus Type 4	31/44	70.4
Adenovirus Type 7	23/29	79.3

Eighty-two paired sera were tested in this evaluation. Fifty-four percent of recruits tested, were susceptible to Type 4 virus (Pre-serum titer <1:4) and 35% were susceptible to Type 7.

MYCOPLASMA INFECTIONS

A summary of Mycoplasma pneumoniae infections over the past 4 years, on a monthly basis, at 6 BCT Forts and the Navy Training Center (NTC) at San Diego is presented in Table 1. These were determined by seroconversions, >4-fold rises by complement-fixation. Figures in the table are presented by seroconversions over number of paired sera tested. During the last 6 months of 1972, mycoplasma CF's were not done, hence the ND in that area. The exception to this was NTC, San Diego. In November of 1972 at NTC San Diego, a large number of mycoplasma infections prompted the performance of CF's. During November and December of that year, NTC experienced more mycoplasma infections (7.1%) than any other training Fort experienced for an entire year (except Fort Knox). Fort Knox experienced the greatest number of infections for the 4-year period, 5.8% (17/293) of samples processed; 7.2% infections in 1973 alone. The grand total of paired sera tested for Mycoplasma pneumoniae from all of the Forts during the 4-year period was 5,842; and of these, 206 were positive, to give 3.5% positive at the 8 stations for the 4-year period.

TABLE 1.

FOURFOLD RISES BY CF TO RPCP PLASMA PRELIMINARY JULY 1970 - JULY 1974

	Indiana					Miami					Fiji					Ord Jct				
	1970	1971	1972	1973	1974	1970	1971	1972	1973	1974	1970	1971	1972	1973	1974	1970	1971	1972	1973	1974
January		0/6	0/0	1/9	2/0		0/19	0/5	0/9	1/11		0/17	0/0	0/4	0/8		1/58	6/48	3/49	3/21
February		0/16	1/27	0/11	0/0		6/70	2/85	0/34	0/21		1/23	0/28	0/6	0/0		3/64	5/63	2/35	1/26
March		0/22	2/81	0/10	0/0		0/93	9/14	6/83	2/22		0/39	0/48	0/19	0/0		5/73	1/78	7/80	0/30
April		0/13	0/35	1/12	0/0		2/85	0/97	1/36	0/18		0/11	1/21	0/24	2/14		2/78	0/22	3/28	0/19
May		0/24	0/36	0/10	0/0		1/88	7/154	3/76	1/95		0/6	1/35	0/12	1/13		1/70	5/68	1/82	1/14
June		0/32	0/0	0/0	1/26		1/86	2/30	1/8	0/25		0/6	0/0	0/0	2/05		2/79	1/11	0/9	0/9
July	0/0	0/0	ND	0/0	0/0	1/33	0/0	ND	0/14	0/27	0/23	0/0	ND	0/2	0/5	0/41	0/0	ND	2/22	0/8
August	0/3	0/0	ND	0/0		0/84	0/84	ND	0/21		0/0	0/0	ND	1/13		0/25	0/98	ND	3/22	
September	1/11	0/2	ND	0/0		0/98	2/96	ND	0/8		0/1	0/0	ND	0/11		0/14	4/81	ND	0/16	
October	1/22	0/0	ND	0/0		1/76	2/35	ND	1/14		0/7	0/2	ND	0/0		1/96	0/57	ND	1/19	
November	1/25	0/19	ND	0/0		1/52	1/33	ND	1/28		2/13	0/5	ND	0/0		0/50	2/45	ND	2/22	
December	0/18	0/19	ND	0/0		0/22	4/67	ND	1/16		0/10	1/21	ND	3/12		0/31	0/60	ND	1/18	
Total	3/79	0/153	3/237	2/52	1/25	3/275	19/972	30/494	14/300	4/374	2/59	2/120	2/132	4/103	5/85	1/237	18/721	10/290	25/362	5/121
% Pos.	3.7	0.0	2.2	3.8	4.0	1.1	2.3	6.6	4.1	2.3	3.7	1.7	1.5	3.9	5.9	0.4	2.5	3.4	6.9	4.1
Total/%		9/996	2.0%				54/1795	3.0%				15/994	3.0%				59/1731	3.4%		

	Ft Dix					Knox					San Diego					Total					Total %
	1970	1971	1972	1973	1974	1970	1971	1972	1973	1974	1970	1971	1972	1973	1974	1970	1971	1972	1973	1974	
January			0/4	0/0	1/5					1/27				8/85	0/29	1/100	0/57	2/154	6/101		19/412	4.6
February			1/92	0/0	0/0					1/19				4/89	0/0	4/173	7/295	6/155	2/66		19/689	2.3
March			1/16	0/15	3/27				13/99	0/0				4/48	0/0	3/207	13/294	30/354	5/81		51/976	5.2
April			0/0	2/33	0/12				1/51	0/23				3/53	0/0	4/185	1/125	11/237	2/75		18/632	2.8
May			0/8	1/37	1/11				0/30	0/0				1/86	0/0	2/138	13/301	6/253	4/93		25/785	3.2
June			0/0	0/2	0/5				0/0	0/17				1/28	0/0	3/163	3/81	2/47	5/125		11/377	2.9
July		0/0	ND	0/7	0/6				0/10	0/0				1/32	0/0	1/97	0/0	ND	3/87	0/46	4/230	1.7
August			ND	0/0					0/8					1/85		0/72	0/102	ND	5/109	—	5/323	1.5
September			ND	0/14					0/0					3/53		1/74	6/109	ND	3/102	—	10/285	3.5
October		0/0	ND	0/14					1/9					1/36		3/181	2/92	ND	4/92	—	9/365	2.5
November		1/20	ND	0/6					0/0				8/105	1/67		4/140	4/120	8/105	4/96	—	20/461	4.3
December		1/15	ND	0/4					0/0				3/69	1/15		0/83	6/172	3/49	6/65		15/369	4.1
Total	0/0	2/35	2/70	3/132	5/63	0/0	0/0	0/0	15/207	2/86	0/0	0/0	11/154	28/395	0/29	9/1605	35/1601	48/1847	92/1791	22/588	
%Pos.	-	5.7	2.9	2.3	7.9	—	—	—	7.2	2.3	—	—	7.1	4.9	0.0	1.4	2.2	3.9	5.1	3.7	
Total/%		12/305	3.9%				17/293	5.8%				44/770	5.8%								

Total Sera Conversions/Total paired Sera 206/5892 = 3.5%

EVALUATION OF MONOVALENT INFLUENZA GROUP B (HONG KONG) VACCINE IN MILITARY RECRUITS

INTRODUCTION

The occurrence of an antigenic shift in the prevalent group B influenza virus first recognized in 1972 required a change in the vaccine to be utilized in the military in the Fall and Winter of 1973-1974. B/HK/5/72 virus was selected as the prototype vaccine strain and two commercial drug companies (Wyeth and Merck) prepared monovalent products. In-vitro evaluation of the potency of these products produced conflicting results and the decision was made early in the Fall of 1973 to test the contigenicity of the vaccines in military recruits.

In collaboration with WRAIR and the H & E Activity at Fort Ord, California two lots of vaccine, one each Wyeth and Merck, were tested and compared to the standard bivalent vaccine containing the B/Mass/1/71 virus for their ability to induce type specific antibody.

MATERIALS AND METHODS

Vaccine:

a. Test Lot #4707G-B/HK/5/72 Manufactured by Merck, Sharpe and Dohme.

b. Test Lot #139501-B/HK/5/72 Manufactured by Wyeth.

c. Control Vaccine Lot #133101-A/England/42/72 and B/Mass/1/71 Manufactured by Wyeth Laboratories.

Subjects and Protocol: The first trial utilized 149 Basic Combat Trainees. A pre-bleed was drawn on 5 November 1973 and vaccine was administered to the experimental groups immediately following the collection of blood for the pre-serum. Three groups were formed from the 149 recruits participating in the study. Group I was a non-immunized control group of 50 people. Group II was comprised of 50 who received the control bivalent vaccine, and Group III, the remaining 49, received the monovalent vaccine prepared by Merck, Sharpe and Dohme. All these groups were bled again 21 days later on 26 November 1973. The second trial was conducted in the same manner for the purpose of evaluating the Wyeth Vaccine. There was a control group (no vaccine) in this trial but no control vaccine group. Pre-bleed and immunization was conducted on 19 December 1973. The post-vaccine bleed was conducted on 18 January 1974.

Viral Serology: Vaccine response was determined by testing pre and post vaccine sera for type specific hemagglutination-inhibition (HI) antibody. A microtiter procedure standard to this laboratory was employed. Sera were treated wiith receptor destroying enzymes to remove non-specific inhibitors. Antigens employed were type specific whole virus in allantoic fluid of embryonated chicken eggs.

RESULTS

The results of the first trial, which involved the Merck, Sharpe and Dohme vaccine, are presented in Table I. This vaccine induced an excellent antibody response producing 4-fold rises in HI antibody titer in all but two of the immunized recruits. A nearly fourteen-fold increase in the geometric mean titer occurred post immunization when measured against either the pre-immunization geometric mean or the 26 November sera of the control group. Fifty-seven percent of the vaccinees showed 16-fold or higher increases in type specific antibody. Surprisingly, this monovalent B-Hong Kong vaccine induced better heterologous B-mass antibody than did the homologous standard bivalent preparation.

Results with the Wyeth monovalent B-Hong Kong vaccine evaluated in the second trial are presented in Table II. Induced HI antibody was not as impressive as was seen with the Merck preparation, but was still very good. The geometric mean titer post immunization was 8-fold higher and 23 of 50 recruits experienced 16-fold or higher rises in HI antibody titer.

It is interesting to note that though these recruits were experiencing some natural infection with a B-mass-like virus during the second trial, these infections did not significantly effect the geometric mean titer to the B-Hong Kong virus.

SUMMARY

Two lots of monovalent B-Hong Kong Influenza Vaccine prepared by different commercial manufacturers were evaluated for antigenicity in Basic Combat Tranees at Fort Ord, California. Both lots were found to be satisfactory and far superior to the Standard Bivalent Vaccine in current use.

TABLE I

EVALUATION OF MERCK, SHARPE AND DOHME MONOVALENT B-HONG KONG INFLUENZA VACCINE

Vaccine Administered	HI Test Antigen	Fold Increase in Titer[1] 0-2	4-8	>16	Total	Geometric Mean[2] 1st Serum	2nd Serum	Four-Fold Rises
Merck Monovalent	B/Mass/1/71	10	21	18	49	23.7	162.3	80%
Lot #4707G	B/HK/5/72	2	19	28	49	6.1	83.5	96%
Standard Bivalent	B/Mass/1/71	21	23	6	50	18.2	32.9	58%
Wyeth Lot #133101	B/HK/5/72	39	9	2	50	5.7	10.0	22%
Control Group	B/Mass/1/71	48	2	0	50	15.8	18.2	4%
No Vaccine	B/HK/5/72	49	1	0	50	5.9	6.2	2%

1. Number of Recruits.

2. Reciprocal HI Titer.

TABLE II

EVALUATION OF WYETH LABORATORIES MONOVALENT B-HONG KONG INFLUENZA VACCINE

Vaccine Administered	HI Test Antigen	Fold Increase in Titer[1]				Geometri Mean[2]		Four-Fold Rises
		0-2	4-8	>16	Total	1st Serum	2nd Serum	
Wyeth Monovalent	B/Mass/1/71	13	20	17	50	19.2	133.6	74%
Lot #139501	B/HK/5/72	12	15	23	50	5.7	47.9	76%
Control Group	B/Mass/1/71	42	8	0	50	22.7	33.4	16%
No Vaccine	B/HK/5/72	49	1	0	50	5.6	5.9	2%

1. Number of Recruits.

2. Reciprocal HI Titer.

INFECTION WITH INFLUENZA A/ENG/42/72 LIKE VIRUS IN TWO GRAVID MILITARY DEPENDENT POPULATIONS

INTRODUCTION

Accounts of induction of congenital anomalies or the increased occurrence of abortions, stillbirths or prematurities as results of maternal infections with influenza A virus have been inconsistent. Beginning about twenty years ago and especially since the introduction of the H_2N_2 strain of influenza A virus in 1957, numerous reports have appeared of the occurrence of excess major CNS anomalies in new borns following influenza epidemics (1-4). At the same time, other studies have found no such association (5-7). One of the latter, which found no increase in major CNS anomalies, did report an increased incidence of cleft lip, oesophageal and anal atreseas and exophales (8). Almost all of the above reports were based on clinical determinations of influenza during periods of accepted outbreaks, but were devoid of supportive laboratory confirmation of infection.

Studies of laboratory supported or proven maternal influenza have in general lent little support to the association between maternal infection and adverse effects on the developing fetus (9-12). The exception to this is a study which did show an increase in abortion, stillbirth or prematurity associated with first trimester infection (13).

The present report presents the findings of 56 cases of serologically confirmed influenza type A infection and 51 cases of serologically presumptive infections in a population of 324 gravida. Fifty-three of the 107 females considered infected were in the first trimester or gestation at the outset of the epidemic that occurred in California in the Winter of 1972 and 1973.

MATERIALS AND METHODS

Background: California had been free of any large outbreak of type A influenza since 1969 and escaped the extensive outbreak of the 1971-1972 season that ran through the remainder of the country (14). In the fall of 1972, the England strain influenza A (H_3N_2) virus appeared in two military installations in Colorado. During November, the virus was reported sporadically throughout the country and by late November the first case had occurred in California. The middle of December saw the appearance of significant numbers of influenza cases in California, documented by both virus isolations and serological means as A/Eng/42/72 like virus, and marked the beginning of a sharp outbreak in the state that peaked between the second and fourth weeks in January 1973 (15, 16). By the middle of February, the group A epidemic was essentially over and the last case recorded in this laboratory as part of the epidemic had an onset during the week ending 17 February 1973. For the purposes of this study, the outbreak in the populations studied was considered to have run from 15 December 1972 to 14 February 1973.

Study Population: The women in this study were dependent wives of military personnel from two installations; March AFB in Riverside County, Ca. and Fort Ord in Monterey County, Ca. All the women were pregnant at the outset of the epidemic. Groups from both stations were similar in age and racial composition, and it was policy at both hospitals to withold influenza virus immunization for pregnant personnel. Vital data on both the mothers and newborns were obtained from hospital delivery and newborn record books.

Serum Collection and Serologic Procedures: Two sera were collected on all participants. The first originated from blood drawn at the first prenatal clinic visit while the second was drawn during the eighth month of pregnancy. All sera were stored at -20°C until testing could be accomplished as paired sera.

The hemagglutination-inhibition (HI) test was utilized as the primary serologic tool in determining influenza A antibody titers (17, 18, 19). Criteria for serologic evidence of infection was a confirmed four-fold or greater rise in type specific antibody. Four-fold rises were confirmed by retesting by HI or by a complement fixation (CF) test (20). Antigens employed were produced in the laboratory by standard techniques (17, 20).

Study Groups: The study population, combined from both installations, was divided into four study groups for analysis purposes. Groups A and B were those women demonstrated to have been infected during the epidemic period (Group A) or those women whose sera eclipsed the epidemic period but escaped infection (Group B). Groups C and D were those women whose first sera were drawn during the epidemic (after 15 December 1972) and did not have a four-fold rise in titer between sera. The second sera of Groups C and D women were drawn after 28 February 1973. Group C women had high titered first serum antibody similar to that in the second sera of Group A women. Group D women had low titered first sera antibody without significant change in titer in the second sera and were thus similar to Group B women.

RESULTS

Group size and range in first serum dates is shown in Table I. Second sera for Groups B, C and D women were all drawn on or after 28 February 1973. The total population studied comprised of 324 persons. A summary of serologic test results for groups and the interpretation of findings is shown in Table II. Groups A and C, comprised of individuals designated infected, totaled 107 of the 324 for an infection rate of 33/100 persons. Other characteristics of the population are shown in Table III. Fifty-three of the 107 infected persons were under 13.3 weeks gestation on 15 December 1972. This compares to 114 of the 217 (52.5%) persons in the non-infected groups. Group C, the high titered group, is significantly different from the remainder of the population in two characteristics. Group C was not randomly distributed with regard to racial composition as tested by chi square ($P<0.005$). In fact, there were an excess of Negroes in this group. Group C is also significantly younger than the population as a whole but this difference was not related to the racial differences. The age disparity but not the racial difference carries over to the combined infected groups A and C, i.e. Persons in the infected groups are significantly younger than persons in non-infected groups ($P<0.01$). The advantage of including Group C and D in the study can be seen for the weeks of gestation data in Table III. Whereas Groups A and B, the known groups, were already nearly half-way through their pregnancy at the start of the outbreak, Groups C and D, the presumptive groups were early in their pregnancies on the 15th of December. This is further demonstrated in the more complete breakout presented in Table IV.

TABLE 1

STUDY GROUPS, SIZE AND DATES OF FIRST SERUM

GROUP DESIGNATION	SIZE	FIRST SERUM DATE REQUIREMENT*	RANGE
A - INFECTED	56	NONE	6/22/72- 2/~~30~~20/73
B - NOT INFECTED	97	<12/15/72	8/10/72-12/12/72
C - HIGH TITERED	51	>12/15/72	1/2/73 - 6/13/73
D - LOW TITERED	120	>12/15/72	12/27/72- 6/5/73

* - by study group definition

TABLE 2

DISTRIBUTION OF HI ANTIBODY AND
GEOMETRIC MEAN TITERS WITHIN STUDY GROUPS

GROUP	A	B	C	D
SEROLOGIC RISE	YES	NO	NO	NO
GEOMETRIC $\bar{X}$				
1ST SERUM	14.1*	19.6	85.1	19.0
2ND SERUM	115.9	22.0	73.3	21.6
% $\leq$1:32				
1ST SERUM	100.0	89.5	0.0	100.0
2ND SERUM	5.3	83.3	15.6	95.0
INFECTION	YES	NO	YES	NO

* - Geometric $\bar{x}$ of Reciprocal Dilution.

TABLE 3

CHARACTERISTICS OF PARTICIPANTS AND
DAYS BETWEEN SERA COLLECTED

GROUP	A	B	C	D	TOTAL
AGE - Years	23.4	24.9	22.4	24.1	23.9
PERCENT CAUCASIAN	85.7	89.6	68.0	85.8	83.9
WKS GESTATION*	19.5	18.6	8.2	7.8	13.2
DAYS BETWEEN SERA	154	168	114	133	144

* - Weeks Gestation at 15 Dec 72

TABLE 4

PERCENT DISTRIBUTION TRIMESTER OF
GESTATION AT THE BEGINNING OF THE EPIDEMIC

GROUP	1ST TRIMESTER	2ND TRIMESTER	3RD TRIMESTER
A	21.4	66.1	12.5
B	13.8	83.0	3.2
C	80.4	19.6	0.0
D	84.2	15.8	0.0

TABLE 5

FINDINGS AT TERM

GROUP	A	B	C	D	TOTAL
WKS GESTATION*	40.0	40.0	40.1	40.2	40.1
BIRTH WT.	3417	3550	3611	3591	3552
PERCENT MALES	39.3	48.4	54.9	53.3	49.7
STILL BIRTHS	0	1	0	1	2
MALFORMATIONS	1	3	0	5	9
APGAR 1 MIN	8.54	8.51	8.47	8.54	8.52
Live Births	56	97+	51	119	323

* - Weeks of Gestation at delivery

+ - 1 Set of Twins.

TABLE 6

WEEKS OF GESTATION AT THE
BEGINNING OF THE EPIDEMIC

	< 20 WEEKS		> 20 WEEKS	
	OBS	EXP	OBS	EXP
INFECTED	74	82	33	25
NOT INFECTED	172	164	42	50

chi square = 5.65 P<0.02

The findings at term are shown in Table V. There was no evidence of prematurity in the infected groups as indicated by either mean weeks of gestation at delivery or mean birthweight at delivery. This conclusion was further strengthened by scattergrams (not shown) of conception dates versus birthweights for individual groups, and breakouts in 2 X 2 contingency tables of weeks of gestation at 15 December 1972 versus high or low birthweight by individual group or combined groups (infected versus non-infected). Group A newborns are slightly smaller than Group B babies, but the difference was not significant. There were four children born of these women that weighed less than 2500 gms. Two were in the infected groups and two were in the non-infected groups.

Two stillbirths and 9 malformations or abnormalities occurred among the 325 births. Both stillbirths and 8 of the 9 abnormalities were in the non-infected groups. Three of the 9 were minor in nature and included one instance of a deformed right ear and two instances of an extra digit on the hand or foot. There were two cases of congenital heart disease; one of a grade 2 murmur in the child shown in Group A and the second in the stillbirth shown in Group B. The only instance of cleft lip or palate occurred in a Group D child.

An as yet unexplained observation is shown in Table 6. The distribution of infected women by weeks of gestation at the beginning of the epidemic was examined and grouped according to the criteria of less than or greater than 20 weeks of gestation at 15 December 1972. This was then compared to the expected frequency based on the distribution for the total population. There were eight fewer women infected in the less than twenty weeks of gestation population than were expected.

The women in this study were also part of a prenatal infections study where retrospective serologic evaluations of paired sera were made for determination of infection rates with the agents of Rubella virus, cytomegalovirus, herpes simplex virus and Toxoplasma gondii. Examination of their records revealed that 10 persons in the group included four in the influenza virus infected groups and six in the not infected groups underwent infection during their pregnancy with one or more of the above agents. All ten of these women had uncomplicated pregnancies and delivered normal children. One of the children born of a Group D mother who underwent a Rubella virus infection developed seizures (hypocalcemic) at two weeks of age. The four women among the influenza virus infected groups underwent infection with cytomegolovirus (two, both from Group C); rubella virus and Toxoplasma gondii (one each both from Group A).

DISCUSSION

The results of this study indicate that fetal anomalies due to infection (with A/Eng/72 influenza virus) during pregnancy are too infrequent to be detectable in a study of this size or if they do occur or exist they are not visible at birth. Korones et. al. in their study of 52 maternal influenza infections with A/Asian/57 virus arrived at similar findings (11). Their study of 1970 involved a primarily Negro population of lower economic status. Our study population was primarily caucasian middle-class and this extends the nature of the data available in the literature. The authors in the Korones' study limited their conclusions to infections during the second and third trimesters of gestation. If we accept the 51 persons in the high-titered group as infected during the epidemic, we can extend the conclusion to include the first trimester gestation. There is little likelihood that the antibody titers of these patients reflected recent-past infections with A/HK/68 virus. As indicated earlier, California had been practically free of A/HK/68 virus infections for two years. Further, a cross check with virus specific antigens and the HI test revealed 16 of 16 persons tested with first serum titers equal to or greater than 1:40. In fact, their titers compared very closely with 23 second serum titers checked by HI of persons from the known infected Group A. It is also unlikely that these patients were infected with A/Eng/72 virus before the epidemic period. The epidemic of 1972-1973 marked the first introduction of this virus into California and our clinical laboratory results indicated that less than 5% of the documented infection with Type A virus within these populations occurred outside of the determined epidemic period. We must, therefore, conclude that the 51 persons of Group C (high titers) were infected with A/Eng/72 virus during the epidemic and that 41 of these persons were in the first trimester of gestation. This would give a total of 53 first trimester infections without incident when coupled with the 12 first trimester infections from Group A (known infected). Conclusions in our study concerning effects of infection on prematurity or spontaneous abortion are limited by the nature of the population. Women who did not reach the eight month of gestation were not included in the study. This design feature was beyond our control. However, a check of records of all births at these installations for 1972 and 1973 indicates the premature birth rate for the first six months of 1973 is similar to or lower than the corresponding rates for 1972. Spontaneous abortion rates were available at only one of the two participating hospitals and these were highly variable over the thirty month period checked. The sex ratio of new borns in Group A, i. e. 39.3% male shown in Table 5 is interesting but not significantly different from the theoretical. Further, when both infected groups were combined, the sex ratio of new borns was comparable to that of the population as a whole.

Hardy's study of 1961 (13), which suggested an association of increased abortion, stillbirth and anomalies associated with first trimester infection, is of interest because the study was performed during the introduction of the H_2N_2 A/Asian/57 virus. This is the only time since virus isolation capability has been developed that a major change in both viral surface antigens, the hemagglutinin (H) and neuraminidase (N), has occurred sumultaneously. The major change that occurred in 1947 involved only the hemagglutinin (H0/34-------> H1/47). Similarly, the change that occurred in 1968, A/Hong Kong/1/68, involved only the hemagglutinin (H_2/57---->H_3/68). The "England" strain of Type A virus of 1972 involved only a minor change in the nemagglutinin and still retains the primary identification of the 1968 virus i.e. A/England/42/72 (H_3N_2). Awareness of this pedigree of the virus inevitably raises the question of the effect of prior antigenic experience of the host on the limitations of the current virus strain's terotogenic capability. There is no question that under certain circumstances influenza virus can be invasive. Transplacental transfer of influenza virus has been shown to occur by Yawn et. al. (21) and the evidence for the invasiveness of the virus has been reviewed by the authors. It seems reasonable to assume that this capability is greatest immediately following a major change in both surface antigens such as occurred in the epidemic of 1957-1959. Hardy's study,though often criticized because the laboratory back-up was based on a single serum antibody determination and the lack of an adequate number of negative controls, did cover the first wave of the 1957 epidemic. The patients of Korones et. al. were drawn over a period of four years from 1959, two years after the first wave, to 1963. Monif's study of 8 patients, though technically complete, was accomplished after the introduction of the A/HK/68 virus (22) and the present study was accomplished 5 years after the introduction of the antigenically similar A/HK/68 virus. Thus, the full answer to the terotogenicity of influenza Type A virus may await the introduction of another major antigenic change in both viral surface antigens such as occurred in 1957.

The results presented in Table 6 showing a skewed distribution of infection associated with later months of gestation suggests the possibility that susceptibility to infection increases with months of pregnancy. Several groups have recently presented evidence that cellular immunity is reduced in pregnancy (23, 24, 25). This study was not designed to confirm or deny this contention, but the data is suggestive and perhaps opens new lines of investigation to answer this important question.

LITERATURE CITED

1. Campbell, W.A.B., Influenza in early pregnancy and effects on the foetus. Lancet 1:173, 1953.

2. Coffey, V.P. and Jessop, W.J.E., Maternal influenza and congenital deformities. A prospective study. Lancet 2:935, 1959.

3. Coffey, V.P. and Jessop, W.J.E., Maternal influenza and congenital deformities. A follow-up study. Lancet 1:748, 1963.

4. Griffith, G.W., Adelstein, A.M., Lambert, P.M. and Wetherall, J.A.S., Influenza and infant mortality. Brit. Med. J. 3:553-556, 1972.

5. Doll, R., Hill, B. A., and Sakula, J., Asian influenza in pregnancy and congenital defects. Brit. J. Prev. Soc. Med. 14:167, 1960.

6. Abramowitz, L.J., The effect of asian influenza on pregnancy. S. Afr. Med. J. 32:1155, 1958.

7. Rogers, C.S., Influenza and congenital abnormalities. Lancet 1:261, 1972.

8. Leck, I., Incidence of malformation following influenza epidemics. Brit. J. Prev. Soc. Med. 17:70, 1970.

9. Walker, W.M. and McKee, A.P., Asian influenza in pregnancy. Relationship to fetal anomalies. Obstet. and Gyn. 13:394, 1959.

10. Wilson, M.G. and Stein, A.M. Teratogenic effects of asian influenza. J.A.M.A. 210:336, 1969.

11. Korones, S.B., Todaro, J., Roane, J.A., and Sever, J.L, Maternal virus infection after the first trimester of pregnancy and status of offspring to four years of age in predominantly Negro population. J. of Ped. 77:245-251, 1970.

12. Elizan, T.S., Ajero-froehlich, L., Fabiyi, A., Ley, A., and Sever, J.L. Viral infections in pregnancy and congenital CNS malformations in man. Archives Neurol. 20:115-119, 1969

13. Hardy, J.M.B., Azarowics, E.N., Mannini, A., Medearis, D.N. and Cooke, R.E., The effect of asian influenza on the outcome of pregnancy. Am. J. Public Health 51:1182, 1961.

14. Chin, J., Magoffin, R.L., and Lennette, E.H., The epidemiology of influenza in California, 1968-1973. California Med. in press.

15. U.S. Department of Health, Education, and Welfare. Public Health Services. Health Services and Mental Health Administration. Morbidity and Mortality Weekly Report. 21:438, 1972.

16. Ibid. 22:134, 1973.

17. Robinson, R.Q. and Dowdle, W.R., Influenza viruses. Diagnostic procedures for viral and rickettsial diseases. Fourth edition edited by E.H. Lennette and J.J. Schmidt. American Public Health Association, Inc. pp. 414-433, 1969.

18. Sever, J.L., Application of a microtechnique to viral serological investigations. J. Immunol. 88:320, 1962.

19. Clarke, D.H. and Casals, J., Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. 7:561-573, 1958.

20. Lennette, E.H., General Principles underlying Laboratory Diagnosis of Viral and Rickettsial Infections. Diagnostic procedures for viral and rickettsial diseases. 3rd edition, edited by E.H. Lennette and N.J. Schmidt. Amer. Pub. Health Assoc., Inc. pp. 1-66, 1964.

21. Yawn, D.H., Pyeatte, J.C., Joseph, J.M., Eichler, S.L., and Garcia-Bunuel, R., Transplacental transfer of influenza virus. J.A.M.A. 216:1022-1023, 1971.

22. Monif, G.R.G., Sowards, D.L., and Eitzman, D.V., Serologic and Immunologic evaluation of neonates following maternal influenza infection during the secodn and third trimesters of gestation. Am. J. Obstet. Gynec. 114:239-242, 1972.

23. Finn, R., St. Hill, C.A., Govan, A.J., Ralfs, I.G. and Gurney, F.J., Immunological responses in pregnancy and survival of fetal homograft. Br. Med. J. 3:150-152, 1972.

24. St. Hill, C.A., Finn, R., And Denye, V., Depression of cellular immunity in pregnancy due to a serum factor. Br. Med. J. 3:513-514, 1973.

25. Thong, U.H., Steele, R.W., Vincent, M.M., Hensen, S.A., and Bellanti, J.A., Impaired in vitro cell-mediated immunity to rubella virus during pregnancy. N. Eng. J. Med. 289:604-606, 1973.

MENINGOCOCCAL POLYSACCHARIDE VACCINE EVALUATIONS AND CARRIER SURVEILLANCE

INTRODUCTION

Since the routine administration of meningococcal "C" vaccine began on 12 October 1971, there have been no meningococcal meningitis disease case caused by sero-group "C" organisms. Eleven (11) cases occurred in 1972, all were caused by sero-group "Y" organisms; 7 cases occurred in 1973, 6 were caused by sero-group "Y" and 1 by sero-group "B" organisms. Two (2) cases occurred during 1974, both were caused by sero-group "B" organisms. Meningococcal disease among recruits has been shown to be due to the lack of significant immune antibody levels. In order to maintain an adequate supply of potent vaccines for continued militay immunizations, these studies are designed to test the stability of vaccines stored at 2-8°C and reconstituted with diluent containing 1:20,000 thimerosal preservative over a 2 year period. This is the first phase of study. Carrier Surveys are performed in conjunction with these field trials.

These studies represent the joint efforts of the US Army Medical Laboratory, Fort Baker, the Health and Environment Activity, Fort Ord, and the Department of Bacterial Diseases, WRAIR.

MATERIALS AND METHODS

Each of 2 lots of Meningococcal polysaccharide "C" vaccine, manufactured by Merck, Sharpe and Dohme Laboratories, lyophilized and reconstituted with 1:20,000 thimerosal diluent preservative, was injected subcutaneously with hypodermic needles and syringes into 2 separate groups (46 and 47 men) of receptionees at Fort Ord. Another lot, manufactured by Squibb Laboratories (Standard Army vaccine) was similarly injected into a third group (43 men), and these were used as controls. Material swabbed from the posterior aspect of the uvula was streaked immediately onto a Columbia Chocolate Agar (CCA) plate (BBL) containing 5% laked sheep bood, 1% isovitalex (BBL), lincomycin (6mcg/ml)and polymyxin B (25 mcg/ml). The plates were incubated immediately at 37°C in a 5 to 10% CO_2 atmosphere 18-20 hours. Standard methods of meningococcal identifications and serogroupings were made. Swabs were taken in similar fashion after two weeks. Blood was collected from each vaccinee at 0 and two weeks, and the serum was used to determine antibody rises to "C" polysaccharide 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 phlebotomies.

RESULTS AND DISCUSSION

Both lots of vaccine caused a favorable number of seroconverters. As indicated by Figure 1, Merck Lot #32412/C-B837 incited 44 of 46 vaccinees to seroconvert, 95.7%; and 44 of the 47 Lot #32413/C-B838 Merck vaccinees seroconverted, 93.8%. The Control Lot, Squibb C-9 seroconverted 43 of 43 vaccinees. The actual geometric mean titers, and titer differences between 0 week and 2 week sera were very favorable. The percent positive carriers at 0 week averaged 28.7% for all vaccine groups; "C" carriers constituted 0.07% of the total (19 B's, 3 C's, 2 29E's, 12 Y's, 2 X's, and 2 non-typeables). After 2 weeks, the percent carriers averaged 36.2% for all vaccine groups; "C" carriers constituted 0.04% of the total (27 B's, 2 C's, 3 29E's, 14 Y's, 1X, and 3 non-typeables). The non-typeable organisms were processed and injected into rabbits for another phase of study designed to identify new serological types, as described under the heading Identification of Undescribed Serogroups of N. meningitidis, which follows.

Two (2) meningococcal meningitis cases have occurred at Fort Ord during the fiscal year. In July 1973, one case occurred in the sixth week BCT, caused by a group B organism; in January 1974 a group B disease was experienced by an AIT. Recently in September 1974, before the completion of this manuscript, a second week BCT experienced disease caused by a group B organism.

Routine monthly carrier surveillance studies were not performed during the year.

SUMMARY

Two lots of meningococcal polysaccharide "C" vaccine that were manufactured by Merck, Sharpe and Dohme were tested for potency in recruits at Fort Ord. The lots had been lyophilized and reconstituted with a 1:20,000 thimerosal preservative diluent. Both lots induced a very favorable antibody response as shown by 95.7% and 93.8% seroconverters. Another meningococcal vaccine lot manufactured by Squibb Laboratories caused 100% seroconverters; this lot was used as a control. Simultaneous meningococcal carrier surveillance indicated a drop in meningococcal "C" carriers two weeks after menigococcal vaccine administration. The predominating carriers were of serological group B.

FIGURE I

VACCINE LOT NO.	PAIRS TESTED	GEOMETRIC MEAN TITERS 0 WEEK TITER	0 WEEK CARRIERS	2 WEEK TITER	2 WEEK CARRIERS	TITER CHANGE	% SEROCONVERSION	
32412/C-B837 Merck	46	1.09	24% (0% C's)	6.13	30% (0% C's)	5.04	44/46	95.7%
32413/C-B838 Merck	47	0.70	31% (0% C's)	5.34	43% (0% C's)	4.64	44/47	93.8%
C-9 Squibb	43	1.67	30% (21% C's)	7.00	36% (12 % C's)	5.33	43/43	100%

IDENTIFICATION OF UNDESCRIBED SEROLOGICAL GROUPS OF *NEISSERIA MENINGITIDIS*

INTRODUCTION

Serological typing procedures for meningococcal organisms have yielded less then totally desirable results in the past. One problem has been the large number of untypeable organisms isolated from meningococcal carriers. At least some of these organisms could be serotypes that have not been previously described. Such organisms encountered by this study group are currently being studied for the probability of identifying new, not previously described serological groups. This is a progress report on these endeavors.

MATERIALS AND METHODS

Two non-typeable (NT) smooth *N. meningitidis* strains that were acquired during carrier surveillances at Fort Ord, labelled #67 and #114, were prepared for inoculations into rabbits by the procedure of the Navy Medical Research Unit #1 (NAMRU #1) Neisseria Repository, Berkely.

Culture Preparations: Each strain was streaked on a Columbia Chocolate Agar (CCA) plate containing 1% Isovitalex. The plates were incubated overnight (16-18 hours) at 37°C in a candle jar. The cells were harvested from the surface of the agar in Trypticase Soy Broth (TSB) and inoculated into 125 ml of TSB to a concentration approximately 10^8 cells/ml when compared with a #3 Mac Farland standard. The flasks were incubated at 36° for 6 hours with continuous agitation.

The cultures were then transferred to centrifuge tubes and packed at 2000 rpm for 15 minutes. The packed cells were washed twice in Hanks Balanced Salt Solution (without glucose or indicators) and resuspended in Hanks' to a concentration of about 10^{10} cells/ml (slightly more turbid than a #10 Mac Farland). Aliquots were dispensed into 12 serum vials and frozen at -64°C, so that a freshly thawed inoculum could be used for each rabbit injection.

Animal Injections: Separate 6-month old rabbits were injected I.V. with 0.5 ml of each suspension once a day for 5 succesive days. A three-day rest period was followed by 1.0 ml injections once a day for five successive days. After five days, the rabbits were bled from the heart, 50 ml per day for three consecutive days.

Agglutination Studies: Stock strains of serogroups A, B, C, D, B, X, Y (E), Z, 29E, and W-135 were obtained from the Neisseria Repository (NAMRU #1). Each strain was reconstituted, confirmed as a meningococcus, and used for agglutination studies. The serogroup of each stock strain was confirmed using antiserum provided by the Neisseria Repository. Cultures #67 and #114 were tested to confirm that they were NT. Each stock Strain and NT Strains 67 and 114 were then tested against the pre-inoculation sera of both rabbits and against the antisera produced against strains 67 and 114.

RESULTS AND DISCUSSION

The results of this testing summarized in Table #1, shows that there was cross-reaction between Group D and the rabbits' sera, both pre-inoculation serum and hyperimmune serum for both smooth strains. The antisera were then titered against their homologous strains (Table II) and the working dilutions determined to be 1:8 for #67 and 1:4 for #114. These were then tested against Group D at their working dilutions, and as Table III shows, there was no cross-reactions with the working dilutions. Table IV summarizes the results of agglutination tests for cultures #67 and #114, showing that with antisera from two independent sources, there was no agglutination with either strain against any of the recognized antisera.

Further studies are in progress which include the following procedures:

a. Agglutination tests with cultures #67 and #114 against antisera of recognized serogroups produced by WRAIR.

b. Testing additional cultures of all recognized serogroups against antisera #67 and #114.

c. Additional carrier surveys at Fort Ord to try and identify NT strains which would then be tested against antisera #67 and #114.

SUMMARY

Two NT strains of N. meningitidis were isolated from carriers at Fort Ord. Preliminary work suggests these to be two strains of a serogroup not previously recognized. A stock strain of N. meningitidis Group D reacted with both antisera before they were diluted. This same strain also reacted with sera from the same rabbits taken before immunization with NT Strains. When the antisera were diluted to the working dilution there was no cross-reaction with Group D. This would inicate that the Group D strain was reacting with a naturally occurring antibody in the rabbits rather than an antibody produced against either NT Strain.

TABLE I

AGGLUTINATION PATTERNS WITH UNDILUTED ANTISERA

CULTURE	Pre Inoculation Serum #67	Pre Inoculation Serum #114	Post Inoculation Serum #67	Post Inoculation Serum #114
#67	-	-	+	+
#114	-	-	+	+
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
D	+	+	+	+
Bo	-	-	-	-
X	-	-	-	-
E (Y)	-	-	-	-
Z	-	-	-	-
29E	-	-	-	-
W-135	-	-	-	-

TABLE II

TITRATION OF ANTISERA

CULTURE	SERUM #67 1:2	1:4	1:8	1:16	1:32	SERUM #114 1:2	1:4	1:8	1:16	1:32
#67	+	+	+	-	weak +	+	+	+	weak +	weak +
#114	+	+	+	-	weak +	+	+	+	weak +	weak +

CULTURE	SERUM #67 Pre-Inoc. Undiluted	Post-Inoc. Undiluted	1:8	1:16	SERUM #114 Pre-Inoc. Unidluted	Post-Inoc. Undiluted	1:4	1:8
D (M-623)	+	+	-	-	+	+	-	-

CULTURE	CDC ANTISERA A	B	C	D	X	Y	Z	NRL ANTISERA A	B	C	D	Bo	X	Y (E)	29E	W135
#67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
#114	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

IDENTIFICATION OF THE VIRAL AND BACTERIAL AGENTS ASSOCIATED WITH GONOCOCCAL AND NON-GONOCOCCAL URETHRITIS

INTRODUCTION

The literature is replete with references concerning the etiologic agents of urethritis. Most have been reports attempting to determine the role of one or two microorganisms in relation to urethritis. Most often no satisfactory control group has been included in the study. The ongoing study presented here is attempting to identify at least the majority of microorganisms (bacterial, viral and parasitological) present in the urethras of males with urethritis as well as those in the urethras of males without urethritis. An attempt is being made also to delineate the normal urethral flora.

MATERIALS AND METHODS

Urethral swabs from cases of urethritis were obtained primarily from patients presently at the NRMC Branch Dispensary of the Alameda Naval Air Station, California. A few were obtained from the Oakland Army Base Dispensary and from Treasure Island Naval Base Dispensary. The vast majority of specimens taken from individuals without urethritis were obtained from personnel undergoing physical examination prior to commencing training at the Nuclear Power School at Mare Island Naval Base, California.

Two urethral swabs were taken from each subject. Swabs were of calcium alginate wool and were inserted approximately 2 cm into the urethra. For transport to the laboratory, each swab was placed in 2.5 ml of Eagles MEM containing 10% fetal Bovine serum, added glucose, glutamine and sodium bicarbonate. The vial intended for viral isolation also contained 100 mg/ml Streptomycin, 50 mg/ml Vancomycin and 2-5 mg/ml Fungazone.

The antibiotic containing media was inoculated into tissue culture for viral isolation. The antibiotic-free media was inoculated onto Mycoplasma isolation media, Trichomonas isolation media, chocolate agar, Thayer-Martin medium, sheep blood agar and Peptone-Starch-Dextrose agar in an effort to isolate as many aerobic bacteria as possible with currently available techniques. Isolation of anaerobic bacteria was not attempted due to failure of other investigators (1) to isolate anaerobes from patients with urethritis.

Procedures for the isolation of Chlamydia were inititated but were discontinued due to technical difficulties. The association of these organisms with urethritis has already been adequately studied (2, 3, 4, 5) so their omission here is not considered detrimental to the study.

Blood was drawn from the majority of individuals in the study at the same time that urethral swabs were taken. Serum was separated and examined for Chlamydial antibodies by complement fixation using a group antigen kindly supplied by Dr. Julius Schachter, Hooper Foundation, University of California at San Francisco.

RESULTS

Mycoplasma: T-strain Mycoplasma were not more frequently isolated from urethritis patients than from sexually active controls (Table I). Nor were they more frequently isolated from cases of non-gonococcal (NGU) than from gonococcal (GU) urethritis (Table II). They were, however, more frequently isolated from sexually active male urethras than from the sexually inactive (Table I).

M. hominis, when no other Mycoplasma was present, was never isolated from the control groups. However, when T-Strain Mycoplasma were present, M. hominis Type I was isolated from 17.2% of sexually active controls, 4.7% of sexually inactive controls and 8.3% of urethritis patients. The latter figure is comparable to the isolation rate of M. hominis Type I when isolated alone from urethritis patients (Table I). The overall isolation of Mycoplasma was very little influenced by the presence or absence of gonococci.

DISCUSSION OF MYCOPLASMA RESULTS

The large colony Mycoplasmas such as M. hominis have been well established as non-pathogenic commensals inhabiting the normal urethra (6-13). A controversy, however, has arisen concerning the implication of the T-Strain Mycoplasma and symptoms of urethritis. Some have reported an association (14-16) while others (17-22) have found no difference between T-Strain isolation rates from patients with urethritis and from the urethras of non-symptomatic controls of comparable age and sexual activity. The results of this study support the latter view. No significant difference was noted between isolation rates from those with urethritis and from those without urethritis. The similarity in isolation rates persisted when patients with GU were compared to those with NGU.

As other workers (23, 24) have demonstrated, colonization of the urethra with T-Strains as well as other Mycoplasma appears to be directly proportional to the degree of sexual activity (See Table I) and not necessarily related to urethritis.

It is interesting that Mycoplasma hominis Type I was not isolated in the absence of other Mycoplasma except in individuals with urethritis. They were, however, recovered from 17.2% of the sexually active control group compared to 8.3% of the urethritis patients when recovered in the presence of T-Strains. This, coupled with the observation that isolation rates of M. hominis Type I are similar in both gonococcal urethritis and non-gonococcal urethritis (See Table II) may indicate that both the presence of T-Strains and the state of the inflamed urethra either enhance the growth or the recovery of M. hominis Type I or both. These observations should be confirmed in another study.

Gram-Positive Cocci: An interesting phenomenon is observed when the isolation rates of gram-positive cocci from the urethras of males with urethritis is compared to those of males without urethritis (Table III). With the exception of the non-hemolytic (gamma) Streptococci and Micrococci the presence of symptoms of urethritis greatly decreases the isolation rates of the gram-positive cocci. The decreased rates in cases of urethritis probably reflects increased non-specific phagocytosis occurring in the inflamed urethras. Dilution of the inoculum by the purulent discharge may also decrease chances of recovery. Other factors such as bacterial interference or other toxic factors in the inflamed urethra may also contribute.

TABLE I

MYCOPLASMA ISOLATION FROM MALE URETHRAS

	No. Patients	T-Strain Mycoplasma Only	Untypeable Mycoplasma hominis	Mycoplasma hominis Type 1	Mycoplasma hominis Type 2	Unidentified Mycoplasma SP	T-Strain M hominis 1	T-Strain M hominis 2	T-Strain Unident Mycoplasma	T-Strain M Orale	No. Mycoplasma
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No (%)	No. (%)	No. (%)	No. (%)	No. (%)
Urethritis Patients	254 (100)	59 (23.2)	1 (.4)	19 (7.5)	2 (0.8)	4 (1.6)	21 (8.3)	1 (0.4)	3 (1.2)	1 (0.4)	143 (56.3)
Sexually Active Controls[a]	64 (100)	19 (29.7)	0 (0)	0 (0)	0 (0)	0 (0)	11 (17.2)	0 (0)	2 (3.1)	0 (0)	32 (50.0)
Sexually Inactive Controls[b]	43 (100)	7 (16.3)	0 (0)	0 (0)	0 (0)	1 (2.3)	2 (4.7)	0 (0)	0 (0)	1 (2.3)	32 (74.4)

[a] No history of Urethritis

[b] Denied sexual activity for 3 months period prior to collection of specimen.

TABLE II

MYCOPLASMA ISOLATION FROM MALE URETHRAS

OF MALES WITH GONOCOCCAL OR NON-GONOCOCCAL URETHRITIS

	No. Patients	T-Strain Mycoplasma Only	Untypeable Mycoplasma hominis	Mycoplasma hominis Type 1	Mycoplasma hominis Type 2	Unidentifies Mycoplasma SP	T-Strain +M hominis 1	T-Strain +M hominis 2	T-Strain +Unident. Mycoplasma	T-Strain +M Orale	No Mycoplasma
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No (%)	No. (%)
Gonococcal	52 (100)	12 (29)	1 (1.9)	4 (7.8)	0 (0)	1 (1.9)	3 (5.8)	0 (0)	2 (38)	0 (0)	28 (53.8)
Non-Gonococcal	200 (100)	47 (20)	0 (0)	17 (8.5)	2 (1)	3 (1.5)	16 (8)	1 (0.5)	1 (0.5)	1 (0.5)	113 (56.5)

TABLE III

ISOLATION RATES OF GRAM-POSITIVE COCCI FROM THE URETHRAS OF MALES WITH OR WITHOUT URETHRITIS

Organisms Isolated	249 Urethritis Patients		107 Normal Controls	
	#[e]	%	#[e]	%
Staphylococcus epidermidis	103	41.4	96	89.7
Staphylococcus aureus[c]	23	9.2	23	21.5
Micrococci[d]	118	47.4	35	32.7
Group B Streptococci[a]	3	1.2	3	2.8
Non-Typeable alpha Streptococci[a]	27	10.8	39	36.4
Non-Typeable beta Streptococci[a]	5	2.0	10	9.3
Non-Typeable gamma Streptococci[a]	8	3.2	4	3.7
Enterococci[a]	20	8.0	25	23.4
Group G Streptococci[a]	0	0.0	1	0.9

[a]Typed by immunofluorescence and Lancefield precipitin Tests. Catalase negative.

[b]Coagulase negative, glucose fermenting, catalase positive.

[c]Coagulase positive, glucose fermenting, catalase positive.

[d]Non-fermenting, cluster-forming, catalase positive.

[e]Number of patients from which organism was isolated.

Coryneform Bacteria: Approximately 65% of all individuals cultured harbored coryneform bacteria in their urethras. Undoubtedly, most of them are part of the normal flora but the possibility exists that some may play a role in urethritis.

Over 22,000 biochemical tests have been performed on 205 urethral isolates. In addition, a battery of 47 tests have been performed on each of 27 ATCC strains of Coryneform (Corynebacterium, Brevibacterium, Arthrobacter, Mycrobacterium, Cellulomonas and Rothia) bacteria and are in progress on 49 other strains. All tests are run in duplicate and equivocal tests are repeated.

When the biochemical tests are completed on the ATCC strains, the results will be compared to see if indeed there are truly distinguishing characters between them in our hands. To help delineate strain differences, vertical gel electrophoresis and perhaps immunoelectrophoresis of cellular poteins are planned. The results on the ATCC strains will be compared to the results on the urethral isolates to precisely identify the urethral isolates. We also plan to utilize a gas chromatograph to perform "fingerprint" cha.-acterization by pyrolysis and characterization by identification of metabolic products released in broth media.

To date, 17 apparently different strains have been isolated and assigned to tentative species or groups. There appear to be mostly Corynebacterium with a few Brevibacterium sp. Four of these were not isolated from normal urethras. However, many isolates have not yet been assigned to any group so no conclusions can be drawn at this time.

Miscellaneous Bacteria: As shown in Table IV, several miscellaneous bacteria and fungi were isolated from urethras. Due to the low isolation rates, no significance can be attached to any of them. A much larger study population might be more revealing.

Trichomonas: No trichomonads were isolated from any of the specimens taken.

Viruses: Herpes virus were isolated from 4 (2%) of 205 individuals with urethritis and from 1 (0.9%) of 106 control. No other likely pathogens were isolated from these patients and the Herpes were probably responsible for the urethral symptoms.

Polio virus was isolated from 2 urethritis cases and Type 7 Adenovirus from 1 case. No other likely pathogens were isolated from the same patients and neither of these two viruses were isolated from control individuals.

There is not sufficients evidence to implicate polio virus and Adenovirus as etiologic agents for urethritis, but the suggestion is there, and it is possible that viral infection accounted for 3.4% of the urethritis cases studied.

Gonococci: Gonococci were isolated from 20% of the urethritis cases studied (50 of 250). No gonococci were isolated from any control individuals.

Chlamydial Antibodies: Since Chlamydia have been shown to be important agents of urethritis (2, 3, 4, 5) it was considered of interest to determine the prevalence of antibody to these agents in the sera of urethritis patients. Since repeat specimens proved very difficult to obtain and titer rises are difficult to demonstrate in chlamydial infections, the results of only one serum per patient is reported here.

Table V compares chlamydial Complement Fixation (CF) titers in males with urethritis to those without urethritis. A CF titer of 1:20 or greater was arbitrarily chosen as the titer indicative of a current or prior chlamydial infection. Approximately 7% more urethritis patients than controls had CF titers of 1:20 or greater.

That significant CF titers to Chlamydia may be more closely related to degree of sexual activity than to urethritis is indicated by the data in Table VI. If it can be assumed that, in general, individuals with a history of urethritis probably have more sexual contacts than sexually active individuals without a history of urethritis then the data in Table VI indicates that the percentage of individuals with significant chlamydial CF titers increases proportionally to the amount of sexual experience. However, the actual number of sexual contacts has not been determined and the number of individuals in each category of the control groups is not large enough to warrant definitive conclusions. It would be interesting to survey a larger population.

TABLE IV

ISOLATION RATES OF MISCELLANEOUS BACTERIA AND FUNGI FROM THE URETHRAS OF MALES WITH OR WITHOUT URETHRITIS

Organism	Urethritis Patients		Normal Controls	
	#[a]	%	#[a]	%
Bacillus licheniformis	2	1.0	0	0.0
Bacillus cereus	1	0.4	1	0.9
Lactobacilli	6	2.4	2	1.9
Bacillus sp.	1	0.4	0	0.0
Neisseria subflava	1	0.4	0	0.0
Moraxella bovis	1	0.4	0	0.0
Moraxella non-liquefaciens	1	0.4	0	0.0
Moraxella phenylpyruvica	2	0.8	0	0.0
Moraxella osloensis	0	0.0	1	0.9
Moraxella kingii	0	0.0	1	0.9
Mima polymorpha	1	0.4	0	0.0
Escherichia coli	3	1.2	4	3.7
Proteus vulgaris	1	0.4	0	0.0
Proteus morganii	0	0.0	1	0.9
Acinetobacter calcoaceticus	0	0.0	1	0.9
Enterobactor aerogenes	0	0.0	4	3.7
Enterobacter cloacae	1	0.4	1	0.9
Pseudomonas aeruginosa	1	0.4	0	0.0

[a]# of urethras from which each organism was isolated.

TABLE V

COMPARISON OF COMPLEMENT FIXATION TITERS TO CHLAMYDIA ANTIGEN IN SERA OF MALES[a] WITH AND WITHOUT URETHRITIS

CF Titer	Urethritis Patients	Normal Controls
<20[b]	80.5%[c]	87.3%[d]
≥20[b]	19.5%	12.7%

[a]Study included 210 urethritis patients and 71 normal controls.

[b]Reciprocal of highest dilution of serum reactive in the CF test.

[c]% of urethritis patients having this titer range.

[d]% of normal controls having this titer range.

TABLE VI

COMPLEMENT FIXATION TITERS TO CHLAMYDIA ANTIGEN IN SERA OF MALES WITHOUT URETHRITIS COMPARED ON BASIS OF SEXUAL ACTIVITY AND HISTORY OF URETHRITIS

		CF TITER	
	No.	<20[a]	>20[a]
Not active[b], No History[c]	29	27(93.1%)	2(6.9%)
Active, No History	35	30(85.7%)	5(14.3%)
Active, History	8	5(62.5%)	3(37.5%)

[a]Reciprocal of highest serum dilution reactive in the CF test.

[b]Had no sexual relations for 3 months prior to collection of serum.

[c]No history of urethritis.

REFERENCES

1. Justesen, T., M.L. Nielsen, and T. Hattel. Med. Microbiol. Immunol. 158:237, 1973.

2. Dunlop, E.M.C., J.J. Hare, S. Darougar, B.R. Jones, and N.S.C. Rice. Infect. Dis. 120:463, 1969.

3. Dunlop, E.M.C., J.D. Vaughan-Jackson, and B.R. Jones. Brit. J. Vener. Dis. 48:425, 1972

4. Oriel, J.D., P. Reeve, P. Powis, A. Miller, and C.S. Nicol. Brit. J. Ver. Dis. 48:429, 1972.

5. Philip, R.N., D.A. Hill, A.B. Greaves, F.B. Gordon, A.L. Quan, R.K. Gerloff, and L.A. Thomas. Brit. J. Vener. Dis. 47:115, 1971.

6. Shepard, M.C. Am. J. Syph. 38:113, 1954.

7. Salaman, M.H. J. Pathol. Bacteriol. 58:31, 1946.

8. Melen, B., B. Linnros. Acta Derm. Venereol. (stockh) 32:77, 1952.

9. Ford, D.K. Arthr. Rheum. 3:395, 1960.

10. Ford, D.K. Br. J. Vener. Dis. 38:22, 1962.

11. Csonka, G.W., R.E.O. Williams. Lancet 1:1292, 1966.

12. Freundt, E.A. Br. J. Vner. Dis. 38:22, 1962

13. Ford, D.K., M. DuVernet. Br. J. Vener. Dis. 39:18, 1963.

14. Sompolinsky, D., A. Harari, F. Solomon, E. Caspi, D. Krakowski, and E. Henig. Israel J. Med. Sci. 9:438, 1972.

15. Shepard, M.C. JAMA 211:1335, 1970.

16. Markham, N.P., J.G. Markham, and E.R. Smith, Br. J. Vener. Dis. 48:200 1972.

17. Handsfield, H.H., et. al.: Etiology and Treatment of Non-Gonococcal Urethritis. Program Twelfth Interscience Conference on Antimicrobial Agents and Chemotherapy.

18. Ingham, H.R., W.V. MacFarlane, J.H. Hale, et. al. Br. J. Vener. Dis. 42:269, 1966.

19. Black, F.T., O.G. Rasmusson. Br. J. Vner. Dis. 44:324, 1968.

20. Fowler, W., R.J. Leeming. Br. J. Vener. Dis. 45:287, 1969.

21. Haas, H., M.L. Dorfman, T.G. Sacks. Br. J. Vener. Dis. 47:131, 1971.

22. Jansson, E., A. Lassus, S. Stubb, et. al.: Br. J. Vener. Dis. 47:122, 1971.

23. McCormack, W.M., P.C. Almeida, P. E. Bailey, E.M. Grady, and Y. Lee. JAMA 221:1375, 1972.

24. Kundsin, R.B., A. Parrino and A. Kirsch. Br. J. Vener. Dis. 49:381, 1973.

RAPID METHODS FOR IDENTIFICATION OF YEASTS AND YEAST-LIKE FUNGI ISOLATED FROM CLINICAL SPECIMENS

INTRODUCTION

The incidence of disease and deaths, due to yeasts and yeast-like fungi has been increasing. This in large part is due to the increased use of broad spectrum antibiotics, cortisone, antineoplastic drugs and immunosuppressants for organ transplant patients.

Methods for sustaining life of very seriously ill patients have also improved greatly. Immunologically compromised and seriously ill patients are succumbing to yeasts that are best described as opportunistic pathogens.

[1]Hammerstein et. al. estimated that 7697 patients with systemic mycotic infections were hospitalized in the United States in 1970 alone with a total hospitalization cost of 9.39 million dollars.

The average clinical laboratory is not equipped or trained to do much more than separate Candida albicans from all other yeasts, which are usually classified as "yeast isolated not Candida albicans."

Classic methods of carbohydrate assimilation and fermentation are very slow and tedious for yeasts. A quick, accurate and convenient method of yeast identification is needed and it is felt that the disc assimilation test technique can provide this service. The goal of this research is to compile about 50,000 test results on about 140 different yeast strains, which will represent about 35 species. All results will be done in triplicate or more, so that a valid data base can be statistically established. This project is about half completed.

MATERIALS AND METHODS

1. Experimental Methods:

Clinically isolated and A.T.C.C. strains of yeasts were inoculated to V-9 agar plates and streaked for isolation. Within 7 days, one well separated pure colony was transferred to 10 ml of isotonic saline. This was well mixed and 0.25 ml was transferred to 10 ml of Y.N.B. and 0.125 ml was transferred to 10 ml of Y.C.B. starvation broths. These "starvation: tubes were incubated for 72 hours at 30°C to reduce the endogenous and exogenous carbon and nitrogen reserves of the yeast cells.

Quad plates of YNB and YCB agar were inoculated using sterile swabs from the corresponding "starvation" broths. Then three of the four segments of each quad plate received one ½" cellulose test disc. The fourth segment served as an inoculated control. Twenty-six sodium salts of organic acids, 17 sugars, 9 polyhydric alcohols, 3 glycosides and one cellulose control disc were placed on inoculated YNB agar. Thirty amino acids of primarily the D configuration, 24 purines, pyrimidines and related compounds, 3 inorganic nitrogen sources and 2 cellulose control discs were placed on inoculated YCB agar. This was a total of 113 test compounds and 3 cellulose controls for each strain tested.

Inoculated plates were then incubated for 5 days at 30°C in a high humidity, circulating air, incubator. Twelve different growth patterns were noted and recorded upon a pre-printed log sheet. These were coded as follows:

N	No growth stimulation.
±	Possible growth stimulation but too weak or too similar to the control to be readable.
±S	Plus or minus to slight growth.
S	Slight growth.
SG	Between slight and good growth.
G	Good growth.
V	Vigorous growth.
IS	Backgound growth inhibited slightly.
IG	Background growth inhibited greatly
SIS	Slight growth stimulation with a zone of inhibition around disc.
GIS	Good growth stimulation with a zone of inhibition around disc.

This data was stored on log sheets as well as transferred in binary form as positive, negative, or positive plus negative to edge punched data cards, which made rapid and in depth review of assimilation data very convenient.

2. Experimental Materials

a. Media: Yeast Nitrogen Base and Yeast Carbon Base Agar in Quad plates with 8.g Ionagar #2 per liter of media. YCB and YNB starvation broth and V-9 agar composed of 350 cc V-8 juice, 20 g dried potatoes, 20 grams agar and 10 grams dried yeast extract per liter of media.

b. Test Reagent Discs: These were prepared by autoclaving 100 each $\frac{1}{2}$ inch purified cellulose "Penicillin" discs in 18 X 150 mm tubes. Then solutions of the carbon source were prepared at a concentration of 50 mgm/ml and 10 ml of solution was added to the sterile discs by using a syringe type filter sterilizer. This amount was uniformly absorbed by the 100 discs. Then the tubes of reagent containing discs were dried by lyophilization resulting in carbohydrate source discs of 5 mgm each. The Nitrogen source discs were prepared in a like manner with the exception that they were prepared at 2.5 mgm/disc. There were 57 carbon source discs including the control and 59 Nitrogen source discs including 2 controls.

c. Yeast Strains Available for Testing:

1. 39 "pedigreed" ATCC strains of all species.
2. 14 clinical C. krusei.
3. 10 clinical C. guilliermondii.
4. 6 clinical C. stellatoidea.
5. 3 clinical C. pseudotropicalis.
6. 11 clinical C. tropicalis.
7. 11 clinical C. parapsilosis.
8. 20 clinical Torulopsis glabrata.
9. 18 clinical Rhodotorula species.
10. 6 clinical Cryptococcus neoformans
11. 8 clinical Cryptococcus species.
12. 12 clinical Saccharomyces species.
13. 100 Candida albicans.
14. 10 clinical varied minor yeast species.

d. Yeast Strains Tested to Date: 190 Complete experiments on various strains of 28 different species of yeasts for a total of over 22,000 test results.

RESULTS AND DISCUSSION

As this project is only about one-half completed, only general comments on recorded experiments will be presented.

It was noted that in 24 experiments on Torulopsis glabrata only the Enedioal sugars D-glucose, D-fructose and D-mannose were constituitively utilized. Trehalose was utilized slowly by what appeared to be an inducible enzymatic process. Trehalose, of course, is hydrolyzed to 2 molecules of D-glucose. In fact, these three sugars were generally well utilized by all yeasts tested and therefore, have little differential taxonomic value.

Many researchers have had difficulty differentiating Candida albicans from the generally less pathogenic C. parapsilosis, C. stellatoidea, and C. tropicalis. To date, it appears that these three species may be merely less pathogenic mutant strains of C. albicans. Recorded results on 46 experiments with C. albicans, 11 experiments with C. parapsilosis, 10 experiments with C. stellatoidea and 8 experiments with C. tropicalis show insufficient differences in assimilation patterns to merit species rank. This topic will be expanded in greater detail in the final published paper. By contrast, C. pseudotropicalis, C. guilliermondii and C. krusei seem to merit species rank by virtue of their differential assimilation results.

Species of Cryptococcus and Rhodotorula produce varying amounts of an intense, brown diffusible pigment when utilizing L or D tryptophan as a sole nitrogen source. This tendency will be pursued further with the intent of producing a differential yeast media with at least Generic separation of isolated clinical yeasts.

Inhibition patterns to toxic sodium salts of organic acids and toxic purines and pyrimidines show some promise in biotyping Candida albicans for epidemiologic investigations. If it is found that certain biotypes are more pathogenic or are carried by certain human hosts, preventive medicine measures can be instituted to reduce the infection of immunologically compromised patients.

Common L configuration amino acids are utilized by most yeasts and their utilization patterns show little taxonomic valve. D configuration amino acids by contrast show good differential taxonomic patterns.

The observation of a zone of inhibition around certain nitrogen discs with stimulated growth beyond this zone bears further analysis. It may be that certain levels are toxic or that ammonia is deaminated and diffuses further in the agar than the toxic intact chemical.

Almost all yeasts are capable of utilizing gaseous ammonia released from urea as a sole nitrogen source. Strains of Cryptococcus and Rhodotorula are rendered more mucoid when urea is utilized as the sole nitrogen source.

Sorbitol, apparently by being metabolized into yeast-toxic sorbic acid, showed resistance patterns with many yeasts.

The non-inhibition of many yeasts by the commonly used preservative sodium benzoate was an interesting and unexpected result. Although results are not as clear cut as with sugars, the assimilation patterns with sodium salts of organic acids showed valuable differential results.

The data base to date is already too large to properly manually analyze all the recorded results. Complete analysis by computer will be necessary to delineate all the similarities and non-similarities of tested strains.

Upon completion of this project, a minimum of 3-4 strains for each species with 3 replicate experiments for each strain will be accomplished. Data will be analyzed by standard numerical taxonomic methods using an electronic computer.

REFERENCES

1. Sabouraudia, Vol. 12 part I, 1974 p. 33-45, "The Incidence of Hospitalized Cases of Systemic Mycotic Infections" by K.J. Hammerman et. al.

2. Wickerham, L.J.: Taxonomy of Yeasts. Technical Bulletin No. 1029. U.S. Department of Agriculture, Washington, D.C. 1951.

3. Hall, C.T., Webb, C.D., Papageorge, C.: Use of an Oxidation-Fermentation Medium in the Identification of Yeasts. H.S.M.H.A. Health Reports, Vol. 87, No. 2, February 1972.

4. The Yeasts. A Taxonomic Review by Lodder, J. and Kreger-Van Rij, N.J.W. North Holland Publishing Co., Amsterdam and Interscience Publishers, Inc., NY 1952.