AD NUMBER

917 539

LIMITATION CHANGES

TO Approved for Public Release:
Distribution Unlimited...
— Code A/1

FROM

N/A

AUTHORITY

Ft. Detrick's SGRD-RMI-S (70-14)
via ltr., Dtd 28 Apr 93.

AD_____

PREVENTION OF INFLUENZA AND OTHER RESPIRATORY DISEASES (U)

ANNUAL PROGRESS REPORT

BY

Gordon Meiklejohn, M.D. and
Theodore C. Eickhoff, M.D.

August, 1973
(For the period 1 June 1972 to 31 May 1973)

Supported by

U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
Office of the Surgeon General, Washington, D.C., 20314
in cooperation with the Commission on Influenza of the
Armed Forces Epidemiological Board

Contract No. DADA 17-71-C-1028
University of Colorado Medical Center
Denver, Colorado 80220

DDC DISTRIBUTION STATEMENT

DISTRIBUTION

PREVENTION OF INFLUENZA AND OTHER RESPIRATORY DISEASES

ANNUAL REPORT

4 copies	HQDA (SGRD-ID) WASH DC 20314
15 copies	Director Walter Reed Army Institute of Research ATTN: Office of Research Management Washington, D.C. 20012
2 copies	Defense Documentation Center ATTN: DDC-TCA Cameron Station Alexandria, VA 22314

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)

Prevention of Influenza and Other Respiratory Diseases

5. TYPE OF REPORT & PERIOD COVERED

Annual Report for period 1 June 1972 to 31 May 1973

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)

Gordon Meiklejohn, M.D. and
Theodore C. Eickhoff, M.D.

8. CONTRACT OR GRANT NUMBER(s)

DADA 17-71-C-1028

9. PERFORMING ORGANIZATION NAME AND ADDRESS

University of Colorado Medical Center
Denver, Colorado 80220

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

U.S. Army Medical Research & Development Command

11. CONTROLLING OFFICE NAME AND ADDRESS

U.S. Army Medical Research & Development Command
Office of the Surgeon General
Washington, D.C. 20314

12. REPORT DATE

August, 1973

13. NUMBER OF PAGES

30

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

" " "

15. SECURITY CLASS. (of this report)

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution limited to U.S. Government agencies only; proprietary information; May 1973 Other request for this document must be referred to the Commanding General, U.S. Army Medical Research and Development Command, ATTN: SGRD-ID, Washington, D.C. 20314

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

SUMMARY

1. Standard military vaccine containing 700 CCA units of A_2/Aichi/68 produced a highly satisfactory homologous response and a somewhat lower response against the new influenza A_2 variant A_2/England/42.

2. In a field trial of vaccine effectiveness, the illness rate was 46.0 per 1000 in unvaccinated men and 18.4 per 1000 in vaccinated men indicating a vaccine efficacy of 61%.

3. Attack rates were far higher among men with titers of 1:8 or less than in those with titers of 1:16 or more when tested for A_2/England/42 antibody. A similar relationship was not observed when tests were done for A_2/Aichi/68 antibody. Influenza A_2 strains were isolated from 87% of 105 serologically confirmed cases. Inoculation of primary Rhesus monkey kidney produced a recovery rate of 75% while chick embryos yielded 67%.

4. The antibody response to older strains of influenza B following vaccination was satisfactory, but antibody to the new B/Hong Kong/72 strain was lacking in all men before and after vaccination.

5. Adenovirus disease was prevalent before the Christmas break but fell off rapidly thereafter and no cases were detected during April and May. Almost all were due to type 7.

6. Evidence that this disappearance of adenovirus disease was caused by administration of vaccine for both type 4 and 7 at Lackland Air Force Base was not convincing. Type 4 disease disappeared before vaccinated men reached Lowry Air Force Base, and type 7 disease was decreasing rapidly prior to that time.

7. Attempts to isolate adenovirus strains from 196 newly arrived men during March and April were uniformly successful. Surprisingly, poliomyelitis virus strains were isolated from 18% of these men presumably as a sequel to the administration of live oral polio vaccine at Lackland Air Force Base.

8. With the exception of the very sharp outbreak of influenza A_2 in November and December of 1972, illness rates for febrile respiratory disease were lower than have been observed in any previous year.

PURPOSES

A. General Aim

The studies carried out during the winter of 1972-73 were a continuation of earlier investigations of the effectiveness of vaccines in reducing the incidence of febrile respiratory disease in newly inducted military personnel. The situation differed from previous years in two respects. First, a marked change in the antigenic composition of the influenza A_2 virus had occurred, and an outbreak of influenza was anticipated. Secondly, for the first time, adenovirus type 4 and 7 vaccines had been administered Base-wide to all men prior to their arrival at Lowry Air Force Base, and it was important to determine the impact of this procedure. Major efforts were directed to studies of these two problems. In addition, observations were continued to determine the importance of other identified causes of respiratory disease in the student personnel at Lowry Air Force Base.

B. Specific Aims

1. Influenza Vaccine

The investigations of the preceding winter (1972-73) had shown that the current military vaccine might provide a high level of protection against the influenza A_2 strains (H_2N_2) which were prevalent during that season. In the meantime, however, new A_2 strains (H_3N_2), known generally as A_2/England/42, had made their appearance in the Far East and had already involved Australia, naval ships in the Pacific and, subsequently, Hawaii in outbreaks of moderate extent. Virus strains had been isolated and vaccine was in the process of preparation, but it was clear in the early fall that there would be no homologous vaccine available in time for testing.

For this reason, the decision was reached that a study should be designed to test the effectiveness of the current vaccine which was prepared from the A_2/Aichi/68 strain (H_3N_2) against challenge with the new A_2/England/42 virus. A large field trial was set up for this purpose just before the arrival of the virus at Lowry Air Force Base during the last part of October of 1972.

2. Adenovirus Disease

Adenovirus disease for many years has been the commonest cause of febrile respiratory disease in the student population at Lowry Air Force Base. A series of vaccine studies there have demonstrated the effectiveness of either inactivated vaccine or live vaccine in sharply reducing the incidence of illness. The present season was unique in that, for the first time, the Air Force had begun the administration of both live type 4 and type 7 vaccines to all recruits at Lackland Air Force Base commencing early in January of 1973. While these vaccines were of less than optimal potency, it was of considerable interest to observe the impact of this new policy on the incidence of adenovirus illness in the student population following their arrival at Lowry Air Force Base some six weeks after receiving the vaccines.

The purpose of these studies was two-fold. The first was to detect any illness caused by adenoviruses and to establish by serologic means and by isolation of virus strains whether they were due to type 7, type 4 or some other type. The second purpose was to determine whether adenovirus strains would continue to circulate in this immunized population. For this reason, throat washings were collected from incoming men over a period of two months during the spring and tested for the presence of virus.

3. Surveillance of Other Respiratory Diseases

There has always been a segment of febrile acute respiratory disease which has not been classified with respect to etiology. Even after infections caused by known agents such as influenza virus, adenoviruses, rubella and streptococci have been identified, 35 - 50% of the illnesses still fall into an unclassified category. Searches have been made for infections due to mycoplasma, coronaviruses and parainfluenza viruses in order to define the roles of these agents and to look for clear-cut outbreaks which might be due to other previously unrecognized infectious agents.

THE INFLUENZA VACCINE FIELD TRIAL

A. Methods and Materials

The population studied was composed of students at Lowry Air Force Base. The median age was 19 years. Most had been in service less than one year and had not received influenza vaccine prior to 1972. Two groups were identified. Group 1 was comprised of 2955 men who had been inducted into the Air Force in the summer of 1972 and had not received vaccine. Group 2 consisted of 979 men who had received Hong Kong influenza vaccine from 4 to 10 months prior to the epidemic. Vaccinated and unvaccinated men were not geographically separated on the Base, and aside from being in different training courses, were randomly mixed in dormitories, messhalls, and other activities.

1. Vaccine

Two vaccines were used in this study. The first, a commercial vaccine available during the first 6 months of 1972, contained 600 C.C.A. (chicken cell agglutinating) units of A_2/Aichi/8/68 antigen and 400 C.C.A. units of B/Massachusetts/3/66 antigen. It was administered by jet gun to all recruits during the first half of 1972. This was the vaccine evaluated in assessment of efficacy. The second vaccine, a commercial ether-split preparation containing 700 C.C.A. units of A_2/Aichi/2/68/X-31, a Hong Kong variant antigen, and 300 C.C.A. units of B/Massachusetts/1/71 antigen. This was administered intramuscularly to a sample group of men prior to the outbreak, and was used in assessing antibody response to vaccination.

Antibody response to vaccination was measured in a sample population before the epidemic began. One hundred and seven unvaccinated men received the 700 C.C.A. unit vaccine. Another group of 64 men who had received the 600 C.C.A. unit vaccine approximately 6 months previously were given a second dose, this time the 700 C.C.A. unit preparation. Hemagglutination inhibiting (H.I.) and complement fixing (C.F.) antibodies were measured in serum drawn prior to vaccination and 2 weeks later.

2. Surveillance of Illness

Surveillance was in general limited to those men with oral temperatures of 99.6° F. or greater who reported to the Base dispensary during daytime sick call. The approximate date of onset was obtained and the nature of symptoms was recorded. Throat washings were obtained with veal infusion broth from those patients who had temperatures of 101° F. or greater. Blood for serologic studies was drawn at that time and 3 weeks later. In 75% of cases throat swabs were obtained for bacteriological study.

3. Virus isolation methods

Throat washings were immediately stored in the freezing compartment of a standard refrigerator until transported to the virus laboratory. Thereafter, they were stored at -20° C. until cultured. The time from obtaining the specimen to inoculation into culture media varied from a few hours to several months. All specimens were tested by inoculation into the amniotic sac of 10-day-old chick embryos, and into primary Rhesus monkey kidney cells. After 48 hours incubation at a temperature between 35 and 36° C. the amniotic and allantoic fluids were tested for the presence of hemagglutinating agents in microtiter using chicken red blood cells. In the tissue culture system areas of hemabsorption were sought following the addition of guinea pig cells. Isolates were identified by macrotiter and microtiter H.I. tests using chicken antisera to A_2/Hong Kong/8/68 and to an A_2/England/72-like strain isolated in Australia (A_2/Victoria/4/72). The antisera were kindly provided by the World Health Organization International Influenza Center for the Americas, Center for Disease Control, Atlanta, Georgia.

4. Determination of Antibody Response

Paired sera were initially tested for complement fixing antibodies to type A and B influenza using allantoic fluid antigen from chick embryos infected with either A_2/Hong Kong/8/68 or B/Massachusetts /8/66 strains. In addition, H.I. tests were performed using A_2/Denver /1F/72, the prototype strain of the epidemic, as well as A_2/Victoria /4/72 and A_2/Hong Kong/8/68 antigens.

All serum pairs were inactivated with receptor destroying enzyme (RDE) according to the Center for Disease Control protocol. Each batch of RDE was checked against sera known to contain high levels of nonspecific inhibitors. Acute and convalescent sera were always tested simultaneously against all the appropriate strains. Serologic tests were carried out by the microtiter method with microtiter and macrotiter virus controls. Paired sera were also tested for adenovirus CF antibodies using adenovirus type 7 as the test antigen.

A four-fold or greater rise in CF or H.I. antibody titers or both was considered diagnostic of influenza. All paired sera in which the H.I. test was negative but the C.F. test was positive or vice versa, and all pairs with two-fold rises, were retested at least twice.

RESULTS

A. Antibody response following vaccination

Table 1 shows the distribution of titers to A_2/Denver/1F/72 following a first or second dose of vaccine.

In the group of 107 unvaccinated men, 85% had H.I. titers of $\leq$ 1:8 before vaccination. Two weeks after vaccination 22% of the group had titers of $\leq$ 1:8. Sixty-six of the 90 men (73%) with pre-vaccination H.I. titers of $\leq$ 1:8 had a four-fold or greater antibody rise. Seven of 12 (70%) who had pre-vaccination titers of $\geq$ 1:16 had a four-fold or greater antibody rise.

In the group of 64 men who had received vaccine, approximately 6 months previously, 36% had titers of $\leq$ 1:8. Two weeks after the second dose, 22% had titers of $\leq$ 1:8. Seven of 23 men (35%) with pre-vaccination titers of $\leq$ 1:8 developed four-fold or greater H.I. antibody rises, whereas 6 of 41 (15%) with pre-vaccination titers of $\geq$ 1:16 had four-fold or greater rises.

To evaluate the potency of the vaccine the homologous antibody response to A_2/Hong Kong/8/68 was also determined. After one dose, the proportion of men in the unvaccinated group with H.I. titers of $\leq$ 1:8 fell from 19% to 7%, and 41% had four-fold or greater rises.

A noteworthy finding was a four-fold or greater rise in influenza A C.F. antibodies in 56% of men receiving one dose of the ether split vaccine, indicating that it contained significant amounts of viral nucleoprotein.

B. The epidemic

The epidemic began late in October, reached a peak on the 16th of November, and had virtually run its course by the end of November. Dispensary visits for febrile respiratory illness, which had been relatively low and constant in October, more than quadrupled during the second and third weeks of November reaching a level of more than 50 per 1000 per week. Illness rates fell sharply in the last week of November, then continued at a low level well into 1973. Figure 1 shows the epidemic curve for all febrile respiratory disease and for influenza in the study group by 8-day periods during November, 1972. Out of 199 men from the study group reporting with febrile respiratory disease, 154 (77%) proved to have influenza.

C. Clinical characteristics

The clinical picture was characterized by the sudden onset of malaise, chilliness, myalgia, headache, pharyngitis, cough and fever. The mean temperature in the unvaccinated men was 101° F. compared to 101.2° F. in the vaccinated men. The median time from onset of symptoms to reporting to the dispensary was one day. Men who were considered too ill to attend their training course were transferred to Fitzsimons General Hospital.

D. Vaccine efficacy

Attack rates for influenza in vaccinated and unvaccinated groups by 8-day periods are shown in Figure 2. The cumulative incidence of

influenza for the two groups is illustrated in Figure 3. One hundred and thirty-six of 2955 unvaccinated men developed influenza, an attack rate of 46.0 per 1000. In the vaccinated group, 18 of 979 acquired the disease for an attack rate of 18.4 per 1000. Vaccine efficacy was calculated by the formula:

$$\frac{\text{Expected ill -- Observed ill}}{\text{Expected ill}} \times 100$$

the null hypothesis being that the illness rate in the vaccine group would be no different from that in the unvaccinated group. The specific overall reduction of influenza in the vaccinated group was 61% ($p=<0.01$).

Forty-eight men were sent to Fitzsimons General Hospital with influenza. Forty-five of 136 cases among unvaccinated men were hospitalized, whereas only 3 of 18 cases among vaccinated men were judged sufficiently ill to require hospitalization. Thus, the rate of hospitalization was approximately twice as high among unvaccinated men as compared to vaccinees.

Table 2 compares the incidence of influenza and other febrile respiratory illness. The attack rates for non-influenzal diseases in vaccinated and unvaccinated men were 10.2 and 11.5 per 1000, respectively and were not significantly different. Considering all febrile respiratory disease, the reduction of illness in the vaccinated group was 50% ($p=<0.01$).

E. Diagnostic tests

Throat washings from 63 patients in whom a diagnosis of influenza had been confirmed by serologic methods were examined for the presence of virus. An influenza virus was recovered on first passage from 51 of the 63 (81%), in all instances antigenically identical to A_2/Denver /1F/72. In addition, throat washings from 22 patients who had failed to show a significant increase in antibody were cultured. None were positive for influenza virus.

A comparison of the C.F. and H.I. tests in diagnosing influenza was made in vaccinated and unvaccinated men. A four-fold or greater rise in the C.F. tests using A_2/Hong Kong/8/68 antigen was found in 89% of cases in vaccinees, and 91% in unvaccinated men. The H.I. test using A_2/Denver/1F/72 antigen detected four-fold or greater rises in antibodies in 94% of vaccinees and 99% of the unvaccinated group. However, when the A_2/Hong Kong/8/68 antigen was used in the H.I. tests, only 67% of vaccinees and 73% of unvaccinated men developed four-fold or greater antibody rises.

F. Acute phase antibody titer related to incidence of influenza

The estimated attack rates in unvaccinated and vaccinated groups specific for different acute phase H.I. antibody levels are shown in Table 3. The difference in attack rates in vaccinees and unvaccinated men with H.I. titers to A_2/Denver/1F/72 of $\leq$ 1:8 compared to those with titers of $\geq$ 1:16 was statistically significant ($p=<0.01$). Among the 5 unvaccinated and 2 vaccinated men with titers of $\geq$ 1:16, the acute phase titers did not exceed 1:64. No illness was encountered in men with higher titers. There was no significant difference in influenza attack rates at any given titer between vaccinated and unvaccinated men.

The presence of H.I. antibody directed at the Hong Kong antigen did not show the same relationship. The influenza attack rate among vaccinated students who had titers to the Hong Kong antigen of $\leq$ 1:8 was 44.2 per 1000, and among those with titers of $\geq$ 1:16 was 47.6 per 1000. Comparable attack rates for unvaccinated students were 16.4 and 18.9 per 1000, respectively. Even among individuals with Hong Kong H.I. titers as high as 1:1024 or more, the illness rates were not different from those with low titers.

G. Estimated overall infection rates in the student population

Eighty-four percent (90/107) of a sample group of 107 unvaccinated men tested prior to the epidemic had H.I. titers of $\leq$ 1:8. In contrast, after the epidemic had subsided, only 50% (33/67) of a sample group of 67 unvaccinated men who were on Base during the epidemic had titers of $\leq$ 1:8. Thus, approximately 34% of susceptibles had been infected with the influenza virus. This is in contrast to the 4.6% rate observed at sick call at the dispensary. The discrepancy is related partly to the inclusion in the study of only those men with an oral temperature of 99.6° F. or greater, and can thus be attributed at least in part to unreported and subclinical illness. Although a few instances of febrile respiratory disease in the study population might have been missed in night or weekend sick call, it is most unlikely that this could have significantly altered the estimate of vaccine effectiveness.

H. Adenovirus and streptococcal disease

Serological evidence of adenovirus disease, usually characterized by moderate to severe pharyngitis, fever, and, occasionally, conjunctivitis was found in 35 of the 199 students examined. In 14 of these, a double infection with influenza was present. Group A streptococci were cultured from the throats of 13 individuals, 7 of whom had concomitant influenza and one of whom had both influenza and adenovirus infection. No influenza B was detected. In only 18 of the 199 students with febrile respiratory illness could an etiologic agent not be determined.

DISCUSSION

Inactivated influenza vaccines of adequate potency have been shown to provide substantial protection during epidemics caused by virus strains identical to or closely related to those contained in the vaccine. When the epidemic strain has differed markedly from that contained in the vaccine, as in 1957 when a major antigenic shift from A_1 to A_2 occurred, the protective effect was eliminated. Protection is affected in variable and less predictable ways by the slow, continuous antigenic drift which is characteristic of influenza A viruses. In early 1957, for example, in the last outbreak of A_1 influenza, caused by the Denver or Nederland type of influenza A_1, a vaccine prepared against an earlier A_1 strain provided a high degree of protection even though there had been a sharp change in the hemagglutinin. On the other hand, when the Hong Kong hemagglutinin replaced the earlier influenza A_2 hemagglutinin in 1968, vaccine effectiveness was sharply reduced. For this reason, it was of particular interest to obtain accurate information on the capacity of Hong Kong type vaccine to protect against the England/72 strains which had shown considerable drift both in hemagglutinin and in neuraminidase antigens.

In the present study, the protective effect of the vaccine was obviously significant but far below the optimal level. The crude incidence of all febrile respiratory disease in the vaccinated group was reduced to one-half of that observed in the unvaccinated group. When the cases of febrile respiratory disease not caused by influenza were removed from the vaccinated and unvaccinated groups, the protective efficacy was calculated to be just over 60%. The occurrence of the outbreak early in the winter season accounted for the fact that the incidence of other diseases was relatively low and, for this reason, influenza accounted for approximately 75% of all illness during the epidemic period.

While the incidence of reportable illness was obviously reduced, it was difficult to obtain a clear picture of whether the severity of the disease was lessened in those vaccinated men who did become ill. When the height of the fever at the time students first reported to the dispensary was compared, it was essentially the same in the two study groups, and the method of follow-up precluded further study. The only suggestion that the disease might be somewhat modified was found in the observation that the rate of hospitalization of men from the dispensary was approximately twice as high in the unvaccinated as in the vaccinated individuals.

The diagnosis of influenza in this study was based on two criteria, clinical and laboratory. The clinical features were the presence of fever in an individual with symptoms of upper respiratory tract illness and/or constitutional symptoms characteristic of influenza. Laboratory criteria were H.I. and C.F. tests on paired sera and the isolation of influenza virus from throat washings. It was of particular importance to confirm the fact that serologic procedures could effectively detect influenza infection in individuals who had received vaccine. The most useful serologic test was the H.I. test, using a virus isolated from the outbreak which demonstrated significant antibody rises in 99% of unvaccinated men and 94% of those who had been vaccinated. Complement fixation tests with A_2/Hong Kong/8/68 antigen showed antibody rises in approximately 90% of the men in each group.

Support for the accuracy of these serological procedures was demonstrated in several ways. First, attempts to isolate influenza virus from individuals who had not shown an antibody increase were unsuccessful in all of 22 throat washings tested. Second, influenza virus was isolated from the throat washings of 81% of the men who had shown an increase in antibody following their illness. Furthermore, the incidence of non-influenzal febrile respiratory diseases was essentially identical in vaccinated and unvaccinated groups. If significant numbers of cases had been missed by serologic methods in the vaccinated group, this would have created an excess number of individuals classified as having had some disease other than influenza.

Cases of influenza were concentrated in the segment of the population which had H.I. antibodies against the epidemic strain of $\leq$ 1:8. Only 7 of the 156 confirmed cases had titers of $\geq$ 1:16. The degree of effectiveness of vaccine does appear to be related to its capacity to produce seroconversion, that is, an increase in titer from $\leq$ 1:8 to $\geq$ 1:16 in the recipients. The percent of vaccinated men who remained seronegative was 36% compared to 84% in the unvaccinated group, a difference of 57%. This figure corresponds closely to the observed reduction in illness of 61%. While it is clear that serum H.I. antibody titers are not in themselves the sole determinant of resistance to influenza, this relationship may be closer and of more predictive value than other parameters such as mean antibody titer levels which have been used in the past. It is of interest that the vaccine under test had been administered approximately 6 months before the outbreak, and that antibody titers were probably already falling. Thus, it is possible that a somewhat higher level of protection might have been observed in men who had received vaccine immediately before the outbreak.

A survey of reports on influenza vaccine effectiveness over the past 30 years must leave the reader confused and uncertain. There have been field trials in which effectiveness of approximately 90% have been reported, and others in which no protection has been observed. There are a number of explanations for these discrepancies. Many of the earlier studies were done with vaccine of inadequate potency; in other instances, significant antigenic drift had occurred in the infecting influenza virus; in still others, the studies were carried out in a manner which precluded obtaining clear-cut data on vaccine effect. Whatever vaccine is to be used in the future, whether improved inactivated aqueous vaccine, adjuvant vaccine or live attenuated virus, it is essential that studies be done in a manner which will provide definitive answers. It, therefore, seems appropriate to review guidelines for adequate vaccine evaluation.

The capacity of the vaccine to evoke antibody response against the epidemic strain must be determined by H.I. test and, perhaps, by other procedures such as neuraminidase antibody determinations. The diagnosis of influenza must be established in all participants in the study or, if this is impossible, in a statistically reliable sample, by laboratory procedures. This can be done by serologic tests, most easily by H.I. tests using the homologous antigen from the epidemic. In those outbreaks caused by virus strains which are readily isolated, the recovery of viruses from the patient may well be a useful procedure. There have been years, however, in which influenza virus isolation has been quite difficult. If laboratory procedures are not used for diagnosis, the results of the trial may be greatly skewed by the simultaneous occurrence of other febrile respiratory diseases. These frequently cannot be distinguished from influenza even by an experienced clinician. They certainly cannot be distinguished by the patient who is being asked to communicate with the investigator by telephone or postcard. When vaccine evaluation is attempted merely on the basis of a crude reduction in respiratory disease rates, the effectiveness of vaccine may be partially or completely obscured by the background of other respiratory diseases.

OTHER OBSERVATIONS ON INFLUENZA VIRUSES

A. Isolation of influenza A_2 from throat washings

Throat washings were collected from 63 men in the study group and from other students reporting to the dispensary with temperatures of 101° F. or higher by having them gargle with 10 ml. of broth and then immediately expectorate the remainder. Washings were initially stored in the freezing compartment of the dispensary refrigerator and, subsequently, stored at -20° C. prior to testing. Specimens were then tested by inoculation into primary Rhesus monkey kidney tissue culture or into ten- to eleven-day-old chick embryos which were then incubated at between 35° and 36° C.

The results are shown in Table 4. Influenza A_2 strains were recovered from 87% of 105 men who showed a significant increase in antibody following infection. Virus strains were recovered in both monkey kidney and chick embryos from 55% of washings. Monkey kidney tissue culture was positive, but the chick embryos were negative in 20%. The monkey kidney tissue culture was negative, and the chick embryo fluids were positive in 12%. It appeared, therefore, that if monkey kidney tissue culture alone had been used, virus strains would have been isolated from 75% of the washings; and, if chick embryos alone had been used, from 67%. In contrast to the results in individuals who had shown antibody increases, the throat washings from 26 men who had shown no increase in antibody for influenza failed to grow virus in each instance. Fourteen of the 26 men had been vaccinated.

The remarkable ease with which strains were isolated and the observation that allantoic fluids frequently contained high hemagglutinating titers within 48 hours of inoculation, led to further exploration of the usefulness of two routes of chick embryo inoculation. Fourteen throat washings from which strains had been isolated were re-tested in chick embryos inoculated by either amniotic or allantoic route. In each case, both amniotic and allantoic fluids were tested for hemagglutination. Amniotic fluids were harvested first with syringe and needle, and every attempt was made to avoid contamination by allantoic fluid which was harvested later. Results are shown in Table 5. In each of the fourteen instances, virus was present in the allantoic fluid regardless of the route of inoculation. The amniotic fluid was positive in only four of fourteen instances when inoculation was done by the allantoic route.

Apart from the differences noted in the behavior of the strains during first passage, these isolates appeared to be homogenous and indistinguishable from the prototype strain which was designated A_2/Denver/1 F/72. The "F" in the designation is used to distinguish this strain from those isolated earlier in 1972 at Lowry Air Force Base during last winter's small outbreak. The majority of the strains were typed as being closely related, if not identical, to A_2/England/42/72 by cross H.I. tests with monovalent antisera provided by the Center for Disease Control.

B. Influenza B

No influenza B was detected until April 30 when the first of three cases was detected, the date of the last being May 30. At the same time, small numbers of cases were reported in the civilian population in Colorado. A single virus strain was isolated in monkey kidney tissue culture and chick embryos and was shown to have antigenic characteristics almost indistinguishable from B/Victoria/70 which, in turn, closely resembles B/Mass/70. At the time when these cases occurred, virtually all men on the Base had been vaccinated, and no inference could be drawn regarding vaccine effectiveness.

Certain observations on the distribution of influenza B antibody and the response to vaccination were obtained by testing the sera of men who had been bled in the fall following first or second injections of the standard military vaccine. The first vaccine used had contained 300 CCA units of strain B/Mass/66, and the second vaccine, which was given to a different group of men, contained 300 CCA units of strain B/Mass/71. Two points of considerable interest are illustrated in Table 6.

The four B strains used in these tests span the period from 1966 to 1972. Prior to vaccination, 36% of the men had titers of less than 1:8 to the B/Mass/66 strain. In tests with B/Victoria/70, 68% had titers of less than 1:8; and in tests with B/Mass/71, 92% had similar titers. With the newly isolated B/Hong Kong/72 strain, none of the men had detectable antibody. It, therefore, appears that there has been a steady diminution in the proportion of individuals with H.I. antibody as the new strains have come along and that the new B/Hong Kong/72 will find a population which is virtually devoid of H.I. antibody.

With regard to antibody response, this was reasonably satisfactory for the three older strains. The somewhat poorer response to B/Mass/71, which was the strain from which the vaccine had been prepared, may have been due in part to the relative non-reactivity of this strain noted both in measuring vaccine response and in testing of paired serums from cases of influenza B. The striking lack of effect of a second injection of vaccine is clearly brought out in this table. Only one of the 25 individuals tested showed a four-fold increase in antibody. Whether a significant effect might have been obtained with an amount of antigen larger than the 300 CCA units used or with adjuvant vaccine, as has been shown in the past, was not determined.

These observations suggest that the population studied may be highly susceptible to infection with the new B/Hong Kong/72 strain and provide support for the recommendation that a monovalent vaccine prepared from the new strain be given as soon as possible.

ADENOVIRUS DISEASE

A. Incidence

Adenovirus disease was present on the Base at the time when studies began on October 20, 1972. The largest numbers of cases were reported during November with a peak of 53 cases. It is of interest that these occurred concurrently with the outbreak of influenza A_2. The number of cases fell somewhat in December due, perhaps, in part to the fact that students were off Base during much of the latter half of the month. Rates in January were low and, thereafter, only two cases were observed in February and March and none in April and May, a situation never observed previously at Lowry Air Force Base. Of the 101 cases diagnosed, 98 were confirmed as being caused by type 7 and one case by type 4. Two cases remained unidentified. In both the latter instances, there was a sharp rise in complement fixing antibody, but no increase in neutralizing antibody for either type 4 or type 7, and virus isolation attempts were unsuccessful. The single case of type 4 occurred in November of 1972.

It is tempting to ascribe the unusually low incidence during the spring to the vaccination program which was carried out at Lackland beginning during the first week of January. This program resulted in an input to Lowry Air Force Base, beginning in mid-February, of men who had uniformly received both types 4 and 7 live oral vaccines. However, it has already been noted that type 4 was absent from the Base throughout December and January and early February even though no vaccine had been administered to the men on the Base at that time. It has been wisely pointed out before that it is impossible to evaluate the effectiveness of a vaccine in preventing disease when no disease occurs.

With regard to type 7, the incidence had already fallen to a miniscule level even before the vaccinated men began to arrive at Lowry and while it is attractive to postulate that there would have been the usual spring outbreak if vaccine had not been administered, there is no conclusive evidence to support such a hypothesis. It was known that both vaccines were of less than optimal potency, and a total elimination of disease by vaccines of low potency has not been observed in the past.

In order to obtain further information on the effect of vaccine on antibody levels, 50 men who had been bled during October and another 50 who were bled during May of 1973 were tested for neutralizing antibody for both types 4 and 7. Results are shown in Table 7. In the group bled before vaccination, 76% had titers of less than 1:4 of neutralizing antibody for type 4 and 64% were negative for type 7.

The group bled in the spring contained 36 men who had been vaccinated prior to coming to Lowry and 14 men remaining from the study group who had remained unvaccinated. In tests for type 4 antibody in vaccinated men, the rate of 77% seronegative was essentially as in the men bled in the fall. Among the unvaccinated men, for reasons unexplained and needing corroboration, 100% were seronegative. There was, thus, no evidence that either vaccination or infection with type 4 virus had had any effect on antibody levels.

The situation with respect to type 7 antibody was somewhat different. In the vaccinated men, the percent of seronegative individuals fell from 64% to 33%, indicating that the vaccine at best had produced seroconversion in about half of the men. In the small group of unvaccinated men who had been on the Base when type 7 was prevalent, the percent of seronegative men had fallen to 22%.

B. Diagnostic methods in adenovirus infections

The diagnosis of an adenovirus infection was confirmed in 103 patients by complement fixation tests, neutralization tests, isolation of virus or a combination of these procedures. Complement fixation tests showed four-fold or greater increases in antibody titer in 91 of the 103 instances (88%). Neutralization tests were positive in 101 of 103 individuals (98%). Seven of these 10 additional individuals had shown two-fold rises in complement fixing antibody tests, and three had shown no changes in titer. Throat washings from 52 of the patients were tested for adenovirus, and 51 proved to be positive (98%). Isolations were attempted in both monkey kidney and hela cells, and the latter proved to be more useful. Fifty of the 51 strains have recovered in hela cells, 34 strains in both hela and monkey kidney, and one strain in monkey kidney but not hela cells.

Recovery of virus frequently required several passages, and difficulty was encountered particularly in the first passage because of the cytotoxic effect of the throat washings or because of difficulty with the hela cells. Slightly less than half the strains were isolated in first or second passage, and the remainder from third and, occasionally, fourth passage.

Comparison of the results of the three methods indicated that neutralization tests were the most satisfactory method for establishing a diagnosis of adenovirus type 7 and, again, showed that complement fixation tests as done in this laboratory were somewhat less sensitive. Isolation of virus strains compared in sensitivity with the neutralization tests but was clearly far more laborious, time consuming and expensive.

C. Attempts to isolate adenovirus from vaccinated men

Beginning in early January, 1973, all men arriving at Lackland Air Force Base received both type 4 and type 7 live oral adenovirus vaccine. These men began arriving at Lowry Air Force Base in mid-February, and from that point on virtually all newly arrived men had received vaccine. In order to determine whether wild adenovirus continued to circulate in the respiratory tract of these individuals, throat washings were collected from samples of approximately 25 men each week commencing on March 5, 1973 and ending on April 23, 1973. The throat washings were inoculated in the human embryonic kidney and into hela cell tissue culture using both macro and micro methods. No adenovirus strains were isolated from the 196 throat washings which were tested.

However, an unexpected observation was made. It was frequently noted that the tissue culture showed cytopathic changes, and it soon became apparent that there was an infectious agent other than adenovirus in a number of the throat washings. This was identified as poliomyelitis virus. The recovery of poliovirus strains ranged from nil to 31% in collections of throat washings made during different weeks, with an average recovery figure of 18.4%. Of the 36 strains, 23 were typed as polio type 1, 12 as polio type 3 and none as polio type 2. One throat washing contained both types 1 and 3. Approximately half the strains were recovered in both human embryonic kidney and hela cells. Sixteen strains were recovered in hela cells but not in monkey kidney. It appeared that the macro method was somewhat more sensitive than the micro method.

Acting on the assumption that these were vaccine strains rather than wild strains, the immunization records of the newly arrived men were examined. It was possible to obtain dates of poliomyelitis vaccination and to estimate the interval between administration of vaccine and the recovery of virus in 19 cases. These data are shown in Table 8. All of the men had been vaccinated more than one week previously, the majority between one and two weeks earlier. Five had been vaccinated 18 or more days before the throat washing was collected, and in one instance the interval was 25 days.

SURVEILLANCE OF FEBRILE RESPIRATORY DISEASE

In addition to the special studies which were carried out in certain segments of the population, a general surveillance was made of the respiratory disease pattern during the period from October 20, 1972 until June 15, 1973. All men reporting from student squadrons with respiratory disease, numbering from 4300 to 4700 men throughout this period, were recorded and

serum specimens were obtained from those who were febrile. Prior to the first of January, this surveillance was limited to men whose reporting temperature was 99.0° F. or higher because the volume of work was such that it was impossible to include all men with temperatures of 99° F. or above as in prior years. Subsequent to the first of January, men with temperatures between 99° F. and 99.6° F. were also included, but they are treated separately in the following discussion.

Table 9 shows by months the number of cases of various identified disease as well as the number of illnesses which remained unclassified. In contrast to most other years, the highest incidence occurred during the pre-Christmas period due mainly to the very sharp outbreak of influenza in November. Thereafter, influenza subsided rapidly, but cases continued to occur in January and February with a single case in March. Adenovirus, likewise, occurred in highest incidence in November and December, fell off in January and, by March, had completely disappeared. This is the only occasion in recent years adenovirus disease has been absent in April and May. Streptococcal disease occurred throughout the winter season but at no time in large numbers. Rubella also was present throughout the study, but the number of cases was small. Mycoplasma infections, as in other years at Lowry Air Force Base, was a relatively uncommon disease. Influenza B occurred only in April and May and would not have been recognized had not serologic tests been carried out. Only 31.5% of the cases remained in the unclassified category. There was no particular accumulation of unclassified cases during any month of the study. Conceivably, tests with coronaviruses or parainfluenza viruses might reduce this number still further.

The influenza A_2 outbreak is reminiscent of the situation which prevailed a number of years ago when influenza vaccine either was not used or the vaccines were relatively ineffective and explosive epidemics occurred. It has been noted earlier that with the strain change of 1972, vaccine effectiveness had been reduced to about 60%. The impact of the influenza outbreak would, presumably, have been even greater if the Hong Kong vaccine had not been used in some 70% of the men on the Base. The peculiar pattern of adenovirus infections has been noted earlier, and attention is again called to the virtual disappearance of type 4, for reasons which are not entirely clear, and the rapid fall off in type 7 cases after January.

In order to put the 1972-73 season into perspective, Figure 4 presents a comparison of the rates of febrile upper respiratory infection in the student population at Lowry Air Force Base during the four years between 1969 and 1973. In the year 1969-70, rates were relatively low in December and January and then rose sharply in February due almost entirely to adenovirus disease which was, at that time, predominantly type 4. Monovalent type 4 vaccine was given shortly after the peak of the outbreak during late March, and the incidence of the disease thereafter fell off steadily, but it is impossible to state whether this was due to vaccine administration or to the usual seasonal pattern. In 1970-71, December was again a light month and began to reach higher levels during late January. At this time, type 4 vaccine again was administered, and rates thereafter remained essentially at a steady level. This was due in large measure to the fact that illness rates were extremely high in the receiving squadron during the period immediately after arrival on the Base before any vaccine effect could have been anticipated. Again, the majority of disease was due to type 4 although small numbers of cases of type 7 occurred during the spring period.

In 1971-72, December was again a low month. In January, there was a very small peak representing the limited outbreak of Hong Kong type influenza A_2 which occurred during a two-week period in January. Adenovirus vaccines for both types 4 and 7 were given at Lackland Air Force Base from the first week of January on to 70% of the men. These men began to arrive at Lowry about the middle of February. Rates remained low throughout the whole remainder of the period of observation though small numbers of cases of both type 4 and type 7 adenovirus disease provided evidence that these viruses were present in the population.

The pattern in 1972-73 was quite different in that the very sharp November peak, comprised in great part of the A_2/England/72 influenza outbreak, was the highest observed during this four-year period. For a similar experience, one has to go back to 1957 when Asian influenza occurred on the Base heavily during September and October and to the 1968 outbreak of Hong Kong influenza which, again, occurred prior to the Christmas break. From February on until June, rates were in general the lowest observed since observations were commenced at Lowry Air Force Base in the early 1950s. This can best be explained by the absence of adenovirus disease from March onward. While it would be pleasant to ascribe this to the use of vaccines in all of the student population, it has been noted earlier that this easy explanation does not fit the situation, and the reason for this absence of disease remains unclear. What is evident, however, is that if influenza and adenovirus disease are brought completely under control, there is reason to believe that extremely low rates of febrile disease can be maintained in the student population throughout the winter season. Hopefully, this goal can be achieved when the vexing problems relating to influenza vaccine are solved and when adenovirus vaccine of potency comparable to the earlier preparations are again made available to the Armed Services.

Investigations at Lowry Air Force Base have been directed toward febrile respiratory disease because it is this segment of respiratory illness which causes interruption of training programs or necessitates hospitalization. Many individuals with U.R.I.s report with temperatures between 99° F. and 99.6° F. Between January and May of this year, for example, 144 men reported ill with temperatures in this range while 234 reported with temperatures of 99.6° F. or higher. Among these 144 men, only 5 cases of influenza A_2 and 2 cases of adenovirus disease were found. In earlier studies of outbreaks of influenza and adenovirus, there have been larger numbers of cases in men with reporting temperatures below 99.6° F., but during this period of observation neither agent was present. A still larger number of men, from three to six times as many, report with respiratory illness without fever. No particular effort has been made to determine the cause of their illness because the present state of knowledge provides little prospect that anything useful could be done to eliminate these nuisance diseases.

TABLE 1 Distribution of Hemagglutination inhibition antibody titers to A_2/Denver/1F/72 in 107 men receiving one dose and in 64 men receiving a second dose* of A_2/Aichi/2/68 vaccine**

	Percent with titer of			
	<8	8	16	≥32
First Vaccination				
Pre	73	11	7	8
Post	22	0	7	70
Second Vaccination				
Pre	28	8	11	53
Post	16	6	9	69

*second injection given approximately 6 months after a primary dose of 600 CCA units of A_2/Aichi/2/68 antigen

**containing 700 CCA units of A_2/Aichi/2/68 antigen

	Vaccinated		Unvaccinated	
	Number of cases	No./1000	Number of cases	No./1000
Influenza	18	18.4	136	46.0
Other Febrile Respiratory Diseases	10	10.2	34	11.5
TOTAL	28	28.6	170	57.5

Overall reduction in total illness = 50%

Specific reduction in influenza = 61%

TABLE 2 Number of cases observed and illness rates (per 1000) for influenza and other febrile respiratory disease, Lowry Air Force Base, November, 1972.

To A$_2$/Denver/1F/72	1:8 or less		1:16 or more	
	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Estimated Total No. of Men*	2482	352	473	627
No. Men Ill	131	16	5	2
No. Ill per 1000	52.8	45.5	10.6	3.2
To A$_2$/Hong Kong/8/68				
Estimated Total No. Men*	1380	183	1575	795
No. Men Ill	61	3	75	15
No. Ill per 1000	44.2	16.4	47.6	18.9

*Extrapolated from samples of 107 unvaccinated and 64 vaccinated men.

Difference in illness rates between men with titers of $\leq$ 1:8 and $\geq$ 1:16 to A$_2$/Denver /1F/72 in either unvaccinated or vaccinated men is significant ($p=<0.01$).

TABLE 3 Comparison of estimated influenza attack rates in vaccinated and unvaccinated men according to acute phase H.I. antibody titer.

Result of Isolation attempt	Percent of virus isolations from Positive cases (105)	Negative cases (26)
M.K. + C.E. +	55	0
M.K. + C.E. -	20	0
M.K. - C.E. +	12	0
M.K. - C.E. -	13	100

TABLE 4

Results of attempts to recover A_2 (H_3N_2) virus by monkey kidney tissue culture and chick embryo inoculation with throat washings from 105 patients with serologically confirmed influenza and 26 patients who showed no increase in influenza A_2 antibody titer.

Route of Inoculation			
Amniotic		Allantoic	
Amniotic Fluid	Allantoic Fluid	Amniotic Fluid	Allantoic Fluid
4/14	14/14	3/14	14/14

TABLE 5

Results of tests for hemagglutinating activity in amniotic and allantoic fluids of chick embryos inoculated with throat washings from 14 serologically confirmed cases by amniotic (stet) or allantoic routes.

Test Strain	Vaccination	Serum Specimen	Percent with titer of ≤ 8	8	16	32	≥64	% with 4 x rise
B/Mass/66	1	Pre-	36	28	28	4	4	
		Post	8	-	32	16	44	60
	2	Pre-	8	4	8	24	56	
		Post	8	-	8	20	64	0
B/Vict./70	1	Pre-	76	12	8	-	4	
		Post	8	4	28	20	40	68
	2	Pre-	12	12	36	12	28	
		Post	12	4	32	24	28	4
B/Mass/71	1	Pre-	92	8	-	-	-	
		Post	28	12	20	24	12	52
	2	Pre-	32	28	24	12	4	
		Post	20	28	32	12	8	4
B/Hong Kong/72	1	Pre-	100	-	-	-	-	
		Post	96	4	-	-	-	4
	2	Pre-	96	4	-	-	-	
		Post	96	4	-	-	-	0

TABLE 6

Increase in H.I. antibody to 4 influenza B strains following first or second injection of vaccine containing 300 C.C.A. units. The first injection was with vaccine containing strain B/Mass/66; the second with vaccine containing B/Mass/71.

Time of Bleeding	Number of Men	Result of Neutralization test for Type 4			Type 7		
		Pos.	Neg.	% Neg.	Pos.	Neg.	% Neg.
October, 1972	50	12	38	76	18	32	64
May, 1973							
Vaccinated	50	11	39	78	35	15	30
Not vaccinated	35	5	30	86	26	9	24

TABLE 7

Comparison of results of neutralization tests for types 4 and 7 adenovirus in two groups of men, one bled at the beginning and one at the end of the 1972-73 study.

Days after Vaccination	Number of virus Isolations
9	2
10	5
11	5
12	1
13	1
18	1
21	2
22	1
25	1

TABLE 8 Interval between administration of poliomyelitis vaccine and subsequent recovery from throat washings.

	Oct. (20-31)	Nov.	Dec. (1-18)	Jan.	Feb.	Mar.	Apr.	May	Total	%
Influenza A	2	222	22	9	4	1	-	-	260	39.0
Influenza B	-	-	-	-	-	-	1	2	3	0.4
Adenovirus	12	53	21	11	2	2	-	-	101	15.2
Streptococcus	-	18	4	18	11	9	6	3	69	10.4
Rubella	-	3	1	12	2	-	-	1	19	2.9
Mycoplasma	-	1	-	-	1	1	1	-	4	0.6
Negative	4	23	46	20	18	25	46	28	210	31.5
TOTAL	18	320	94	70	38	38	54	34	666	

TABLE 9 Number of cases of different febrile upper respiratory infections in whole student population by months.

TOTAL OBSERVED
FEBRILE RESPIRATORY DISEASE AND INFLUENZA
INFLUENZA STUDY GROUP, NOVEMBER, 1972

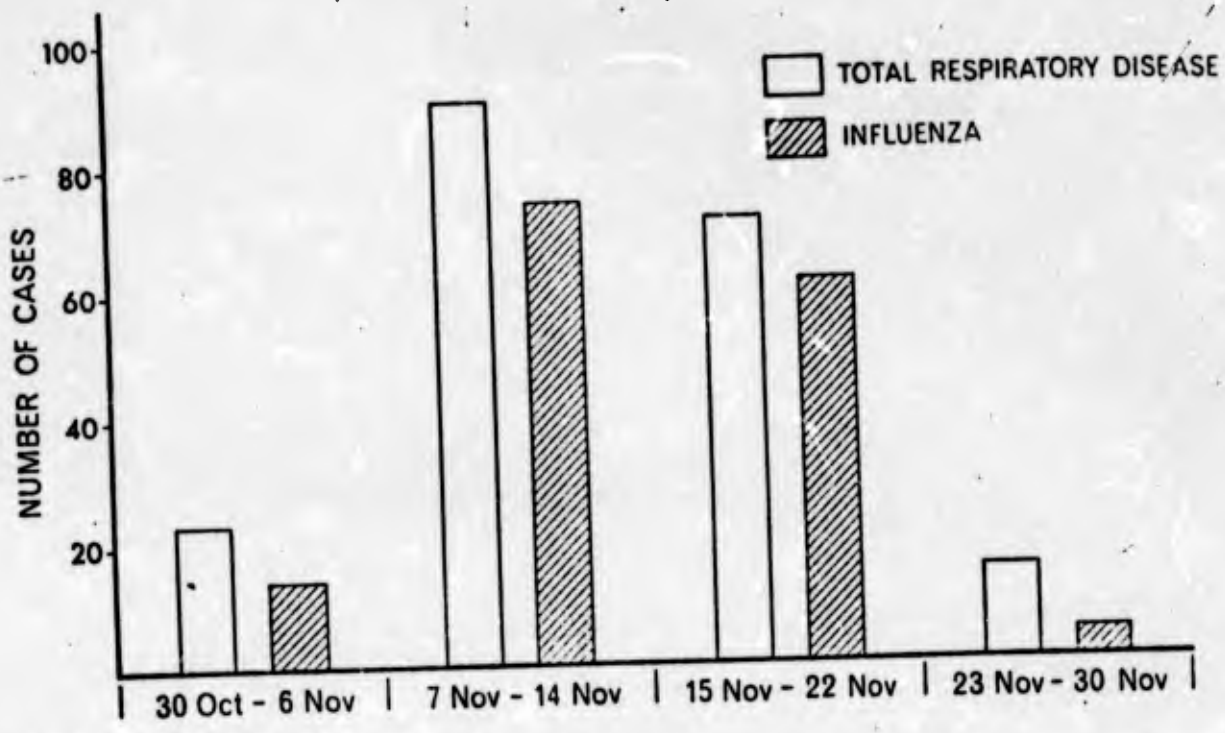


FIGURE 1

ATTACK RATES FOR INFLUENZA IN
VACCINATED AND UNVACCINATED MEN
INFLUENZA STUDY GROUP, NOVEMBER, 1972

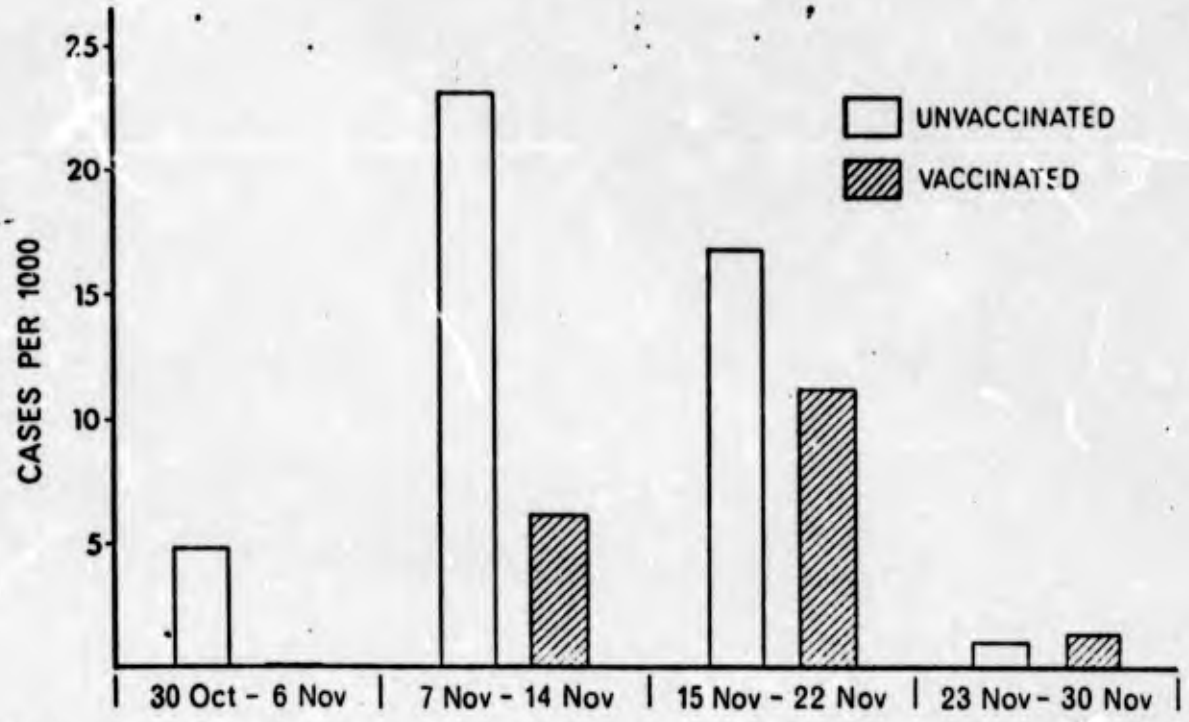


FIGURE 2

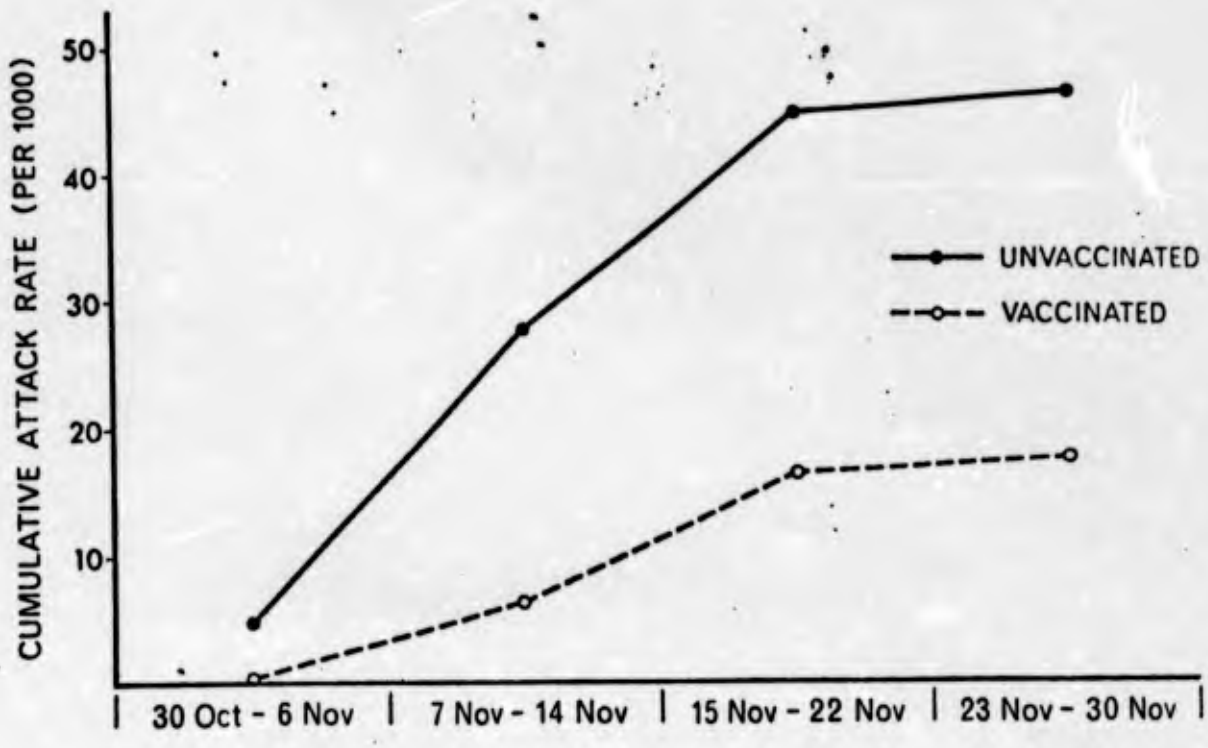
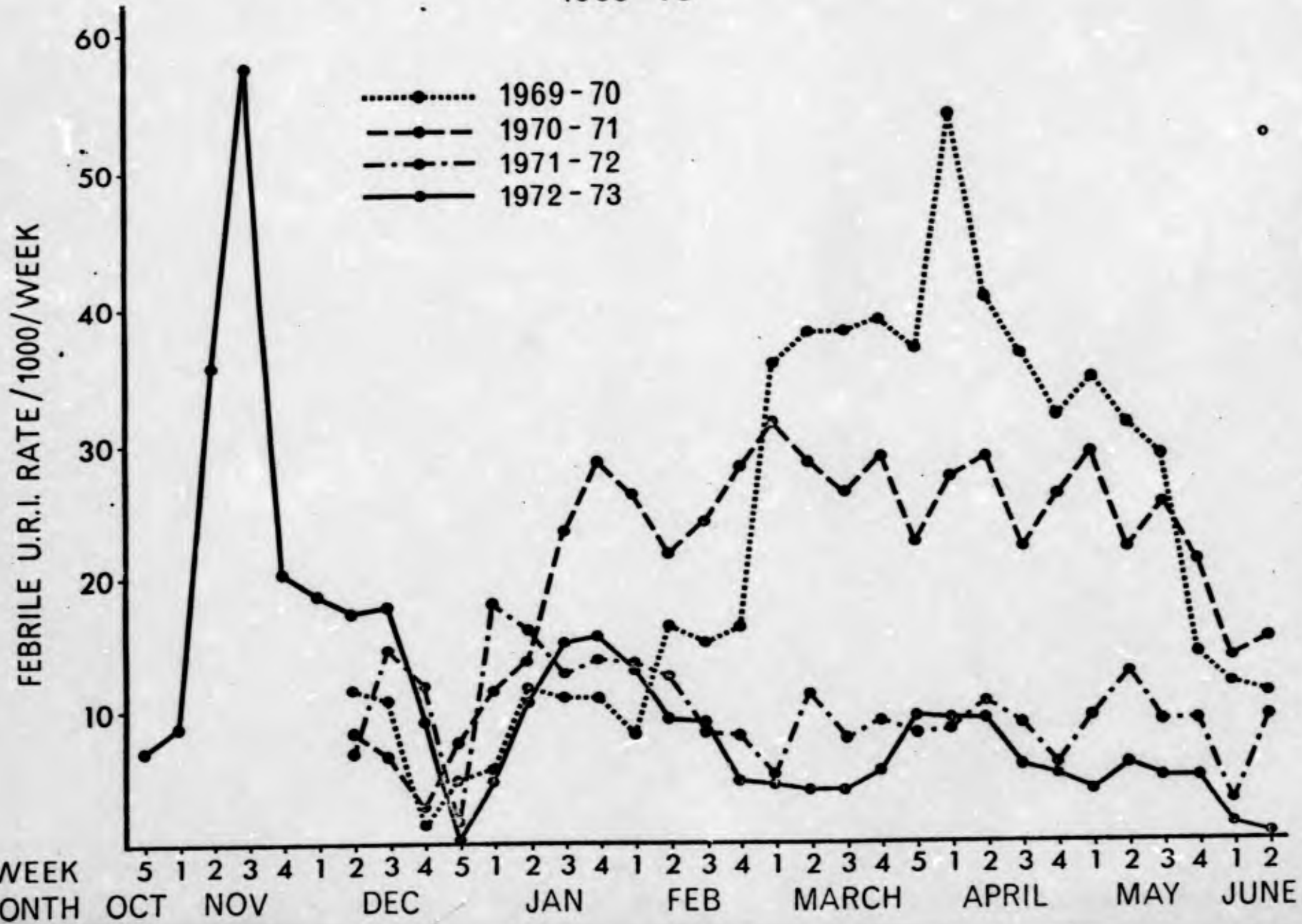

CUMULATIVE INFLUENZA ATTACK RATES
IN UNVACCINATED AND VACCINATED MEN
CUMULATIVE ATTACK RATE (PER 1000)
50
40
30
20
10
UNVACCINATED
VACCINATED
30 Oct - 6 Nov
7 Nov - 14 Nov
15 Nov - 22 Nov
23 Nov - 30 Nov

FIGURE 3

WEEKLY RATES OF FEBRILE UPPER RESPIRATORY INFECTION, STUDY POPULATION, LOWRY AIR FORCE BASE, 1969-73



ACKNOWLEDGEMENTS

The authors are grateful to Colonel Robert A. Watson, Director, Base Medical Services, Lowry Air Force Base, for support and assistance in the conduct of this study; to Mrs. Pat Graves, Research Virologist, and Mrs. Jan Hopkins, Research Technologist, for technical assistance; and to Mrs. Viola De Tuerk for secretarial assistance.