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PREVENTION OF INFLUENZA AND OTHER RESPIRATORY DISEASES (U)

ANNUAL PROGRESS REPORT

BY

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

August, 1976
(For the period 1 June 1975 to 1 November 1976)

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

a) During an influenza A/Victoria/75 outbreak in the Denver area in February and March of 1976 the illness rate in vaccinated students at Lowry AFB was only 0.8%.

b) The attack rate varied inversely with the level of HI antibody for A/Victoria/75, with highest rates in persons having titers of 8 or less and almost no illness in those with titers of 64 or higher.

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c) Overall febrile respiratory disease rates remained very low. Only 4 cases of adenovirus disease were detected throughout the period from 15 November 1975 to 15 May 1976.

d) In a trial designed to compare the vaccines prepared by four manufacturers, each at dosage levels of 200, 400 or 800 CCA units, the Merck, Sharp and Dohme whole virus vaccine was most effective. At 200 CCA units it produced seroconversion in 68% of persons while the other three products fell below 44%, at 400 CCA units the Merrell and Wyeth vaccines were more satisfactory; with seroconversion rates of 58% and 65%. These two vaccines failed to produce higher seroconversion rates with 800 CCA unit doses; the seroconversion with Merck, Sharp and Dohme vaccine at the 800 CCA unit dose rose to 78%.

e) Most of the reactions observed 24 and 48 hours after vaccination were mirer, and none were alarming. Merck vaccine at the 400, and particularly at the 800 CCA U dose caused more fever and severe systemic reactions than did other vaccines at any of the doses tested.

f) HI antibody for A/NJ/76 in titers higher than 16 was not found except in individuals over 30 and a few younger persons with known pig contact.

g) A second injection of vaccine containing A/NJ/76 resulted in seroconversion in all of 48 vaccinees.

h) The 1976 formula military vaccine effected seroconversion for A/NJ/76 in 55% of recipients. Antibody levels for A/Victoria/75 and B/HK/72 were highly satisfactory.

i) In a civilian trial in the fall of 1976 a split virus vaccine (Parke-Davis) produced an antibody response comparable to that of a whole virus vaccine (Merck), but rates of local, systemic and febrile reactions were significantly higher with the whole virus vaccine.

j) The A/NJ/76 vaccines evoked an A/PR8/34 antibody response in a high proportion of recipients. With the Merck vaccine the proportions developing antibody and the antibody titers were higher for A/PR8/34 than to the A/NJ/76 strain.

k) An earlier recombinant, X-42 (H Eq1 N2) from which the parent A/PC/74 and A/PR8/34 had presumably been eliminated produced an HI antibody response for the latter viruses in 67% and 40% respectively, of seronegative persons.

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4. Summary

1. Introduction

The studies carried out during the contract period 1 June 1975 to November 1976 are presented in two sections. The first deals with observations made during the winter season of 1975-76 when the student population of Lowry Air Force Base, which had previously received military vaccine was challenged by influenza A/Victoria/75 during an outbreak in the Denver area. The second section deals with extensive studies to evaluate the effectiveness and reactogenicity of a number of lots of vaccine prepared against the swine influenza strain A/NJ/76. The third describes some unexpected observations on recombinant vaccines. Originator Supplied Keywords include:

2. Observations during the 1975-76 winter season

a) Objectives

(See DD1473)

1) To obtain serologic data which might be useful in the prediction and enhancement of influenza vaccine effectiveness.

2) To monitor the incidence of viral respiratory disease in the student population at Lowry AFB, which had received standard bivalent influenza vaccine and oral vaccine against adenoviruses Type 4 and 7

b) Material and Methods

1) Population studied

The student population numbered between 2900 and 3200 during the winter season. Following a period of approximately 6 weeks at Lackland AFB students were enrolled in training courses of variable length at Lowry AFB, some of only a few weeks, others of many months duration.

2) Vaccines

Vaccines had been given at Lackland AFB during the period between induction into the Air Force and transfer to Lowry AFB. Bivalent influenza vaccine containing 350 CCA units of A/PC/4/73, 350 CCA units of A/Scot/74 and 500 CCA units of B/HK/5/72 and oral live Type 4 and Type 7 vaccines had been given to virtually all the student population.

3) Surveillance

Most students reporting to the dispensary with symptoms of a respiratory infection and a temperature of 99.6°F or higher were seen at the Influenza Surveillance Office. Clinical data were recorded and throat washings were collected for virus isolation attempts. Paired serum specimens were collected when consent was obtained (specimens were actually obtained from 86%). Bacterial cultures were carried out by the dispensary laboratory.

4) Virus isolation

Throat washings were collected in PBS and held at 4°C if they were to be inoculated on the same day. Otherwise they were held

at -10°C during the few days prior to inoculation. All were tested in Rhesus monkey kidney and HeLa cell tissue culture and in chick embryos. Hemadsorbing or hemagglutinating isolates were identified with appropriate influenza antisera. Parainfluenza virues were typed with specific fluorescein tagged antibody.

5) Serologic procedures

All serum pairs were run in complement fixation tests with influenza A and B allantoic fluid antigens and in hemagglutination-inhibition tests with influenza A/PC/1/73, A/Scot/74, A/Victoria/3/75 and B/HK/2/76. A few neuraminidase inhibition tests and neutralization tests in chick embryos were run using standard procedures.

c) Results

1) Occurrence of febrile respiratory disease

The incidence of febrile respiratory disease remained very low in 1975-76 as it had in 1974-75. Between 1 November 1975 and 31 December 1975 specimens were collected from 64 patients. Five virus strains were isolated, 4 of parainfluenza 1 and 1 of parainfluenza 2. No adenovirus disease was detected.

Between 1 January 1976 and 15 May 1976 specimens were collected from 155 patients. Influenza A, (A/Victoria/75) was the cause of 29 illnesses between 20 January and 2 April, with most cases occurring during the latter half of February and first half of March. This outbreak coincided with a sharp but limited outbreak in the surrounding civilian community. Influenza B was not detected at Lowry AFB when scattered cases occurred in the area during the late spring.

2) Isolation of influenza viruses

Influenza A strains resembling A/Victoria/3/75 were isolated from 28 patients. Serum pairs were available from 24 of these patients and all but one showed a 4-fold increase in antibody titer in CF tests or in HI tests with one of the 3 influenza A (H_3N_2) strains. The sensitivity of chick embryos and RMK tissue culture as isolation methods was compared with washings from 24 persons who showed antibody increases; when both media were used virus was isolated from 22 of 24 (91%). Using chick embryos alone the isolation rate was 21 of 24 (88%) and with RMK tissue culture the rate again was 21 of 24 (88%). It thus appeared that, as with other strains in the Hong Kong family, chick embryos or RMK tissue cultures were highly sensitive means for virus isolation and were comparable in sensitivity.

3) Serologic tests

Influenza A HI antibody levels in this population were almost uniformly elevated, reflecting the fact that most persons have been infected with one of the members of the influenza H_3N_2 family during the past 7 years or had received A/PC/1/73 vaccine. Among

the 155 persons seen with febrile respiratory disease which was not influenza, 23% had titers of 8 or less for A/Victoria/3/75. The corresponding figures for A/PC/73 and A/Scot/75 were 10% and 12%. The same situation held for B/HK/5/72 with only 9% of persons having titers of 8 or less. Complement fixing antibody titers for adenovirus were also generally less than 8, reflecting the absence of adenovirus disease and the well recognized failure of oral adenovirus vaccines to produce significant increases in complement fixing antibody titers.

In view of the continuing interest in the relationship between HI antibody titer level and the incidence of influenzal illness, an estimate was made of the incidence of influenza in individuals with different antibody levels. It was assumed that the antibody levels determined in the 107 persons who did not have influenza during the period between 1 January and 15 May were representative of the whole student population. The percentages observed were extrapolated to the approximately 3200 students (Table 1). Even though the number of cases was small there was an obvious decrease in incidence as the titer rose. The illness rate was 2.1% among persons with titers of 8 or less, 0.8% in those with titers of 16 or 32, and only 0.1% in those with titers of 64 or more. Thus, there was a major difference in incidence between those with titers of 8 or less and those with titers of 64 or more.

It is also of interest that influenza spread poorly and infected only a very small proportion of the estimated 880 persons who had A/Victoria/3/75 antibody titers of 8 or less. This suggests that other factors, possibly neuraminidase antibody, may have contributed to the limitation of illness. Whatever the explanation, this vaccinated military group experienced a very low influenza illness rate of 0.8%, even though the disease was prevalent in the surrounding civilian community for a period of approximately two and one half months.

4) Incidence of other respiratory diseases

The low overall incidence of febrile respiratory disease is shown in Table 2. In only 3 of the 33 weeks of the studies did the rate/1000/week exceed 10. The highest rates of 12.3 and 12.9 coincided with largest number of cases of influenza. However, without laboratory diagnosis this would hardly have been recognizable as an epidemic peak.

An etiologic breakdown of the febrile respiratory diseases present on the base throughout the 1975-76 season was made by combining laboratory and clinical diagnoses. Streptococcal pharyngitis was responsible for 19% of dispensary visits, influenza A for 13%, rubella for 7%, rubeola for 3%, adenovirus for 2% and parainfluenza viruses for 2%. The etiology remained unknown in 54% of the patients.

3. Observations on swine influenza vaccines

a) Introduction

The isolation of influenza A strains resembling swine influenza A and

the evidence that this virus had spread to large numbers of men resulted in an abrupt change in activities at Lowry AFB. A number of experimental vaccines were tested at Lowry AFB as part of a national program. These studies together with evaluation of response to later vaccines and a continuing surveillance for swine influenza have occupied most of the efforts of the laboratory during the balance of the contract period.

b) Objectives

1) To determine what CCA unitage of vaccine and what method of vaccine preparation would best evoke a satisfactory antibody response.

2) To assess the frequency and severity of systemic and local reactions to different amounts and types of vaccine.

3) To compare the effectiveness of single and double vaccination schedules.

4) To search for evidence of present or past infection with swine influenza virus.

c) Material and methods

1) Vaccines

Four manufacturers, each using its own method of preparation, provided vaccines to the Bureau of Biologics, each in doses of 200, 400 and 800 CCA units. Three manufacturers used a virus strain (X53A) which was a recombinant of A/NJ/3/76 with A/PR8/34. The precise identity of the swine-like virus used by the fourth producer, Parke-Davis, is at this time still uncertain. The Merck Sharp and Dohme and Merrell-National vaccines were whole-virus (W) vaccines, while the Parke-Davis and Wyeth vaccines were split-virus (S) vaccines. The vaccines were provided under code along with a placebo solution.

2) Volunteers

Following an educational and publicity campaign at Lowry AFB and with excellent support from both the medical and administrative personnel, 272 individuals volunteered to receive vaccine, to cooperate in the evaluation of reactions, and to give pre- and post-vaccination blood specimens. All signed approved informed consent forms. This group was then randomly assigned to 13 groups of approximately 20 persons. The members of each group then received one of the 12 vaccines or the placebo solution.

Two vaccines which were provided in the fall of 1976 for an evaluation of reactogenicity were tested in sophomore medical students, medical technology students and blood bank personnel at the University of Colorado Medical Center. Informed consent forms were obtained from all participants. The source and composition of these vaccines and of the military vaccines used in the fall of 1976 are listed in Table 3.

3) Assessment of reactions to vaccines

All volunteers had their oral temperatures recorded prior to vaccination using an Ivac portable digital readout thermometer, and those with temperatures of $\geq 99^{0}$ F were excluded. Volunteers returned at approximately 24 and 48 hours after vaccination; at both these follow-up visits oral temperatures were again recorded, and the participants were specifically questioned regarding local symptoms of pain or burning, and systemic symptoms of malaise, headache, feverishness, and upset stomach. In addition the injection site was examined for tenderness, redness and induration. All responses and reactions were graded on a scale of 0 to 3 plus, 1+ considered mild, 2+ considered moderate, and 3+ considered severe.

4) Measurement of antibody response

In accordance with the protocol for the national program all sera were tested at the Center for Disease Control. However, the sera were also run at the University of Colorado. Results checked very well. The viruses used in HI tests were A/NJ/8/76, A/Swine/1976/31 and A/Victoria/3/76. For reasons to be described later, tests were subsequently run with A/PR8/34 and A/Ann Arbor/2/57 (H_1N_1). During the fall of 1976, when other bivalent commercial vaccines were given to large civilian groups and standard military vaccines were given to personnel at Lowry AFB, serum specimens were collected from these groups as well.

d) Results

1) Observations in military personnel

(a) Antibody levels before vaccination

Tests for antibody against A/NJ/76 showed that almost all of the 272 persons were seronegative. When the age distribution of the group was examined it was obvious that older individuals were more likely to have antibody than younger persons. This presumably reflected the first influenza infection which these persons had experienced. Results are summarized in Table 4.

Only six of the persons below the age of 30 had measureable levels of A/NJ/76. The titers of these individuals were, respectively 8, 8, 16, 128, 128 and 256. The four persons with higher titers were interviewed and all had a history of swine contact. One had grown up on a pig farm in Iowa, another in Minnesota. One, from Indianapolis had frequently visited pig farms. The fourth had, as a child, had often played with pigs at a children's zoo in Florida. Six of the 13 persons between 30 and 45 years of age had A/NJ/76 antibody, possibly as a result of prior infection with H_1N_1 or H_0N_1 strains or of receiving vaccine with a swine component.

A parallel relationship existed when the sera were tested

against A/PR8/34. Only 10.5% of persons 19 and under had titers of 8 or higher, and the titers of these persons were uniformly low. In the 20 to 29 year age group the percentage with titers of 8 or more was 19.0%, and a few had high titers. Among the 13 persons with ages between 30 and 45, 11 (84.6%) had antibody for PR8, often with high titer (Table 5).

Antibodies to A/Victoria/76 were tested in order to determine the effectiveness of the 1975 military vaccine which almost all of the persons had received. This vaccine contained 700 CCA units of influenza A, equally divided between A/Port Chalmers/73 and A/Scotland/74. Results are shown in Table 6. The relatively close relationship between A/Victoria/76 and the earlier strains is apparent. Forty-eight percent of the persons had titers of 64 or higher and only 19.4% had titers of 8 or less.

(b) Antibody response following experimental A/NJ/76 vaccines

Vaccines from four manufacturers were tested, each at a dose of 200, 400 or 800 CCA units. The results were surprising and somewhat disconcerting (Table 6). These are expressed in terms of the percent of persons under 25 years of age who seroconverted following vaccination. The few individuals who had preexisting antibody have been excluded. The Merck vaccine was clearly superior, with seroconversion rates of 68% at the 200 and 400 CCA unit potency and 78% at 800 CCA units. Merrell-National, the other whole virus vaccine, was somewhat less effective but performed better at the 200 CCA unit level then either of the split-virus vaccines. The Parke-Davis vaccine, perhaps as a result of its composition, was clearly the least effective. No explanation is provided for the drop in seroconversion rates of two of the vaccines at the 800 CCA unit level. A similar observation was made in the larger CDC studies.

The paired sera were also tested for antibody against A/Victoria/75, A/Ann Arbor/57 (H_1N_1), and PR8. Antibody levels to A/Victoria/75 were already elevated as a result of previous vaccination. Only 1 significant antibody rise was found among 272 serum pairs tested. Among 21 persons who were seronegative for A/Ann Arbor/57 there were 2 persons with antibody increases of 4- or 8-fold (10%). Increases in A/PR8/34 were numerous. These are described in a subsequent section.

(c) Systemic and local reactions following vaccination

All save 35 of the 298 (89%) recipients of vaccine returned 24 hours after vaccination for follow-up evaluation of local and systemic reactions, and all save 39 (87%) returned for the 48-hour evaluation. Overall results are shown in Table 7. Because of the small numbers of participants in each manufacturer-dose category, tests of significance were not carried out. Inspection of the table, however, suggests that the Merck,

Sharp and Dohme product at the 800 CCA unit dose, and to a lesser extent at the 400 CCA unit dose, was clearly more productive of fever and systemic reactions than were other vaccines at any dosage level tested. Approximately 20% of volunteers who received MSD vaccine at the 400 or 800 CCA unit dose had fever $\geq$99.5°F, although these were most common at 24 hours and most had subsided by 48 hours. More common, however, were systemic reactions of malaise, headache and feverishness, which, at the 800 CCA unit dosage, tended to be moderate or severe in character. In the pooled national data, MSD vaccine at both the 800 and 400 CCA unit dosage were significantly more reactogenic than all other products and doses. Other significant differences were not detected. With few exceptions, reactions observed were neither severe nor resulted in loss of duty.

(d) Effectiveness of a second injection of A/NJ/76 vaccine

It has been generally believed that a second injection of aqueous influenza vaccine adds little to the response which follows the first injection. This is true for individuals who develop moderate or high levels of antibody following the first injection, though not for those who develop no antibody after one injection. These are usually few in number in years when there is only modest antigenic drift. In years when a new influenza family appears and the whole population is without antibody it is difficult to obtain a satisfactory response with a single injection unless the amount of antigen is very large. In 1957, the year of the Asian pandemic, it was shown that a vaccine containing 400 CCA units caused seroconversion in 60% of persons vaccinated. When vaccine containing 200 CCA units was given twice at an interval of 6 weeks the seroconversion rate rose to 85%.

In the fall of 1976 there were still 48 persons at Lowry AFB who had received one of the 12 experimental vaccines during the spring. The overall seroconversion rate in this heterogenous group had been only 42%. When these individuals received the routine military vaccine and were bled 2 to 3 weeks later, the effect of the second injection was striking. All individuals raised their antibody titers to 16 or more and the majority had titers of 64 or higher (Table 8).

(e) A/PR8/34 antibody response to A/NJ/76 vaccines

Because earlier studies with recombinant vaccines had produced unexpected HI antibody responses, the sera of all recipients of the 12 experimental vaccines were tested against A/PR8/34. Some A/PR8/34 antibody response was expected because a moderately strong cross between A/Swine/1976/31 and A/PR8/76 has been recognized from the time when the latter was first isolated. For this reason it was not surprising that the Parke-Davis vaccine, prepared from a seed virus of uncertain identity evoked some antibody response in many individuals. The fact that titers to the two strains were equally high was

unexpected. The Wyeth vaccine was far less effective in producing an A/PR8/34 antibody response. The Merrell-National vaccine was more effective and in many persons the A/PR8/34 titer exceeded the A/NJ/76 titer (Table 9).

The Merck vaccine produced the most surprising results. The proportion of persons who seroconverted and the level of A/PR8/34 antibody was higher than that of A/NJ/76. In many instances the A/PR8/34 antibody of individuals exceeded the A/NJ/76 titer by 8, 16 or even 32-fold. A number of persons who showed no response to A/NJ/76 had a marked increase in A/PR8/34 antibody. It appeared, in fact, that the experimental vaccine was behaving like a bivalent vaccine. The significance of these findings is still not clear and work is in progress to determine whether this HI antibody is also neutralizing antibody. These investigations are being undertaken in collaboration with Dr. Kilbourne.

(f) Antibody response to standard military vaccine in fall of 1976

Sera were collected from 74 student volunteers before and approximately 3 weeks after they received their routine influenza immunization in early November 1976. Each person had received in one arm two injections by jet gun of 0.5 ml, each containing bivalent Merck whole virus vaccine with 200 CCA units of A/NJ/76 and 200 CCA units of A/Victoria/75 and in the other arm one injection of Wyeth split virus vaccine containing 500 CCA units of B/HK/72. Results are presented in Table 10.

None of the 74 persons had antibody for A/NJ/76 before vaccination. After vaccination 55% had titers of 16 or higher but relatively few had markedly elevated titers. The response appeared to be less favorable than that observed in the experimental study, when 68% of persons developed titers of 16 or higher.

The response to A/Victoria/75 and B/HK/72 was influenced by the fact that almost all men had previously received vaccine containing A/PC/73, A/Scotland/74 and B/HK/72. The distribution of prevaccination antibody titers was relatively high. The only significant change in antibody levels was observed in those individuals whose titer was 8 or less. The percent of such persons was reduced from 20% to 11% in the case of A/Victoria/75 and from 2% to 0% in the case of B/HK/72.

The levels of antibody for A/NJ/76 suggest that protection would be of relatively low order in the event of an outbreak of A/NJ/76, probably in the range of 60%. On the other hand outbreaks due to A/Victoria/76 or B/HK/72 would be expected to cause even less illness than in 1975-76, when attack rates were in the range of 1%. Further antigenic drift would, naturally, tend to reduce vaccine effectiveness.

(g) Reactions following standard military vaccine

The number of dispensary visits rose sharply on the days

which followed the administration of the influenza vaccines to different squadrons. Records were kept of the numbers of men who had fever at the time of their dispensary visit, usually 15 to 24 hours after being given vaccine. Among 2897 students followed in this way, 173 (6.0%) had fevers of 99°F or higher (Table 11). Temperatures of 100°F or higher were observed in 3.8%. Two persons had temperatures of 103°F or higher. These figures are presumably minimal, since many persons with fever undoubtedly did not report and large numbers of men who reported without fever are not included.

2) Observations in civilians

(a) Antibody status before vaccination

Because all personnel at Lowry AFB had previously received military vaccine there was no opportunity then to assess antibody levels in unvaccinated persons for current influenza A and B strains. For this reason sera were collected from 122 medical and medical technology students and from the staff of the blood bank. Most of these persons then received either Merck or Parke-Davis bivalent vaccine containing 200 CCA units each of A/NJ/76 and A/Victoria/75. Post-vaccination sera were collected 3 weeks later. These individuals were included in a large study designed to assess reaction rates in hospital and medical center personnel following administration of commercial civilian vaccine.

Results are summarized in Table 12. The most notable findings with the prevaccination sera of persons who received no vaccine the year before were the relatively high proportion of persons who had titers of less than 8, namely 74% for A/Victoria/75 and 68% for B/HK/72. Among persons who had received civilian vaccine one year before the comparable figures were 40% and 12%. While both viruses have caused outbreaks in the Denver area it appears that a large proportion of this population has escaped infection. It seems likely that, if either virus is reintroduced the population will be somewhat vulnerable.

(b) Comparison of response to split and whole virus vaccines

It was concluded on the basis of the experimental vaccine trials that split virus vaccines were considerably less effective than whole virus vaccines in evoking an antibody response to A/NJ/76 in children and young adults. The civilian reaction study had utilized both a split and a whole virus vaccine and serum pairs were available from 42 persons who received the former and 57 who received the latter. There were persons under 30 years of age. Persons between 25 and 30 were included after it was shown that their response was similar to that of persons under 25. Persons over 30, responded far more favorably.

A comparison of the response to the two vaccines is shown in Table 13. The seroconversion rates and distribution of post-vaccination antibody titers after the whole virus vaccine are slightly lower than those observed with the same dose of vaccine

in the earlier trials. In contrast, the response to the split virus vaccine is far better and is in all respects as good as the response to the whole virus vaccine. With both vaccines the seroconversion rate was 60%, a figure comparable to that observed in military personnel who received vaccine of double the potency (400 CCA units). Relatively few persons showed markedly elevated titers.

The response to A/Victoria/75 was highly satisfactory (Table 12). Following the whole virus vaccine 96% of previously seronegative persons had titers of 16 or greater. With the split virus vaccine the comparable figure was 85%. The enhanced response, when compared to that observed with A/NJ/76, suggests that even these individuals who had titers of less than 8 before vaccination, had probably been infected over the past 8 years by some member of the A/Hong Kong family.

These observations suggest that it is unwise to pass judgement on the relative value of different methods of vaccine production on the basis of comparing single lots. Lot to lot variation has been notorious in the past and, even though potency standardization procedures have been markedly improved, the ultimate test for vaccines lies in their effectiveness in the human host.

(c) Reactions following vaccination

In the swine influenza vaccination program for personnel of the University of Colorado Medical Center and the Denver Veterans Administration Hospital, personnel were given either a split-virus bivalent vaccine (Parke-Davis Lot #913340A) or a whole-virus bivalent vaccine (Merck, Sharp and Dohme Lot #4835G) on an alternating basis. Both vaccines contained 200 CCA U each of A/NJ/8/76 and A/Victoria/3/75 per 0.5 ml dose. Vaccines were administered by jet-injector. Employees were asked to volunteer to complete a vaccine reaction form similar to that used earlier in the initial evaluation of A/NJ/76 vaccines at Lowry AFB. In addition, nursing personnel were asked to record oral temperatures at 8, 12, 24 and 48 hours after vaccination. Reaction forms were returned by 2117 of 3000 (71%) of employees at the UCMC, and by 950 of 1100 (88%) of employees at the DVAH.

The mass campaign, carried out during a 3-day period, did not adversely affect absenteeism, but the whole-virus vaccine caused significantly more local, systemic and febrile reactions than did the split-virus vaccine. Not unexpectedly, these differences were most pronounced in the younger age groups, i.e., personnel and students less than 25 years of age.

Of interest, the frequency of oral temperatures $\geq 37.5^{o}C$ among 220 nurses who received split-virus vaccines was 4.3%, 2.7%, 2.5%, and 2.1% at 8, 12, 24, and 48 hours, respectively; the corresponding figures among 232 nurses who received whole-virus vaccines were 4.2%, 15.1%, 12.6%, and 4.3% (Table 14). The differences at 12 and 24 hours were highly significant, but

had returned to "normal" levels at 48 hours. The febrile responses were concentrated in younger age groups; a significant frequency of temperature elevations did not occur in nurses ≥40 years of age, regardless of the vaccine received.

The vast majority of reactions were minor and easily tolerated; none were life-threatening or alarming.

(d) Persistence of antibody one year after vaccination

Twenty-five of the UCMC students who were vaccinated in 1976 had received vaccine containing A/PC/73, A/Scotland/74, and B/HK/72 one year earlier. Seventeen of these persons had shown an increase in antibody titer in tests with A/Victoria/75. When the prevaccination sera from these 17 persons were tested in 1976 all but one still had antibody for A/Victoria/75. In 2 instances the titer increased 2-fold; in 5 the titer was unchanged; in 8 there was a 2-fold drop; in 1 a 4-fold drop and in 1 an 8-fold fall in titer.

These data are consistent with earlier observations on the persistence of HI antibody following aqueous vaccines. They suggest that, if the rise in titer is sufficiently large, individuals may carry over enough antibody with their second year to provide some protection.

3) Further observations on recombinant vaccines

With the collaboration of Dr. Kilbourne a number of observations have been made on recombinant vaccines. Our initial interest was to determine the effectiveness of neuraminidase vaccines in producing antibody and in preventing clinical influenza. More questions have been raised than answers found.

We observed that when the recombinant X-42 (H Eq1 N_2) was put into humans it produced a good neuraminidase response and a bizarre HI antibody response. The parents of this recombinant were A/Equi 1 and A/PC/73 and it had subsequently been recombined with A/PR8/34 to obtain better growth in chick embryo. Only 14% of persons showed an increase in HI antibody in tests with X-42 or A Equi 1, which was the hemagglutinin of the vaccine strain. Sixty-seven percent of persons who were seronegative for A/PC/73 (one of the eliminated hemagglutinins) showed an increase in HI titer for A/PC/73. Forty-one percent showed an increase in HI antibody for A/PR8/34 (another eliminated hemagglutinin).

Subsequently we have noted, quite unexpectedly that certain A/NJ/76 vaccines produce a higher HI response to A/PR8/34 than to the vaccine strain A/NJ/76. This might be explained on the basis of the known relationship between swine and A/PR8/34 or by differences in avidity, but this explanation fails to account for numerous instances in which there has been no increase in HI antibody for A/NJ/76 coincident with a rise in A/PR8/34 antibody to levels as high as 1024.

Dr. Kilbourne has postulated that this HI factor is not associated with neutralizing capacity and we are in the process of investigating this hypothesis. In any event, if we are to rely on recombinant vaccines in future years, it is important to obtain a better understanding of these manufactured vaccines.

4. Summary

a) During an influenza A/Victoria/75 outbreak in the Denver area in February and March of 1976 the illness rate in vaccinated students at Lowry AFB was only 0.8%.

b) The attack rate varied inversely with the level of HI antibody for A/Victoria/75, with highest rates in persons having titers of 8 or less and almost no illness in those with titers of 64 or higher.

c) Overall febrile respiratory disease rates remained very low. Only 4 cases of adenovirus disease were detected throughout the period from 15 November 1975 to 15 May 1976.

d) In a trial designed to compare the vaccines prepared by four manufacturers, each at dosage levels of 200, 400 or 800 CCA units, the Merck, Sharp and Dohme whole virus vaccine was most effective. At 200 CCA units it produced seroconversion in 68% of persons while the other three products fell below 44%, at 400 CCA units the Merrell and Wyeth vaccines were more satisfactory; with seroconversion rates of 58% and 65%. These two vaccines failed to produce higher seroconversion rates with 800 CCA unit doses; the seroconversion with Merck, Sharp and Dohme vaccine at the 800 CCA unit dose rose to 78%.

e) Most of the reactions observed 24 and 48 hours after vaccination were minor, and none were alarming. Merck vaccine at the 400, and particularly at the 800 CCA U dose caused more fever and severe systemic reactions than did other vaccines at any of the doses tested.

f) HI antibody for A/NJ/76 in titers higher than 16 was not found except in individuals over 30 and a few younger persons with known pig contact.

g) A second injection of vaccine containing A/NJ/76 resulted in seroconversion in all of 48 vaccinees.

h) The 1976 formula military vaccine effected seroconversion for A/NJ/76 in 55% of recipients. Antibody levels for A/Victoria/75 and B/HK/72 were highly satisfactory.

i) In a civilian trial in the fall of 1976 a split virus vaccine (Parke-Davis) produced an antibody response comparable to that of a whole virus vaccine (Merck), but rates of local, systemic and febrile reactions were significantly higher with the whole virus vaccine.

j) The A/NJ/76 vaccines evoked an A/PR8/34 antibody response in a high proportion of recipients. With the Merck vaccine the proportions developing antibody and the antibody titers were higher for A/PR8/34 than to the A/NJ/76 strain.

k) An earlier recombinant, X-42 (H Eql N_2) from which the parent A/PC/74 and A/PR8/34 had presumably been eliminated produced an HI antibody response for the latter viruses in 67% and 40% respectively, of sero-negative persons.

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TABLE 1

Estimated attack rates of influenza among individuals with differing levels of HI antibody for A/Victoria/3/75

HI titer range	Percent with titers of	Estimated No.* with titers of	Observed number of cases	Estimated attack rate
8 or less	22	704	15	2.1
16 to 32	28	896	7	0.8
Over 64	50	1600	2	0.1
TOTAL	100	3200	24	0.8

*Based on extrapolation of the titers of the 107 persons tested to the whole population of 3200.

TABLE 2

Incidence of febrile respiratory disease in student population at Lowry Air Force Base, 1975-76

Week of	Rate/1000/week	Week of	Rate/1000/week
13 October	2.9	2 February	4.5
20 October	2.9	9 February	9.3*
27 October	5.2	16 February	12.3*
3 November	6.8	23 February	11.9*
10 November	7.1	1 March	7.1
17 November	5.8	8 March	9.3*
24 November	6.8	15 March	8.7
1 December	5.5	22 March	9.3
8 December	7.4	29 March	5.8
15 December	3.9	5 April	5.2
22 December	---	12 April	5.5
29 December	1.0	19 April	6.1
5 January	6.1	26 April	4.2
12 January	4.5	3 May	5.2
19 January	9.3	10 May	4.8
26 January	11.3	17 May	5.2
		24 May	6.5

*Weeks with largest number of cases of influenza.

TABLE 3

Source, type, potency and lot number of the 17 vaccines studied

Manufacturer	Vaccine Type	Potency (CCA)	Virus Strain	Lot Number
Merck Sharp and Dohme	W	200	A/NJ/8/76	Provided
	W	400	A/NJ/8/76	Under
	W	800	A/NJ/8/76	Code
Merrill National	W	200	A/NJ/8/76	Provided
	W	400	A/NJ/8/76	Under
	W	800	A/NJ/8/76	Code
Wyeth	S	200	A/NJ/8/76	Provided
	S	400	A/NJ/8/76	Under
	S	800	A/NJ/8/76	Code
Parke Davis	S	200	A/NJ/8/76	Provided
	S	400	A/NJ/8/76	Under
	S	800	A/NJ/8/76	Code
Merck Sharp and Dohme (Bivalent)	W	400 400	A/NJ/8/76 A/Victoria/3/75	4834G
Merck Sharp and Dohme (Bivalent)	W	200 200	A/NJ/8/76 A/Victoria/3/75	4335G
Parke Davis (Bivalent)	S	200 200	A/NJ/8/76 A/Victoria/3/75	913340A
Wyeth	S	500	B/HK/5/72	173701

TABLE 4

Incidence of HI antibody for A/NJ/76 and A/PR8/34 in pre-vaccination sera in different age groups

Age	Year of Birth	Exposed to Hemagglutinin	No. of Persons	HI antibody for A/NJ/76		HI antibody for A/PR8/34	
				No.	%	No.	%
17-19	1957-59	H_2	133	4*	3.0	14	10.5
20-29	1947-57	H_2, H_1	126	2	1.6	24	19.0
30-45	1931-47	H_2, H_1, H_0	13	6	46.2	11	84.6

TABLE 5

Relationship between age and level of HI antibody for A/PR8/34

Age	Exposure to Strains	No. of persons with titers of							
		<8	8	16	32	64	128	256	512
17-18	H_2	57	5	2	--	--	--	--	--
19-29	H_2, H_1	165	10	11	8	--	3	1	--
30-35	H_2, H_1, H_0	2	1	2	--	2	3	3	--

TABLE 6

Comparison of results of HI tests for A/NJ/8/76 with paired sera from groups of 17 to 24 persons under 25 years of age.

Numbers indicate percent of persons showing ≥ 4-fold increase in titer.

	Number of CCA units		
Manufacturer	200	400	800
Merck, Sharp & Dohme	68	68	78
Wyeth	17	65	50
Merrill-National	44	58	35
Parke-Davis	26	25	44

TABLE 7

Reactions observed among Recipients of A/NJ/76 vaccines
at Lowry Air Force Base, May 1976

Dose (CCAU)	Placebo	MSD			P-D			Wyeth			M-N		
		200	400	800	200	400	800	200	400	800	200	400	800
N=	21	20	24	24	22	21	21	22	21	21	20	20	18
Fever													
99.5-100	-	2	3	2	-	-	-	-	-	-	2	-	-
>100	1	-	2	2	1	-	-	-	-	-	1	-	1
Local	1	-	-	1	2	1	2	-	1	4	-	1	-
Systemic	1	4	8	15	5	1	2	1	1	3	3	2	6
+	-	2	2	3	5	1	1	1	1	-	-	2	2
++	-	2	4	6	-	-	1	-	-	3	1	-	2
+++	1	-	2	6	-	-	-	-	-	-	2	-	2

TABLE 8

Distribution of HI antibody titers for A/NJ/8/76 of 48 persons following first (Post-1) and second (Post-2) injection of vaccine.

	No. of persons with HI titers of									
Serum	<8	8	16	32	64	128	256	512	1024	% with titer 16 or more
Pre-	48	--	--	--	--	--	--	--	--	0
Post-1*	22	5	9	5	4	--	2	--	1	42
Post-2**	--	--	7	12	9	9	8	1	2	100

*Received any one of 12 experimental vaccines and were bled 3 weeks thereafter.

**Received second injection of vaccine 6 months later. Bled 2-3 weeks after vaccine was given.

TABLE 9

Comparison of post-vaccination HI antibody to A/NJ/76 and PR8 of persons under 25 years of age and seronegative before vaccination: (groups numbered 45 to 59 persons)

Source of Vaccine	Test Strain	% of persons with titers of: 8 or less	16-32	64-128	256-512	1024 or more	% with 4-fold rise
M.S.D.	A/NJ/76	28	48	11	9	4	72
	PR8	16	11	19	33	20	83
M.N.	A/NJ/76	58	22	11	4	4	40
	PR8	67	4	16	9	4	33
Wyeth	A/NJ/76	54	17	14	5	10	42
	PR8	78	8	7	5	2	22
P.D.	A/NJ/76	73	2	15	6	4	27
	PR8	67	13	8	4	8	33

TABLE 10

Distribution of pre- and post- vaccination HI antibody titers for A/NJ/76, A/Victoria/75 and B/HK/72 of 74 students at Lowry AFB who received standard military vaccine in November 1976. Most persons had previously received 1975 formula vaccine.

Test Strain	Serum Specimen	Percent with HI antibody titer of									% with 4-fold rise
		<8	8	16	32	64	128	256	512	1024	
A/NJ/76	Pre-	74	—	—	—	—	—	—	—	—	
	Post-	34	11	20	8	9	4	1	5	5	55
A/Victoria/75	Pre-	20	—	12	11	27	9	7	4	9	
	Post-	11	—	7	4	23	22	15	4	15	30
B/HK/72	Pre-	1	1	11	18	14	19	15	14	8	
	Post-	—	—	4	16	11	24	20	16	8	12

TABLE 11

Number of dispensary visits for febrile reactions on day following administration of standard military vaccine

Number Vaccinated	2897	
Number with fever of		
$99-99^8$	63	(2.2%)
$100-100^8$	65	(2.2%)
$101-101^8$	37	(1.3%)
$102-102^8$*	8	(0.3%)

*Includes 2 persons with temperatures over 103°.

TABLE 12

Comparison of pre-vaccination and post-vaccination HI antibody titers following injection of 1976 civilian vaccine containing 200 CCA units of A/Victoria/75 and A/NJ/76 in 75 unvaccinated students with those of 25 students vaccinated in 1975.

Test Strain	Vaccinated in 1975	Serum Specimen	Percent with HI antibody titer of									% with 4X rise
			<8	8	16	32	64	128	256	512	1024	
A/Victoria/75	0	Pre-	61	13	21	—	3	2	—	—	—	
		Post-	3	5	15	15	19	15	10	6	13	82
A/Victoria/75	+	Pre-	36	16	32	4	8	4	—	—	—	
		Post-	8	4	24	28	20	8	—	—	8	40
B/HK/72	0	Pre-	53	15	15	5	4	1	4	1	1	
		Post-	53	15	11	9	4	1	4	1	1	0
B/HK/76	+	Pre-	12	28	—	28	16	12	—	4	—	
		Post-	12	28	—	24	20	12	—	4	—	0

TABLE 13

HI antibody titers for A/NJ/76 of University of Colorado Medical Center students and technicians following administration of commercial split or whole virus vaccines containing 200 CCA units of A/NJ/76

Type of Vaccine	Specimen	Percent of persons with titers of									% with 4X rise
		<8	8	16	32	64	128	256	512	1024	
Split (P.D.) (42)	Pre-	100	—	—	—	—	—	—	—	—	
	Post-	36	5	12	5	17	12	5	5	5	60
Whole (M.S.D.) (57)	Pre-	100	—	—	—	—	—	—	—	—	
	Post-	28	12	19	9	12	5	5	4	5	60

TABLE 14

Febrile reactions to bivalent vaccines containing 200 CCA units each of A/NJ/8/76 and A/Victoria/3/75 in Nursing Personnel at the University of Colorado Medical Center and Denver Veterans Administration Hospital

A. Percent Fever ≥ 37.5°C by vaccine type

Interval	Split-virus vaccine (n= 220)	Whole-virus vaccine (n= 232)
8 hours	4.3	4.2
12 hours	2.7	15.1
24 hours	2.5	12.6
48 hours	2.1	4.3

B. Percent Fever ≥ 37.5°C by age group

Interval	≤ 25 (n= 64)	26-39 (n= 224)	≥ 40 (n= 163)
8 hours	7.7	5.0	1.8
12 hours	18.8	9.3	5.2
24 hours	14.1	7.8	4.7
48 hours	1.5	3.0	4.1

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