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A CLINICAL, EPIDEMIOLOGICAL AND IMMUNOLOGICAL EVALUATION OF VACCINATION AGAINST EPIDEMIC INFLUENZA ^{1, 2}

By

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the autumn of 1943 a field trial of the efficacy of vaccination against influenza was undertaken at the University of Michigan as part of a more extensive program conducted by the Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army. A brief account has been reported of the clinical results observed by the various investigating groups during the epidemic of influenza A that followed shortly after vaccination (1). The purpose of this report is to present details of the study at the University of Michigan, including clinical and epidemiological observations as well as laboratory studies. Full recognition has been

given, in the analysis of the data, to the need for a correlated clinical, epidemiological and immunological approach for a thorough understanding of the complex factors involved in immunity to influenza and a critical evaluation of the efficacy of vaccination.

MATERIALS AND METHODS

Vaccine. The vaccine employed (1) in the present study contained the viruses of influenza A and B recovered and concentrated (2) from the extra-embryonic fluids of infected chick embryos. The type A component included the PR8 (3) and Weiss (4) strains; for the type B component the Lee strain (5) was used. The Weiss strain, isolated from throat garglings of a patient seen in May, 1943, was included in the vaccine since preliminary study indicated distinct antigenic difference, when compared with the PR8 strain. The presence of this virus in the population at the time suggested the possibility that an outbreak during the winter months might be caused by a virus closely related antigenically to the one in circulation during the spring.

Control material consisted of physiological solution of sodium chloride and formalin and preservative in the concentrations present in the vaccine.

Subjects. The individuals participating in the study numbered 1,776 men in 8 companies of the 3651st Service Unit of the A.S.T.P. at the University of Michigan. The men ranged in age

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TABLE I
Distribution of men according to companies, places of residence and activities

Company	Strength *	Location of quarters	Location of mess	University studies
A	239	Dormitory A	Dorm. A	Language
B	34	Fraternity house 1	Union	Engineering
	30	2		
	33	3		
C	112	Dormitory B	Union	Language
	44	Fraternity house 4		
	45	5		
	40	6		
D	263	Dormitory A	Dorm. A	Language
E	328	Dormitory A	Dorm. A	Engineering
F	40	Fraternity house 7	Union	Language
	66	8		
	40	9		
	50	10		
	30	11		
G	282	Dormitory C	Dorm. C	Medicine
	37	Fraternity house 12	Dorm. C	Dentistry
Sta. Comp.	63	Private rooms and apartments	Union or Dorm. A	None

* At start of study.

from 18 to 47 years; 56 per cent were between the ages of 18 and 22, and 90 per cent between the ages of 18 and 32. The mean age of the group was 22.7 years.

The men in all but one of the companies were housed together in the university dormitories and fraternity houses. They slept in double-decker beds and in rooms occupied by 2 to 6 men. The strength of each company, places of residence and mess hall, and activities are shown in table 1.

Divided according to place of residence, 830 men (46.8 per cent of the total group) were quartered in one large dormitory, with 3 entrances but with common corridors and recreation rooms; 564 men (27.5 per cent) were distrib-

uted in 11 fraternity houses and a small dormitory; 319 men in Company G (15.9 per cent of the total group), were housed in a small dormitory and a fraternity house; and 63 (3.5 per cent) lived in private homes. Divided according to the places at which meals were taken, 48 per cent ate in one hall, 34 per cent in another, and the remaining 18 per cent in a third mess hall.

The activities of Companies A to G were such that the men in each were together for the greater part of the day, either in classes, study hall, dining hall, quarters, or at drill. Consequently their activities were largely those of a closed unit. In the course of the investigation only 35 men were lost to the study; 19 in the control, and 16 in the

ence and activities

tion of mess	University studies
A	Language
	Engineering
	Language
A	Language
A	Engineering
	Language
C	Medicine
C	Dentistry
or Dorm. A	None

vaccinated groups. These removals were scattered through the various companies and 6 in each group were moved out before the epidemic began. The mean weekly strengths have been used in calculating rates.

Vaccination. The same two lots of influenza virus vaccine employed by the other groups of investigators, each prepared by different manufacturers, were pooled in equal quantities immediately before use, and inoculations were carried out according to the schedule below. The men in each company were alphabetically and alternate

individuals were given subcutaneously 1 ml of vaccine or 1 ml of control solution. The men did not know which material they had received. Uninoculated individuals were not considered controls. The dates of inoculation and the number of vaccinated and control subjects in each company are shown in table 2. By following this plan, any variations between companies would be eliminated from influencing results, as might be the case if entire companies were vaccinated and others kept as controls.

At the time of inoculation, and again 2 weeks later, blood for serological study was obtained from approximately 10 per cent of the vaccinated subjects and 20 per cent of the controls. Every tenth vaccinated person and every fifth control, based upon alphabetical arrangement, was selected for the sample group. The same individuals were again bled at the conclusion of the study, several weeks after subsidence of the epidemic.

Records. The two standard record forms used by each of the groups of investigators were employed. These were: (a) a master card containing the identifying data and vaccination record of each subject and (b) an illness record

form. On the reverse side of the master card provision was made for summarizing the results of the laboratory tests.

Master cards were prepared in advance and the date of injection and material received were entered at the time of inoculation. The master file, containing the record of vaccination, was removed from the place of attendance at sick call (Student Health Service) and was not consulted until the study was completed. Clinical evaluations were made without knowledge as to whether the patient reporting to sick call was in the vaccinated or control group.

In the case of patients seen only in the dispensary, the forms used for recording the clinical data were completed daily from the "buck-slips." In the case of hospitalized patients, more detailed records of history were obtained and physical examinations were made. This information was supplemented by clinical and laboratory data recorded on the hospital chart.

Clinical observations. Sick call was held at specified times each morning in the Student Health Service. In addition, individuals who became ill at other times of the day or night either reported

TABLE 2
Schedule of inoculations

Dates	Companies	Number receiving	
		Virus vaccine	Control solution
10-25-43	Sta. Comp.	33	30
10-26-43	A	122	117
	B	48	49
10-27-43	C	120	121
10-28-43	D	132	131
10-29-43	E	163	165
11- 1-43	F	114	112
11- 2-43	G	156	163
Totals		888	888

unity houses and a small men in Company G (of the total group), were 11 dormitory and a fra- and 63 (3.5 per cent) homes. Divided accord- s at which meals were ent ate in one hall, 34 her, and the remaining third mess hall.

of Companies A to G he men in each were to- reater part of the day, study hall, dining hall, t drill. Consequently were largely those of a he course of the investi- men were lost to the control, and 16 in the

or were visited in quarters. Unless admitted to hospital, they were referred to sick call the following day. The great majority of the patients on sick call as well as at other times were seen by one of us (W. J. M., Jr.) in his capacity as contract surgeon. In addition all patients who were hospitalized were closely observed and followed by at least one of us. Because of the fact that all individuals with illness of any kind reported to the same dispensary, not only were those cases of respiratory disease requiring hospitalization observed, but the milder forms as well. In this respect, the cooperation of the officers and men was extremely good. Consequently, an opportunity was afforded for clinical and laboratory studies upon cases of respiratory disease that ordinarily do not come to the attention of physicians because of the absence of fever and mildness of symptoms. In the text, the latter are referred to as the dispensary group, as distinguished from the more typical cases in the hospitalized group.

Records were kept of the daily attendance at sick call, at which time temperatures were taken and brief notations made of the chief symptoms and prominent features of the examination. At the same time the physician's clinical impression was recorded, using the following terminology: influenza; local respiratory infection (nasopharyngitis, tonsillitis, pharyngitis, sinusitis, etc.); common cold (rhinitis); a few cases as pneumonia unrelated to the epidemic, (measles, infectious mononucleosis). The men were requested to return to sick call daily until symptoms improved or temperature fell below 99 F. In this way the duration of illness in the mild cases could be followed.

Except in a few instances, all men who were found to have a temperature of

100 or more were hospitalized. It was possible to study the latter group much more carefully. Histories and physical examinations were more detailed; temperatures were recorded every 4 hours; leucocyte counts were obtained at least once in almost all instances; and roentgenograms of the chest were made whenever indicated. The administration of antipyretics, but not analgesics, was withheld to avoid influencing the temperature response to infection. Sulfonamides were used only in the presence of frank bacterial pneumonia. The patients were visited daily while in the hospital and then followed in the dispensary if necessary.

For laboratory diagnosis, throat washings, taken during the acute phase of illness, and acute-phase and convalescent blood samples were obtained from all the hospitalized patients and from a sampling of those seen only in the dispensary.

Serological tests. For measuring the concentration of influenza antibody in serum the erythrocyte agglutination-inhibition reaction was employed (6). The procedure followed has been described (7). In selected instances the neutralization test in mice was used (8).

For the erythrocyte agglutination-inhibition tests a single pool of each antigen was used throughout. The histories of the strains since last ferret passage are as follows: PR8 (type A), 70 mouse passages, 717 tissue culture transfers and 43 egg passages; Weiss (type A), 32 mouse and 5 egg passages; Lee (type B), 137 mouse and 38 egg passages. In the neutralization test, the virus used was fresh mouse-passage material.

Tests for the presence of virus in throat washings. Throat washings were obtained as early as possible during the acute phase of illness by having the pa-

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ment gargle about 20 ml of beef heart
infusion broth in 2 or 3 portions. These
were then stored in stoppered tubes in
a dry-ice box. A variety of methods for
detecting the presence of virus was em-
ployed.

The usual methods for isolation of
virus in mice and ferrets were used.
However, the identification of the in-
fectious agent was hastened by subin-
oculation of suspensions of infected
ferret tissue into the allantoic sac of
chick embryos, and virus in the allan-
toic fluid of infected embryos was iden-
tified by the agglutination-inhibition
reaction using known antisera. In this
manner influenza A virus was isolated
from throat washings of two patients
before the epidemic became promi-
nent (4).

Attempts were made to detect and
identify virus by testing for the devel-
opment of type-specific immunity in
mice 2 to 3 weeks after intranasal in-
oculation with patients' throat wash-
ings. In order to accentuate the effect
of virus that might be present in their
lungs (9), the mice were again anes-
thetized and given an intranasal instil-
lation of sterile broth on the third day
following the inoculation of throat wash-
ings. In 5 of 50 trials immunity was
demonstrated in throat washings which
had been stored for 4 months.

An additional method employed,
which proved to be simpler and more
rapid, involved the inoculation of un-
treated throat washings directly into the
chorio-allantoic sac of chick embryos
(10). For this purpose the frozen
specimen of throat washings was al-
lowed to thaw slowly at room tempera-
ture until 2 to 3 ml of liquid appeared.
A sufficient quantity for inoculating 4
to 6 eggs with 0.1 ml was removed.
Since the fluid had been allowed to
freeze slowly and thaw slowly the ma-

terial that first separated upon thawing
represented a concentrate of the solutes
and particles present in suspension.
No effort was made to remove or kill the
bacteria in the inoculum. Embryonated
eggs in the tenth to twelfth days of de-
velopment were injected intra-allanto-
ically and then incubated for 2 days.
Allantoic fluid without erythrocytes was
aspirated from all eggs, including those
in which the embryo had died as a result
of the bacterial contamination. The
fluids were tested for the presence of
virus by mixing, in a small test-tube,
0.5 ml with an equal volume of a 0.25
per cent suspension of chicken erythro-
cytes, allowing the cells to settle at re-
frigerator temperature and then ob-
serving the pattern of the sedimented
cells. In many instances the presence
of influenza virus hemagglutinin was de-
tected by the latter procedure when the
usual method for observing agglutina-
tion grossly in a Petri dish was negative.
Upon subsequent passage the hemagglu-
tinin titer increased and the agglutina-
tion was promptly demonstrable grossly.
Certain of the more heavily contami-
nated fluids agglutinated the erythro-
cytes, as indicated by the pattern
of the cell sediment, but this could be
distinguished from the agglutination
pattern produced in the presence of
virus. In general, 5 serial passages
from a specimen of throat washings
were made in eggs and gross agglutina-
tion became apparent in the majority of
positives in the third and fourth trans-
fers; in two instances the gross agglu-
tination was seen in the first eggs in-
oculated. Bacteriostatic agents were
not used with any regularity for the
serial transfers since dilution of the in-
oculum 100 to 1,000 times usually re-
duced the concentration of bacteria
sufficiently to permit survival of the
embryo in the presence of bacterial

multiplication. Fluids from dead embryos were passed if the tube agglutination test was positive, except in instances in which decomposition had occurred, indicating early death. Ultimately, fluids were filtered to remove bacteria and to permit the isolation of virus in pure culture.

Virus was isolated from 8 of 18 specimens tested in this manner. In 3 others definite evidence of the presence of virus was obtained but the virus was not finally established in eggs. These results are in accord with those obtained by Rickard, Thigpen and Crowley (11).

RESULTS

Serological response to vaccination. The antibody responses of large groups of individuals to vaccination with a preparation similar to the one employed in this study have previously been made (12). The present vaccine differs in one respect from the vaccine used previously in that the type A component includes two strains, PR8 and Weiss, rather than PR8 alone.

For estimating the antigenic activity of the vaccine, blood for serological study was obtained from 82 men representing 9.2 per cent of the vaccinated group. The distribution of antibody titers measured by agglutination-inhibition for the 3 separate antigens, before and 2 weeks after vaccination, are shown in table 3.

It is readily evident that following vaccination antibody titers of the group were raised to levels above those of the majority of individuals before vaccination. With the PR8 strain of type A virus, antibody titers of 128 or above were observed in 22 per cent before vaccination and in 95 per cent after vaccination; with the Weiss strain of type A virus, titers of 256 or more were found in 26 per cent before vaccination

and in 88 per cent after vaccination; with the Lee strain of type B virus, titers of 128 or more were observed in 12 per cent before and 89 per cent after vaccination. The vaccine failed to evoke any demonstrable increase in titer to these antigens in 9 per cent, 10 per cent and 6 per cent of instances, respectively; while 4-fold or greater changes occurred in 70 per cent, 70 per cent and 83 per cent, respectively. The failures of response were essentially limited to individuals with high titers before inoculation. The increase in mean antibody titer of the group for the PR8 strain was 5.9-fold, for Weiss strain, 5.8-fold, and for Lee strain 8.7-fold.

It should be noted that, numerically, the mean antibody titers for the Weiss strain of type A virus, both before and after vaccination, were approximately twice those obtained with the PR8 strain. There are unknown factors which appear to influence the inhibition reaction and until these are understood it would be hazardous to accept, without reservation, the differences in the values obtained with the PR8 and Weiss strains as indicative of a difference in concentration of antibody for the respective antigens, particularly since the use of different lots of antigen of the same strain may result in different titers. This applies not only to the procedure herein employed but is noted in the method described by Hirst (13). For this reason a single preparation of antigen was used for each strain throughout the present study.

In the study conducted in 1942-1943, referred to above, in which a similar vaccine, containing only the PR8 and Lee strains of influenza virus, was tested for its capacity to induce a rise in antibody in human beings, no significant difference was apparent in the antibody responses to the type A and type

TABLE 3
Antibody response to vaccination

Antigen	Antibody titers*	Prevacc.		2 weeks post-vacc.		Relation of prevaccination titer to degree of antibody change						
		No.	Per cent	No.	Per cent	None	2X	4X	8X	16X	32X	64X+
PR8 (Type A)	<32	15	18	1	1	1	0	3	4	3	2	2
	32	16	20	0	0	0	0	4	5	5	1	1
	64	33	40	3	4	0	10	13	5	5	0	0
	128	14	17	21	26	3	7	3	0	1	0	0
	256	4	5	31	38	3	0	1	0	0	0	0
	512			15	18							
	1,024			9	11							
	2,048			2	2							
	Totals	82	100	82	100	Number 7	17	24	14	14	3	3
	Geom. Mean		52		308	Per cent 9	21	29	17	17	4	4
Weiss (Type A)	<32	7	9	1	1	1	0	1	3	2	0	0
	32	11	13	0	0	0	0	2	4	2	1	2
	64	19	23	1	1	0	2	2	5	4	3	3
	128	24	29	8	10	1	6	11	4	1	1	0
	256	12	15	16	20	2	5	3	1	1	0	0
	512	6	7	25	30	2	3	0	0	1	0	0
	1,024	3	4	17	21	2	0	1	0	0	0	0
	2,048			6	7							
	4,096			7	9							
	8,192			1	1							
Totals	82	100	82	100	Number 8	16	20	17	11	5	5	
Geom. Mean		100		576	Per cent 10	20	24	21	13	6	6	
Lee (Type B)	<32	26	32	1	1	1	0	1	4	8	6	6
	32	15	18	0	0	0	1	1	6	3	4	0
	64	17	21	2	2	0	1	5	8	2	1	0
	128	14	17	6	7	0	3	5	4	2	0	0
	256	7	9	24	29	2	4	1	0	0	0	0
	512	1	1	27	33	1	0	0	0	0	0	0
	1,024	2	2	16	20	1	0	1	0	0	0	0
	2,048			5	6							
	4,096			1	1							
	Totals	82	100	82	100	Number 5	9	14	22	15	11	6
Geom. Mean		51		440	Per cent 6	11	17	27	18	13	6	

* Measured by the agglutination-inhibition reaction.

B components of the vaccine. In the present study the mean increase in antibody titer for the type A viruses present in the vaccine was significantly lower than for the type B virus. The reason for this difference is suggested by a

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comparison of the potencies of the vaccines as determined by immunization tests in mice which indicated that the potency of the type A component of one of the lots included in the pool for vaccination was considerably substandard (14). It may be that the differences in serological response to type A in the 2 years is related to differences in potency of vaccine employed in the two studies.

The serological observations in the present experiments, extended for a period of 3 months, confirm the earlier findings (12) that the level of antibodies resulting from vaccination was essentially sustained over this interval. Since the titers to type A are influenced by the epidemic occurrence only the titers to type B can be properly interpreted. In this case, among 70 vaccinated individuals, 19 showed a twofold decrease in titer from that observed 2 weeks after vaccination; the remainder showed no change.

Reactions to administration of vaccine. Information concerning reactions resulting from the injection of vaccine was obtained from the record of attendance at sick call and from surveys made in the barracks 24 hours after inoculation.

A total of 50 men (1.46 per cent of the vaccinated group) reported to sick

call with complaints referable to the injection. All had received the virus vaccine. Headache, generalized aching and chilliness were the most prominent complaints. Temperatures of 100 or more were observed in 9; the highest temperature was 101.5. Symptoms generally began 6 to 12 hours following the inoculation and persisted for not more than 12 hours.

In the surveys made in the barracks, 624 men were seen. Of these 312 had received the vaccine and 312 the control material. No reactions were observed in any of the individuals who had received the control inoculation. The tabulation of reactions among the vaccinated group is shown in table 4.

The systemic symptoms recorded during the survey of the barracks were not of sufficient severity or duration to induce the men to report to sick call. The local reactions consisted of tender areas of redness and induration. These varied in size from just visible redness to areas of 40 to 50 millimeters in diameter. They were similar to the reactions noted in the studies of vaccination in 1942-1943 (12).

Epidemiological observations. A study of the incidence and nature of respiratory disease occurring in the A.S.T.P. unit at the University of Michigan was begun before the groups were vaccinated. The number of afebrile colds and other types of localized infections was not unusual. The earliest cases of influenza were diagnosed 10 days after completion of the vaccination program. The first patient with symptoms suggestive of influenza appeared at sick call on November 10th, and another who warranted a definite diagnosis was seen on November 12th. Not until November 15th did several cases appear daily. It is interesting to note (figure 1) that during the 3-week period end-

TABLE 4

Reactions following vaccination recorded during survey in barracks 24 hours after inoculation

Company	Number of vaccinated individuals seen	Systemic symptoms		Local reactions	
		No.	Per cent	No.	Per cent
C	101	37	37	83	83
F	65	22	34	45	69
G	146	87	60	100	68
Totals	312	146	48	228	73

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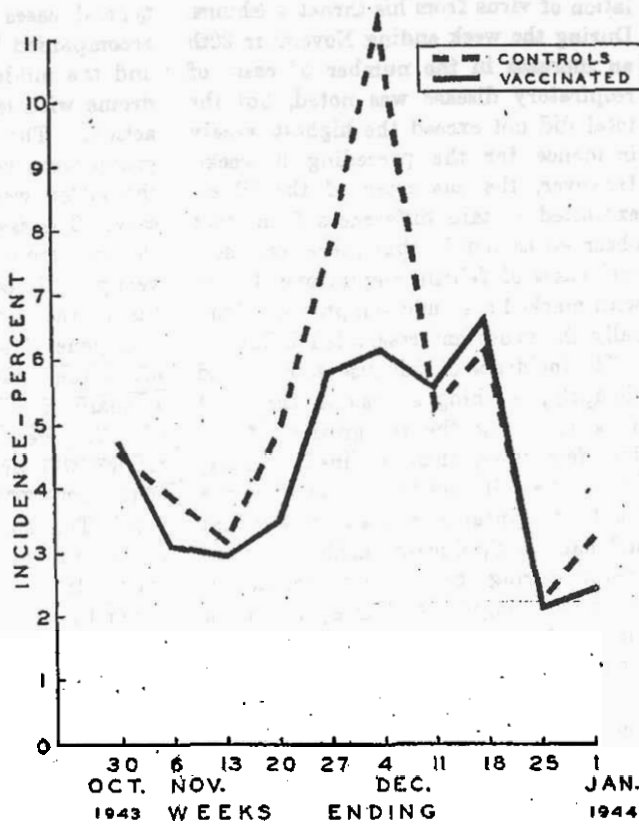


FIGURE 1. Weekly incidence of respiratory disease in the University of Michigan A.S.T.P. unit, from October 25th, 1943, to January 1st, 1944.

ing November 13th, a gradual decline in total incidence of respiratory disease was observed, reaching a low point in the week ending November 18th although, as was subsequently demonstrated, the virus of influenza A had appeared in the population during that period. There is serological evidence to indicate that the virus was in circulation before it became evident clinically or epidemiologically. Of the group of 134 persons who were bled before and 2 weeks after inoculation with salt solution, one individual exhibited an increase in antibody titer to the type A virus, but no change in type B anti-

body titer. This individual had not reported to sick call at any time, suggesting that a subclinical infection had occurred between October 28th and November 4th. Thus, the virus was in circulation for at least 1 week before it became evident clinically and about 2 weeks before an epidemic was apparent. That influenza was prevalent in other locations at the same time was suggested by the clinical diagnosis of influenza on November 12th in a man who developed symptoms en route to Ann Arbor after 5 days at Fort Sheridan. Confirmation of the diagnosis was subsequently obtained by serological means and by iso-

lation of virus from his throat washings. During the week ending November 20th an increase in the number of cases of respiratory disease was noted, but the total did not exceed the highest weekly incidence for the preceding 3 weeks. However, the character of the illness exhibited certain differences from that observed earlier in that there were several cases of febrile respiratory disease with marked systemic symptoms. Clinically the syndrome resembled influenza.

The incidence of this disease increased abruptly, reaching a peak in the third week following the recognition of the first few cases, then declined sharply, only to be followed by a secondary rise due to its appearance in a company that had had no significant incidence of influenza during the major prevalence in the other companies. After the week ending December 18th, cases appeared sporadically until early January. The last case was seen on January 13th in a man who was exposed while on furlough in Wisconsin.

Coincident with the increase in incidence of influenza there occurred a rise in the number of cases diagnosed as colds. After the onset of influenza the curve describing the incidence of colds tended to parallel the curve of incidence of influenza and reached a peak at the same time. This suggested that a proportion of the colds were mild forms of influenza. On the other hand the incidence of cases diagnosed as local respiratory infections was not strikingly influenced.

Clinical observations

The general clinical pictures of the respiratory diseases prevalent during the period of study and the diagnostic criteria employed were as follows:

Influenza. As stated earlier, a distinction was made between the more severe,

typical cases of influenza which were accompanied by fever of 100 F or more and the milder forms of the same syndrome with lesser degrees of febrile reaction. The patients in the former group were hospitalized, while those in the latter were treated in the dispensary. The essential distinction between the two groups was based upon the severity of illness, one index of which was the degree of fever.

In general, symptoms began abruptly; often the exact hour could be stated. In many, the sudden onset of symptoms of fever, generalized aching and prostration was preceded by a dry, hacking cough with retrosternal tickling or rawness. The cough was the most constant complaint referable to the respiratory tract. In some, dryness or a raw feeling of the throat was the complaint, and in a small number, nasal symptoms were present. In one instance the illness began abruptly with nausea and vomiting accompanied by slight nasal irritation but with no other symptoms of respiratory tract involvement. The picture was chiefly that of an infection of the intermediate portions of the respiratory tract with marked systemic reaction.

Physical examination at the onset of illness generally revealed varying degrees of prostration, flushed skin and little evidence of infection in the respiratory tract other than some enlargement of the lymphoid follicles on the posterior pharyngeal wall, some injection of the vessels and congestion of the nasopharyngeal mucosa. In a few instances examination of the chest by auscultation revealed inconstant clicks in localized areas.

On the average, the duration of illness among hospitalized patients, as measured by the persistence of temperature of 99 or more, was 2 to 4 days.

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fluenza which were of 100 F or more. Some of the same syndromes were observed in the former group, while those in the latter were distinguished in the dispensary. The distinction between the two groups was based upon the severity index of which was

onset of symptoms was abrupt, and could be stated by a dry, hacking cough and nasal tickling or rawness. The most constant complaint was the respiratory distress or a raw feeling in the throat, and nasal symptoms were present. In one instance the illness was accompanied by slight nasal involvement. The clinical picture was that of an infection in the upper portions of the respiratory tract with marked systemic

involvement at the onset of illness. The fever revealed varying degrees of intensity, flushed skin and tachycardia in the respiratory group. In some enlarged lymphoid follicles on the pharyngeal wall, some injected conjunctiva and congestion of the nasal mucosa. In a few instances rattling in the chest by rales and inconstant clicks.

The duration of illness in hospitalized patients, as determined by the persistence of temperature, was 2 to 4 days.

Weakness was present for variable periods of time and in one instance the patient was readmitted for "post-influenza asthenia." Cough persisted for several days to several weeks. Pneumonia was seen as a complication in 4 cases. The distribution of leukocyte counts taken within a day or two of onset of illness in 89 hospitalized patients was as follows: 12 per cent were less than 5,000, 52 per cent were between 5,000 and 8,000, 30 per cent were between 8,000 and 11,000, and 6 per cent were between 11,000 and 14,000. There was no evidence of any difference in distribution of leukocyte counts in the 70 controls and 19 vaccinated individuals included in this tabulation.

The clinical picture presented by the patients included in the dispensary group in whom a diagnosis of influenza was made, apart from the severity of symptoms and the degree of febrile reaction, resembled the syndrome described above. In approximately 60 per cent of this group, temperatures at sick call were not above 98.8 F. Most of the remainder exhibited a temperature between 98.8 and 100 F on only 1 day. In a few, temperatures of 99 F or more persisted as long as 3 days. Three vaccinated and 6 control subjects with temperatures which reached above 100 F were confined to quarters and, since less completely studied, have been considered in the dispensary group, although more properly comparable with the hospitalized influenza patients.

Local respiratory infection. The diagnosis of local respiratory infection was reserved for those cases presenting evidence of more or less localized infection, such as sinusitis, pharyngitis, tonsillitis, etc. Only 4 such patients were hospitalized because of the presence of a temperature of 100 F or more; the majority of patients with this diag-

nosis were seen only in the dispensary. In general, they did not complain of systemic symptoms.

Common colds. In this category were included those cases in which the symptoms and signs of infection were chiefly those of rhinitis. Here again, fever and systemic symptoms were absent at the time the diagnosis was made.

Comparison of incidence of respiratory disease in control and vaccinated subjects

In the preliminary report (1) of the clinical evaluation of subcutaneous vaccination against influenza was included a comparison of the incidence of hospitalized cases of influenza in control and vaccinated groups under observation at the University of Michigan. In the present report the incidence of all forms of respiratory disease is considered, including the milder cases studied in the dispensary as well as those hospitalized. The data first to be considered are based entirely upon clinical diagnosis, without reference to the results of etiological studies carried out in the laboratory.

The weekly incidence of all forms of respiratory disease in control and vaccinated groups throughout the period of observation from October 25th, 1943, to January 1st, 1944, is shown in figure 2 and table 5. During this period, a portion of which was occupied by the epidemic, the cumulative incidence of all forms of respiratory disease in the unvaccinated group was 51.9 per cent, while in the vaccinated half of the population the incidence was 43.1 per cent. Considering only the period from November 7th to January 1st, in which the virus of influenza was known to be in operation, the incidence of respiratory infection among controls and vaccinated was 43.6 per cent and 35.3 per cent, re-

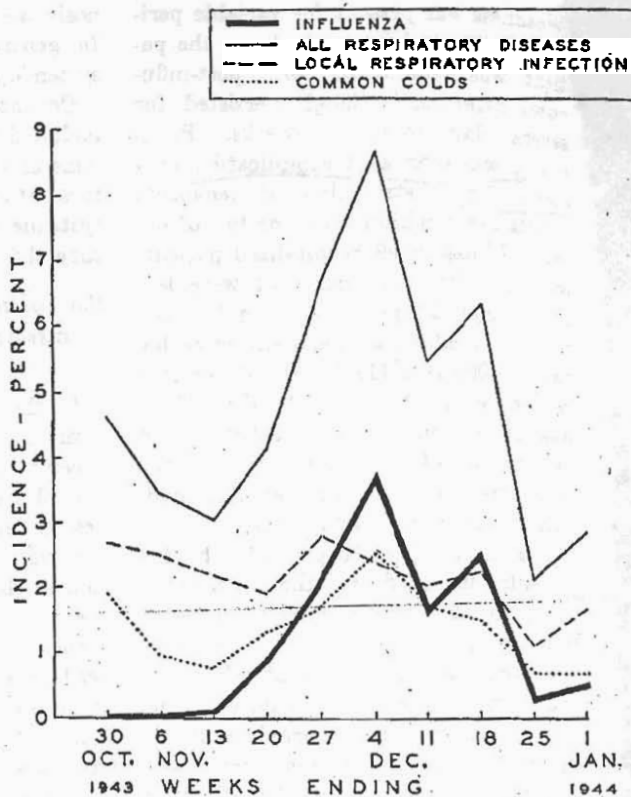


FIGURE 2. Weekly incidence of respiratory disease in the population under study.

spectively. When the clinical data are divided into the several diagnostic categories it is seen, in figure 3 and table 5, that the difference between vaccinated and controls was quite evident in the groups of cases with the diagnosis of influenza; whereas no significant differences were evident between controls and vaccinated when the diagnosis was not influenza.

It should be noted that in the groups of cases diagnosed clinically as influenza the greatest difference in incidence between the test groups occurred among those who experienced the most severe infections; i.e., the hospitalized group. There were 75 cases in the control group, an incidence of 8.58 per cent,

and 20 cases in the vaccinated group, an incidence of 2.27 per cent. Thus, 79 per cent of the cases occurred in the control group. A significant but less striking difference was evident in the group of 112 patients considered to have influenza but treated in the dispensary. Of these, 70 (62.5 per cent of the total) were in the control group, an incidence of 8.04 per cent, and 42 in the vaccinated group, an incidence of 4.78 per cent.

Roentgenograms of the chests were done in 28 of the 75 controls and 11 of the 20 vaccinated individuals who were hospitalized. Clinically and roentgenologically, pneumonia was seen as a complication of influenza in 4 of the con-

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lation under study.

the vaccinated group, 2.27 per cent. Thus, 79 cases occurred in the A significant but less was evident in the patients considered to be treated in the dispensary (62.5 per cent of the control group, an incidence of 4.78

s of the chests were 75 controls and 11 of individuals who were roentgenologically and roentgenologically was seen as a complication in 4 of the con-

EVALUATION OF INFLUENZA VACCINE

TABLE 5
Weekly incidence of respiratory disease in control and vaccinated groups

Week ending	Mean strength		Influenza						Local respiratory infection		Common cold		Total respiratory disease			
	Cont.	Vacc.	Hospitalized cases			Dispensary cases			Cont.	Vacc.	Cont.	Vacc.	Cont.	Vacc.		
			No.	Per cent	No.	Per cent	No.	Per cent								
10-30-43	888	888	0	0.00	0	0.00	0	0.00	0	0.00	3.04	2.63	1.46	2.36	4.50	4.99
11- 6-43	884	884	0	0.00	0	0.00	0	0.00	0	0.00	2.83	2.15	1.02	0.90	3.85	3.05
11-13-43	882	882	2	0.23	0	0.00	0	0.00	0	0.00	2.72	1.70	0.33	1.25	3.28	2.95
11-20-43	874	878	9	1.03	0	0.00	3	0.34	3	0.34	1.95	1.94	1.49	1.25	4.81	3.53
11-27-43	874	877	20	2.29	7	0.80	4	0.45	4	0.45	2.86	2.74	1.60	1.82	7.55	5.81
12- 4-43	873	876	35	4.00	1	0.11	10	2.18	10	1.14	2.41	2.40	2.63	2.51	11.22	6.16
12-11-43	871	876	4	0.46	7	0.80	13	1.48	5	0.57	1.61	2.51	1.72	1.71	5.27	5.59
12-18-43	869	873	4	0.46	5	0.56	20	2.32	14	1.60	2.19	2.41	1.04	2.06	6.01	6.63
12-25-43	869	872	0	0.00	0	0.00	3	0.34	3	0.34	1.27	1.03	0.58	0.80	2.19	2.17
1- 1-44	869	872	1	0.11	0	0.00	5	0.58	3	0.34	1.73	1.61	0.81	0.46	3.23	2.41
11-13-43 to 1- 1-44 incl.	875	878	75	8.58	20	2.27	70	8.04	48	4.78	16.74	16.34	10.20	11.86	43.56	35.25

Epidemic period

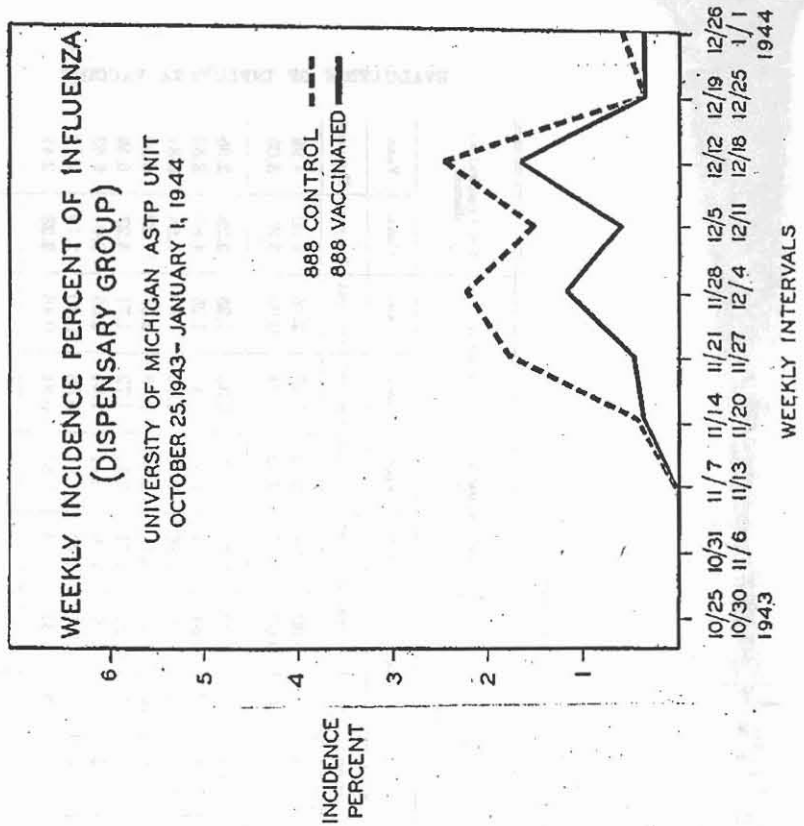
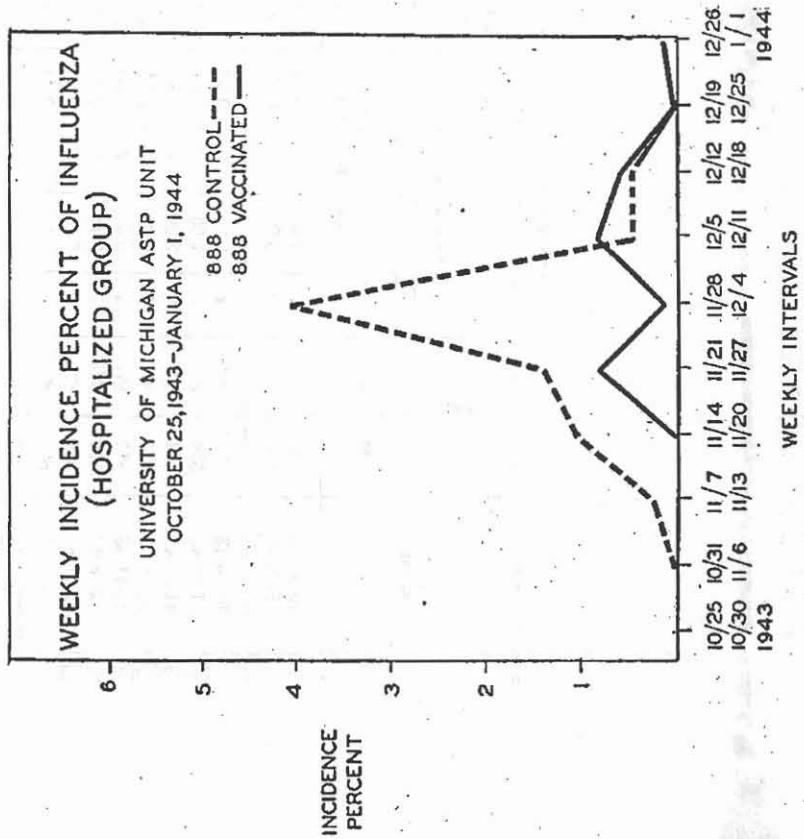


FIGURE 3. Weekly incidence, among controls and vaccinated individuals, of (a) influenza admitted to hospital; (b) mild influenza seen in the dispensary; (c) local respiratory infections; and (d) common colds.

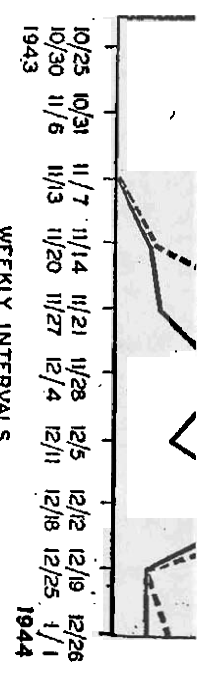
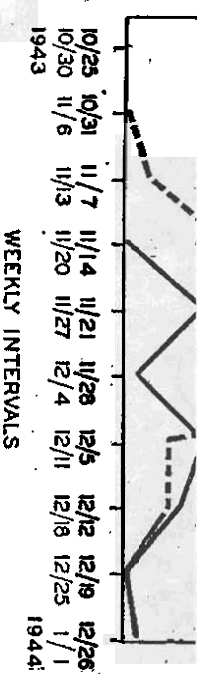


FIGURE 3. Weekly incidence, among controls and vaccinated individuals, of (a) influenza admitted to hospital; (b) mild influenza seen in the dispensary; (c) local respiratory infections; and (d) common colds.

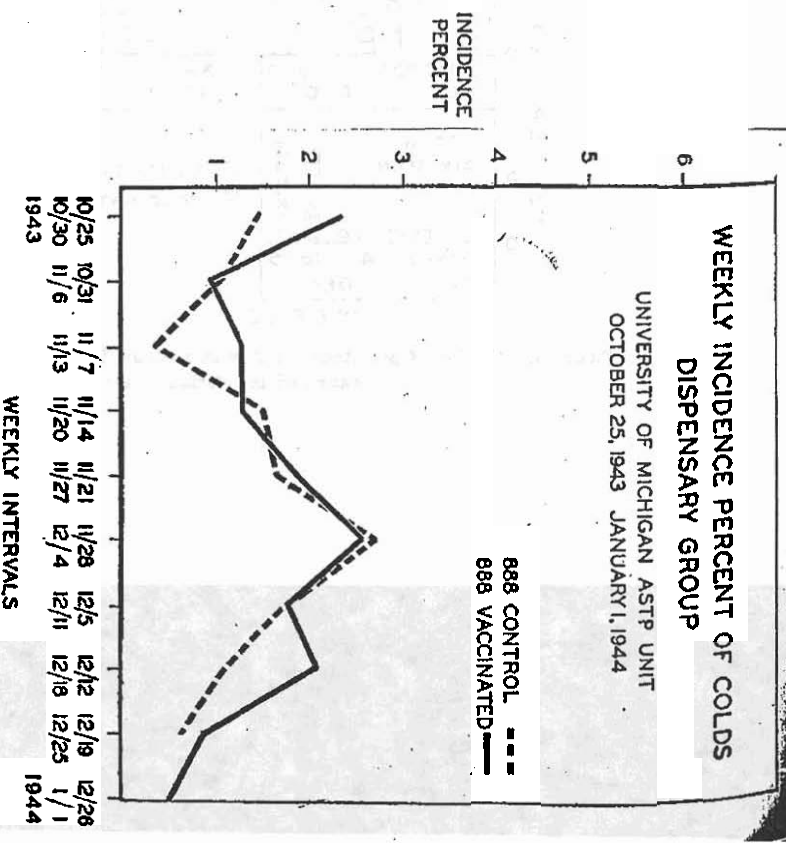
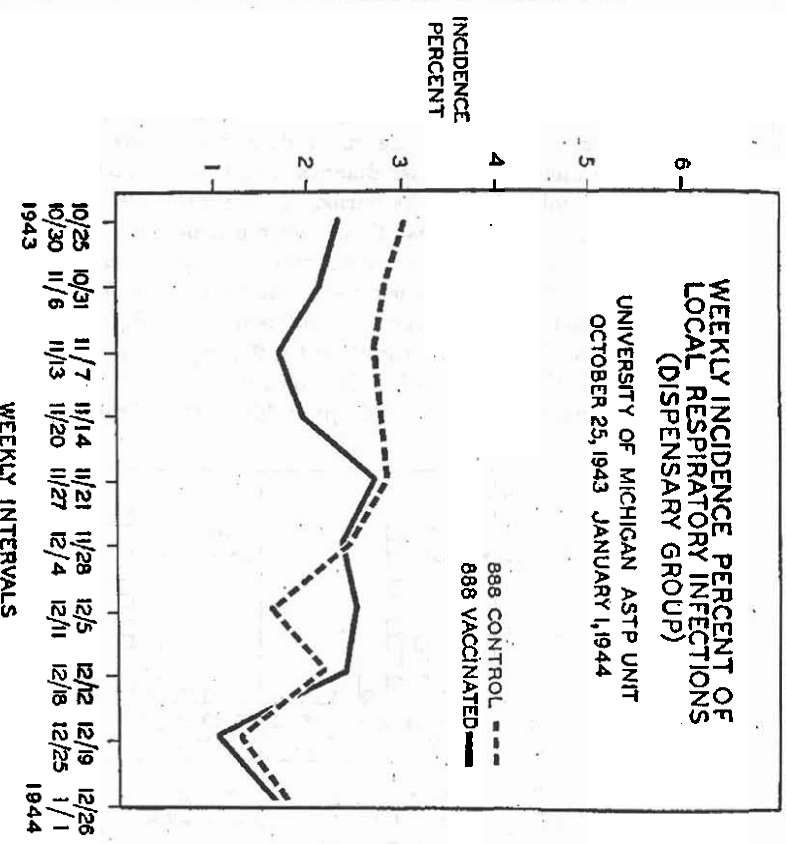


FIGURE 3. Weekly incidence, among controls and vaccinated individuals, of (a) influenza admitted to hospital; (b) mild influenza seen in the dispensary; (c) local respiratory infections; and (d) common colds.

trols and in none of the vaccinated patients.

It is clear from figure 3 and table 5, showing the weekly incidence of local respiratory infections, that no significant increase in the number of these ailments occurred during the influenza prevalence. Furthermore, there was no significant difference between vaccinated and control groups. However, from the shape of the curve describing the incidence of cases diagnosed as common colds during the period of observation, it would appear that a certain proportion of these cases occurring during the influenza outbreak were due to infection by the virus of influenza A. Since there was no significant difference in incidence of colds in control and vaccinated groups, it is probable that

equal proportions of infections so diagnosed but etiologically due to influenza virus were present in both groups.

Although every effort was made in setting up the study to reduce the number of variables to a minimum, the influence of certain factors could not be estimated until the epidemic was in progress. An analysis of the number of persons hospitalized for influenza in the different companies has revealed considerable variation and confirms the value of the arrangement of the study, whereby equal numbers of controls and vaccinated persons were included in each company and each place of residence, rather than vaccinating all in one group and using another for control. It is seen from the data given in figure 4 that the outbreak of influenza

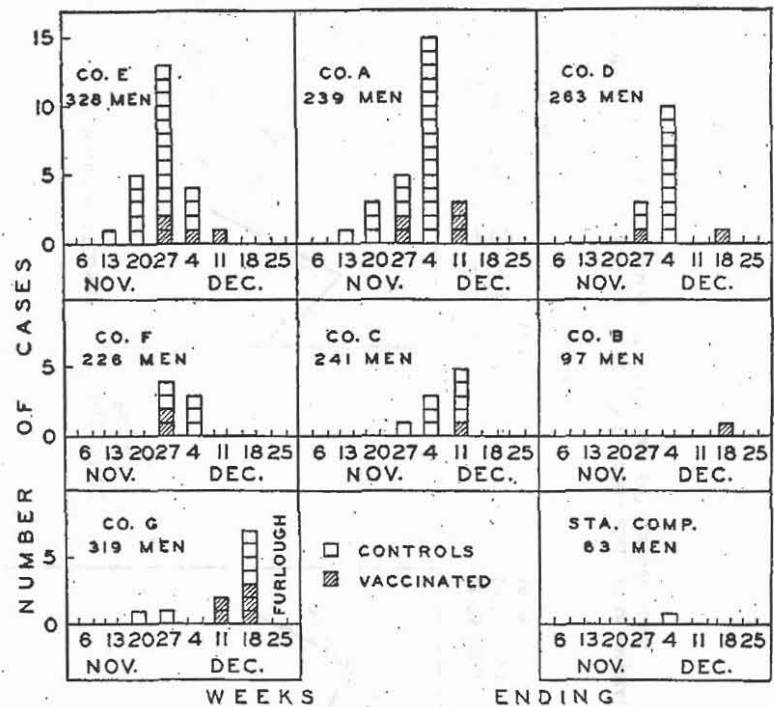


FIGURE 4. Number of hospitalized influenza patients in control and vaccinated groups, in each company and in relation to the epidemic period.

TABLE 6
Cumulative incidence of respiratory disease among controls and vaccinated in companies grouped according to residence

Diagnosis	Group I*		Group II*		Group III†	
	Control (410) ‡ (per cent)	Vaccinated (414) (per cent)	Control (301) (per cent)	Vaccinated (308) (per cent)	Control (163) (per cent)	Vaccinated (155) (per cent)
Influenza (hospital)	13.39	2.66	4.65	1.31	3.70	3.23
Influenza (dispensary)	8.57	6.05	8.96	3.55	4.34	3.24
Local respiratory infection	20.77	17.21	15.89	17.83	6.16	9.69
Common cold	11.24	14.30	10.62	11.99	6.77	5.82
Total respiratory disease	53.97	40.22	40.12	34.78	20.97	21.95

* Figures include observations during interval of November 7th, 1943 through January 1st, 1944.

† Figures include observations during interval of November 7th, 1943 through December 18th, 1943, at which time group III was given a furlough.

‡ Figures in parentheses denote mean strength.

was first recognized in Company E, reached a peak at the end of the second week (November 20th to 27th) and declined at the same rate at which it increased, with occasional cases occurring in the last 2 weeks of the 5-week prevalence. The disease appeared in Company A at approximately the same time it was recognized in Company E, but did not reach a peak until a week later (November 28th to December 4th). Although cases did not begin to appear in Companies D, C and F until a week after they were first noted in Companies E and A, the peak coincided in time with that of Company A. The data showing the case incidence of influenza in Company G were somewhat at variance with the others. Although occasional cases of influenza occurred during the period of prevalence in the rest of the population, not until the general incidence of disease was sharply declining was there a significant increase in incidence in Company G. At this point the men in this company went on furlough. Apart from variations in the epidemic picture in the different companies, figure 4 also reveals the varia-

tions in the attack rates among controls and vaccinated in the different companies.

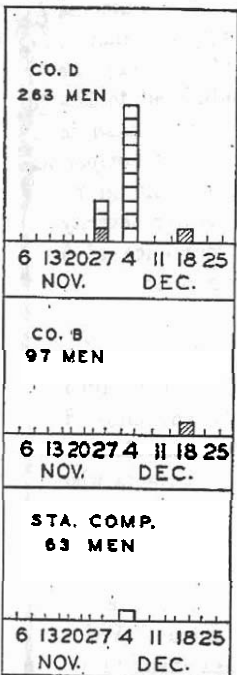
It is readily apparent that the incidence of influenza was significantly greater in Companies E, A and D. From the differences in appearance of the data shown in figure 4, the companies seem to fall into 3 groups (table 6).

Group I, with the highest incidence of illness, includes Companies A, D and E. The 830 men in this group were housed in dormitory A and they took their meals in the dining hall in the same building.

Group II, with a lower incidence includes Companies B, C, F and station complement. The 627 men in this group were quartered in 11 fraternity houses in groups of 30 to 66 and in dormitory B housing 112 men; the 63 men in the station complement lived individually in rooming houses or apartments. All of the men in this group were served their meals in the cafeteria of the Union.

Group III is one company of 319 men composed of medical and dental students. The 282 medical students were

ortions of infections so diagnosed etiologically due to influenza present in both groups. Every effort was made in the study to reduce the number of variables to a minimum, the influence of certain factors could not be eliminated until the epidemic was over. An analysis of the number of men hospitalized for influenza in the different companies has revealed considerable variation and confirms the value of the arrangement of the study. Equal numbers of controls and vaccinated persons were included in each company and each place of residence. The results show that the incidence of influenza was higher in the control than in the vaccinated groups in every company and using another control group in Company G. As seen from the data given in figure 4, the epidemic picture in the different companies at the outbreak of influenza



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l and vaccinated groups, in each 3 period.

quartered in dormitory C at some distance from the main campus and took their meals in the same building. The 37 dental students lived in a fraternity house but were served their meals with the medical students.

A comparison of the 3 groups, shown in table 6, suggests that housing of large numbers of men together may have influenced the incidence of influenza. In group I it is likely that greater opportunity for continuous exposure was had in a single large building with common corridors and recreation rooms, in contrast to group II in which the opportunity for exposure was reduced by the distribution of men in smaller groups living in widely separated quarters. The course of the epidemic in group III could not be ascertained since the men in Company G were given a furlough on December 18th, at the time the epidemic was apparently mounting. Direct questioning shortly after return on December 28th elicited no evidence of additional illness among the men during the furlough. While this was somewhat surprising it indicates that a group on furlough cannot be considered comparable to groups in residence under usual conditions and suggests that dispersion of the group may have averted a sharp outbreak.

It is also seen in table 6, that the effect of vaccination was most marked in group I in which the total incidence, of hospitalized cases was greatest. Attack rates of 13.4 per cent and 2.7 per cent were observed among control and vaccinated individuals, respectively.

Analysis of the data in terms of the smaller subdivisions, therefore, tends to give a somewhat different view of the epidemic than when it is considered in the composite sense. With some groups the temptation arises to suggest that vaccination of half the unit had re-

sulted in almost complete protection of the entire group. The question also arises whether the incidence observed in the total control group is representative of that which might be expected in an entirely untreated population.

In this connection it is of interest to note the degree of illness encountered in a company of the enlisted reserve corps of the A.S.T.P., none of whom was vaccinated. Nevertheless, careful observations were made, since these men were seen at sick call together with those in the study and were not distinguished in any way except at the time of compiling the records. During the interval of November 21st to December 18th, 20 of the 104 men in this company were hospitalized with a diagnosis of influenza. Blood for serological study was obtained from 18, and all but two showed serological evidence of influenza A infection. Thus, it appears that in this totally unvaccinated company the incidence of influenza admitted to hospital was considerably higher than in any of the other companies of comparable size. The disease in this group appeared also to be of greater severity as evidenced by the occurrence of 5 cases of pneumonia among them.

Clinical and serological correlations

Throughout the period of observation blood for serological study was obtained during the acute and convalescent phases of illness from all patients who were hospitalized and from a random sample of patients seen in the dispensary. The acute and convalescent sera were compared with respect to titer of antibody for both the PR8 and Weiss strains of type A influenza virus in an effort to establish an etiological diagnosis. The results of the serological studies in the various clinical groups

TABLE 7

Correlation between clinical diagnosis and results of serological tests in control and vaccinated subjects

Diagnosis	Group	Total number of patients*	Number of patients studied serologically	Increase in antibody titer for Type A influenza virus in convalescence											
				Weiss strain						PR8 strain					
				4-fold		2-fold		None		4-fold		2-fold		None	
				No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Influenza (hospitalized)	Control	75	75	65	87	2	3	8	10	42	56	18	24	15	20
	Vacc.	20	20	10	50	5	25	5	25	5	25	2	10	13	65
Influenza (dispensary)	Control	70	62	37	60	3	5	22	35	25	40	11	18	26	42
	Vacc.	42	34	5	15	1	3	28	82	2	6	3	9	29	85
Local resp. infection (dispensary)	Control	146	24	6	25	1	4	17	71	2	8	3	12	19	80
	Vacc.	143	24	2	8	0	0	22	92	0	0	2	8	22	92
Common cold (dispensary)	Control	89	15	6	40	0	0	9	60	4	27	1	6	10	67
	Vacc.	105	21	4	19	0	0	17	81	1	5	2	10	18	85

* Total number of patients seen between November 7th, 1943, and January 1st, 1944.

are presented in table 7 and figure 5. The findings in control and vaccinated subjects are shown separately. No positive serological tests to type B influenza virus were obtained.

Several trends are revealed: (a) a high proportion of positive serological reactions in the groups of cases in which a clinical diagnosis of influenza was made. The proportion of positive serological reactions was highest in the hospitalized influenza group; (b) a greater proportion of positive serological reactions in control than in vaccinated subjects; (c) greater sensitivity of the Weiss than of the PR8 strain in revealing quantitative changes in the agglutinin-inhibition titers of acute-phase and convalescent sera.

The relative reactivity of the Weiss and PR8 antigens used in the agglutination-inhibition test for serological diagnosis of influenza A are shown in

table 7. Except in the hospitalized group, no significant differences in the percentage of positive serological reactors is revealed by the two antigens, when a twofold or greater change in titer is considered. However, when a positive reaction is considered to be a 4-fold or greater increase in antibody titer in convalescence, then the Weiss antigen appears to be more sensitive. Parallel variations in serological response to the respective strains were not encountered in a small number of sera studied by the neutralization test in mice. That the PR8 and Weiss strains are different antigenically, although both are strains of type A influenza virus, has been demonstrated by cross immunization tests in mice and cross serological tests in ferrets and rabbits. These observations support those of Magill and Sugg (15) which indicated a need for consideration of antigenic dif-

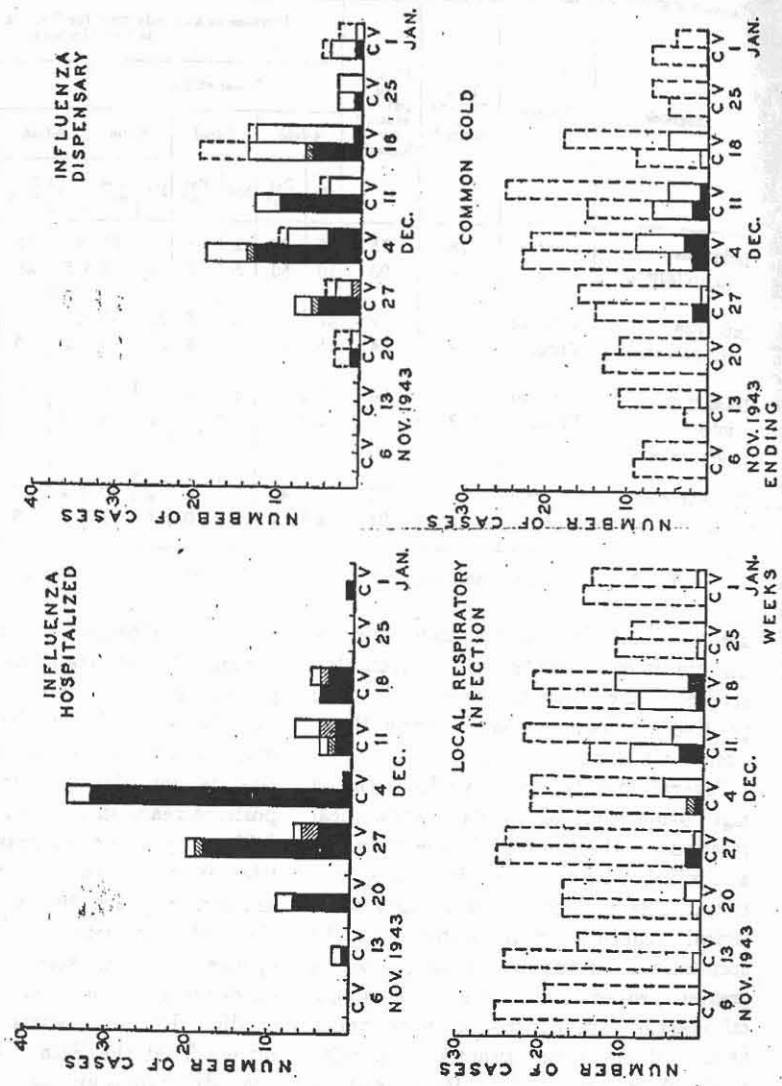


FIGURE 5. Correlation between clinical diagnosis and results of serological tests in control and vaccinated individuals, in relation to the epidemic period. The dotted lines indicate the number of cases in which blood for serological study was not obtained. The solid black column indicates that a 4-fold or greater increase in antibody titer was observed when acute-phase and convalescent sera were tested with the Weiss strain of type A influenza virus in the agglutination-inhibition test; the shaded portions of the columns indicate two-fold increases; and the white areas indicate no change. The letters C and V, beneath each column refer to controls and vaccinated individuals, respectively.

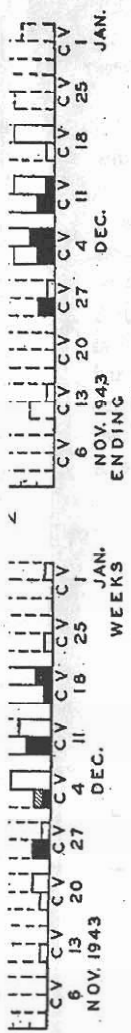


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ferences among strains of the type A group of influenza viruses in serological diagnosis.

The findings recorded in table 7 and figure 5 indicate that among hospitalized patients, from all of whom blood was obtained, serological data agreed with the clinical diagnosis in 90 per cent of the cases in the control group and 75 per cent of the cases in the vaccinated group, when a reproducible twofold change in titer is taken to indicate a positive serological reaction. It is likely, particularly among the vaccinated subjects, that many of the cases in which serological confirmation of the clinical diagnosis of influenza was not obtained were due to influenza virus infection without demonstrable serological response. This phenomenon has been pointed out previously (16-19). Moreover, virus was isolated from throat washings of one vaccinated individual who exhibited no change in antibody titer. The fact that there was no significant change in the number of serologically negative cases at different intervals throughout the epidemic period (figure 5) and the high order of correlation between clinical and laboratory diagnosis suggest that few, if any, cases of other etiology were included in the group of patients hospitalized with a diagnosis of influenza.

The dispensary group with a diagnosis of influenza comprised 112 individuals in 96 of whom serological tests were made. Sixty-five per cent of the control subjects but only 18 per cent of the vaccinated patients exhibited rises in antibody titer. Toward the end of the epidemic period a slight increase occurred in the proportion of serologically negative cases. A certain number of these patients did not report until symptoms had persisted for a week; they may ac-

count for some of the failures to demonstrate serological changes. The probability is clear that a number of cases called influenza may have been due to some other agent. If it be assumed that a clinical diagnosis of influenza was etiologically correct in the same proportion of vaccinated subjects and controls, then only two-thirds of the 112 cases would be considered influenza and the incidence of mild influenza among the controls and vaccinated subjects would be proportionately reduced. If, however, it be assumed that the actual number of noninfluenzal cases in the vaccinated individuals in the dispensary group equalled that noted in the controls, it would result in a greater proportionate reduction in incidence of influenza among the vaccinated than among the controls.

Serological tests were done on a relatively small sample of those in whom a diagnosis of local respiratory infection or common cold was made. The overall incidence of patients with diagnosis of local respiratory infection or common cold was essentially the same in the vaccinated and control groups. The serological results obtained in a small sample of these patients demonstrated that at the height of the epidemic a proportion of each group had influenza A. These findings suggest that influenza virus may appear jointly with other agents which cause localized infections of the respiratory tract. While it is generally considered that bacterial infections are superimposed upon virus infections, the evidence suggests, at least in some cases, that the virus infection occurred simultaneously with, or was a "complication" of, a localized infection of the type generally ascribed to bacterial invasion.

Relationship between serum antibody level and frequency of infection

The data accumulated in the course of these studies have been analyzed to determine whether any relationship exists between serum antibody titer and severity and frequency of infection in the course of the outbreak.

For this purpose the information gathered from the group of patients hospitalized with a diagnosis of influenza was studied since it represented a homogeneous group etiologically and these patients were studied most carefully. The patients in this group exhibited a clear-cut and characteristic illness generally considered typical of influenza. Moreover, blood for serolog-

ical study was obtained from all subjects early during the acute phase of illness, as opposed to the incomplete sampling at irregular periods after onset in the other 3 groups that have been considered.

Figure 6 shows the relationship that was observed between the titer of antibody in serum taken at the onset of symptoms and the maximum temperature recorded in the course of illness. The symbols indicate the serological reaction to infection in each subject. Antibody titers for the PR8 and Weiss strains are shown in separate charts and control and vaccinated subjects are compared. The relationships have been analyzed in terms of the two type A

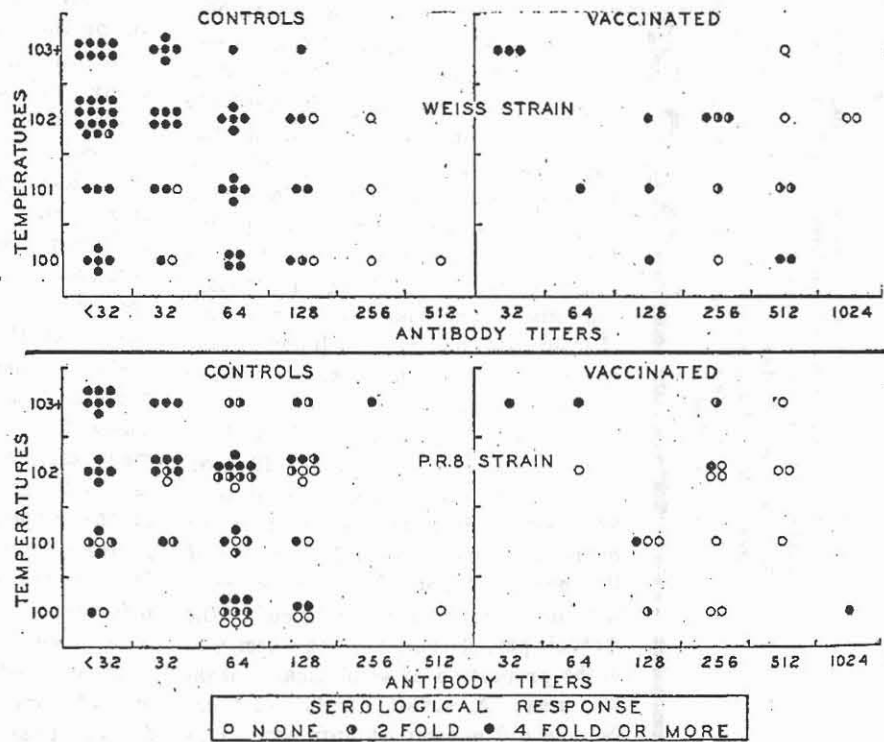
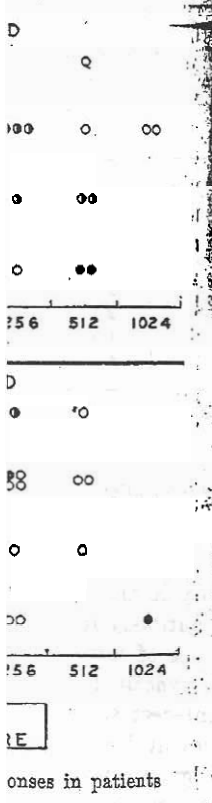


FIGURE 6. Relation of acute-phase titers to febrile and serological responses in patients hospitalized with diagnosis of influenza.

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relationship that
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strains since the serological results ob-
 tained with the two were different.

Among the control subjects who be-
 came ill, temperatures of 102 F or
 higher were observed in a greater pro-
 portion of those with antibody titers of
 32 or less than in those with antibody
 titers of 64 or greater. The tendency
 toward greater frequency of more se-
 vere illnesses in individuals with the
 lowest levels of antibody was more
 prominent in determinations made with
 the Weiss strain than in those done with
 the PR8 strain. The usual level of anti-
 bodies in patients from the vaccinated
 group was higher than in those from the
 control group, but the numbers of anti-
 body titers at the different levels among
 patients previously vaccinated were too
 few to furnish sufficient data for analy-
 sis.

In order to determine whether any
 relationship between antibody level and
 frequency of infection exists it is neces-
 sary to relate the distribution of anti-
 body titers in the group of individuals
 who became ill to the distribution of
 antibody titers in the population of
 which they are a part. The relation-
 ships are shown in table 8.

The distribution of antibody titers in
 the unvaccinated population was de-
 termined from the initial bleedings ob-
 tained from 246 individuals 2 to 3 weeks
 before onset of the epidemic and before
 vaccination was done. These repre-
 sented 162 of the control sample group
 and 82 of the vaccinated sample group.
 The distribution of antibody titers in
 those taken sick was determined from
 the antibody titers in the acute phase
 sera of the 75 control subjects who were
 hospitalized for influenza; the few
 cases in which serological confirmation
 of the clinical diagnosis was not ob-
 tained were not excluded. In a similar
 manner, the distribution of antibody

titers in the vaccinated population was
 estimated from the bleedings obtained
 from 82 individuals 2 weeks following
 vaccination, or 1 to 2 weeks before onset
 of the epidemic. The distribution of
 titers among vaccinated individuals who
 subsequently developed influenza was
 determined from the acute-phase titers
 of those who were hospitalized; as in the
 controls, serologically negative cases
 were not excluded. Estimates of the
 number of controls and vaccinated sub-
 jects with antibody titers at the differ-
 ent levels were made by applying the
 distribution found in the samples to the
 respective total populations.

Considering titers to the Weiss strain
 it is readily apparent from the data that
 a significantly higher incidence of in-
 fection occurred among individuals with
 antibody titers in the lower range. Of
 the 75 cases in the control group, 62 (82
 per cent) occurred among the 48 per
 cent of the population with antibody
 titers of 64 or less. It is seen, more-
 over, that as the concentration of serum
 antibody increases, the incidence of dis-
 ease diminishes progressively. Apart
 from the consistency of the trend, the
 significance of the observation is height-
 ened by the numbers of individuals in-
 volved. Thus, of an estimated number
 of 119 persons in the control group hav-
 ing antibody titers of less than 32, 31
 were hospitalized for influenza; while of
 an estimated 126 controls with antibody
 titers of 256, three were hospitalized. In
 groups of corresponding size at the dif-
 ferent levels from less than 32 to 512
 the progressive decline in incidence was
 observed. When estimations of inci-
 dence of infection in relation to the dif-
 ferent antibody levels measured against
 the PR8 antigen were made, the trend
 was less pronounced.

Since only 21 of 888 vaccinated sub-
 jects were estimated to have titers of less

TABLE 8

Frequency of influenza among individuals with different levels of serum antibody

Antibody titers	Controls					Vaccinated				
	Distribution in sample groups		Estimated numbers in control population*	Number of hospitalized cases among controls	Case incidence (per cent)	Distribution in sample groups		Estimated numbers in vaccinated population*	Number of hospitalized cases among vaccinated	Case incidence (per cent)
	No.	Per cent				No.	Per cent			
A. As measured with the Weiss strain of type A influenza virus										
<32	55	13.4	119	31	26.1					
32	50	12.2	108	16	14.8	2†	2.4†	21†	4†	20.0†
64	54	22.0	196	15	7.7					
128	72	29.3	260	9	3.5	8	9.9	87	3	3.4
256	35	14.2	126	3	2.4	16	19.5	173	5	2.9
512	17	6.9	61	1	1.6	25	30.5	272	6	2.2
1,024	5	2.0	18	0	0	17	20.7	184	2	1.1
2,048						6	7.3	64	0	0
4,096						7	8.5	76	0	0
8,192						1	1.2	11	0	0
Totals	246	100	888	75	8.6	82	100	888	20	2.3
B. As measured with the PR8 strain of type A influenza virus										
<32	29	20.1	179	19	10.6					
32	21	14.6	129	12	9.3	4†	4.9†	44†	3†	6.8†
64	57	39.6	352	26	7.4					
128	28	19.4	172	15	8.7	21	25.6	227	4	1.8
256	7	4.9	44	1	2.3	31	37.8	336	8	2.4
512	2	1.4	12	0	0	15	18.3	163	4	2.4
1,024						9	11.0	98	1	1.0
2,048						2	2.4	21	0	0
Totals	144†	100	888	75	8.6	82	100	888	20	2.3

* Based upon population of 888.

† Because of small numbers, data from antibody titers of <32 to 64 in vaccinated population are grouped together.

‡ Antibody titers were determined with the PR8 strain in only 144 of the 246 sera obtained.

than 128 to the Weiss strain, the data below this level were insufficient for analysis. However, in the range of titers of 128 or greater, not only was the inverse relation between antibody level and frequency of influenza observed, but the frequencies of infection at the different levels of antibody were similar in both the vaccinated and con-

trol subjects. This similarity in the relationship between level of antibody for the Weiss strain and incidence of infection, in both control and vaccinated subjects, is of particular interest since the amount of antibody present in the individuals of the control group presumably reflects the residual immune reaction to previous natural exposures,

levels of serum antibody

Vaccinated			
	Estimated numbers in vaccinated population*	Number of hospitalized cases among vaccinated	Case incidence (per cent)

influenza virus

4†	21†	4†	20.0†
3	87	3	3.4
5	173	5	2.9
5	272	6	2.2
7	184	2	1.1
3	64	0	0
5	76	0	0
2	11	0	0
	888	20	2.3

influenza virus

4†	44†	3†	6.8†
	227	4	1.8
	336	8	2.4
	163	4	2.4
	98	1	1.0
	21	0	0
	888	20	2.3

64 in vaccinated population are

44 of the 246 sera obtained.

This similarity in the level of antibody for in and incidence of infection control and vaccinated particular interest since antibody present in the control group prevents the residual immune response previous natural exposures,

while the antibody titers in the vaccinated subjects were artificially raised by subcutaneous vaccination. From these data it appears that, in terms of serum antibody concentration, subcutaneous vaccination conferred essentially the same benefit as previous exposure to natural infection.

When the frequency of infection in vaccinated individuals is considered in relation to the levels of antibody for the PR8 strain, the same uniform trend noted in relation to the levels of antibody for the Weiss strain was not observed. A titer of 128 is the only level at which there are large enough numbers of control and vaccinated subjects for proper comparison. The frequency of infection among an estimated 172 controls at this level was 8.7 per cent and among 227 vaccinated subjects was 1.8 per cent. This suggests that the level of antibody for type A virus, as measured by the PR8 strain, does not have the same significance in both groups. The results might be interpreted to mean that vaccination confers some benefit beyond that reflected in the antibody. However, in view of the clear-cut relationship between incidence and antibody titer measured with the Weiss strain, it is suggested that the difference in incidence between controls and vaccinated subjects with titers of 128 to the PR8 strain occurred because the vaccinated subjects are benefited by the simultaneous increase in titer to the Weiss strain included in the vaccine.

These data, together with the fact already referred to that the antibody responses to the epidemic disease were much greater when measured with the Weiss antigen, suggest that strain specificity within the type A group of influenza viruses may be of considerable importance in the problem of human vaccination.

The data in table 8, showing the variations in incidence of influenza in relation to the different levels of antibody for the Weiss strain, are illustrated graphically in figure 7. It is seen that in the control group the incidence of influenza drops sharply from 26 per cent at antibody titers of less than 32 to 3.5 per cent at titers of 128. In the vaccinated group, however, the number of individuals with antibody titers below 128 were too few for interpretation. As the concentrations of serum antibody increase from 128 to 1,024 in both control and vaccinated groups the curves describing the frequency of infection slope gradually to approximately 1 per cent. Although none of the controls had antibody titers above 1,024, among 151 vaccinated persons with antibody titers of 2,048 or greater the incidence of influenza was zero. These observations suggest the existence of critical antibody zones, above which the probability of an individual developing influenza during an epidemic is reduced significantly.

When the curves showing the probability of contracting influenza are considered in relation to the antibody distribution curves of the control and vaccinated populations, estimated from the respective sample groups, certain practical implications of these interpretations become evident. The observations shown in figure 7 and table 9 suggest that the beneficial effect of vaccination was associated with a shift in antibody titers of the vaccinated individuals from the zone in which the probability of infection is high to zones in which the probability of developing influenza comparable in severity to that seen in the present hospitalized series is lower. It is of interest to note the effect of the epidemic upon the distribution of titers in the unvaccinated group in re-

lation to 3 different antibody zones. It is seen in table 9 that in the course of the epidemic the proportion of individuals with antibody titers in the lowest zone, in which susceptibility appeared

to be greatest, was reduced from 48 per cent to 25 per cent. Vaccination, however, reduced the proportion of individuals with the lowest antibody titers to only 2 per cent, and in the course of

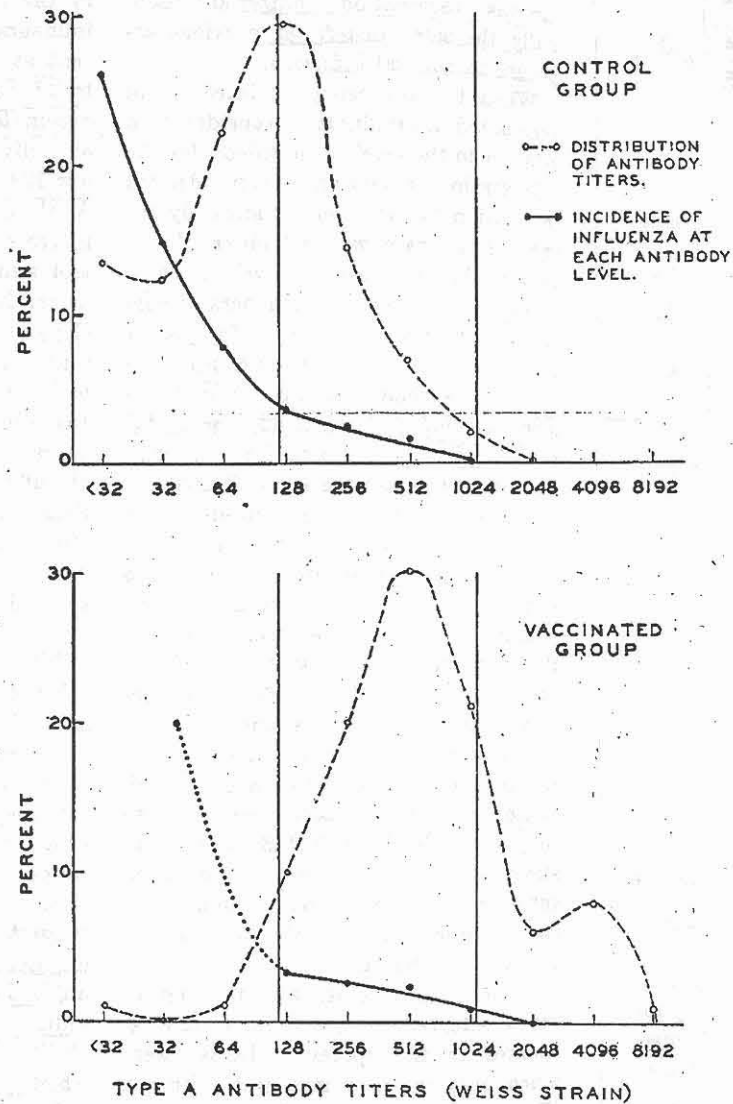


FIGURE 7. Curves showing the distribution of hospitalized influenza patients in relation to the various levels of antibody in control and vaccinated groups; and the curves describing the distribution of individuals with different levels of serum antibody in control and vaccinated groups in relation to 3 antibody zones which appear to be associated with different degrees of susceptibility. The vertical lines separate the 3 antibody zones.

TABLE 9

Percentage distribution of individuals, before and after the epidemic, in relation to zones of serum antibody concentration associated with different frequencies of illness

Antibody zone (Weiss strain)	Frequency of illness*	Control population †		Vaccinated population †		Cont. and vacc. combined	
		Before	After	Before	After	Before	After
<32 to 64	26.1 to 7.7	47.6	25.4	2.4	2.8	25.0	14.1
128 to 1,024	3.5 to 1.0	52.4	70.1	80.6	85.8	66.5	77.9
2,048 or above	0	0	4.5	17.0	11.4	7.5	8.0

* Hospitalized influenza.

† Estimated from bleedings of sample groups before and after epidemic. Postepidemic bleedings were obtained on January 26th, 1944; in the vaccinated group this represented the 3-month post-vaccination bleeding.

the epidemic the serological status of the vaccinated group did not change appreciably. Comparatively, vaccination was more efficient in decreasing the concentration of individuals with low antibody titers than was exposure of the population to the epidemic itself.

When the control and vaccinated groups are considered together as a single population, it is seen that the 25 per cent of individuals in the antibody zone with presumably highest susceptibility at the start of the epidemic was reduced to approximately 14 per cent at the termination of the outbreak. If the distribution of antibody titers in the general population was the same as that of the controls, in which 48 per cent had antibody titers in the lower zone, it is of interest that the epidemic extended into the study group although only 25 per cent of the total population belonged in the low antibody range in which the frequency of infection had been the highest. At the end of the epidemic only 14 per cent of the total population had antibody titers in the lowest zone.

Incidence of subclinical infection

In order to obtain some estimate of the incidence of asymptomatic infection

in the course of the epidemic, bleedings were obtained, after the outbreak was over, from the same individuals included in the sample groups which have been described. Of 162 in the control sample group who were bled before the onset of the epidemic, 138 were again bled after the subsidence of the outbreak. Similarly, bleedings were obtained from 70 of the 82 persons in the vaccinated sample group. The paired sera were tested for change in titer to type A and type B influenza viruses. Since the degree of serological change was more marked when measured with the Weiss than with the PR8 strain of type A virus, all sera were tested with the Weiss antigen, and approximately half were also tested with PR8 antigen.

Of the 138 controls, from whom bleedings had been obtained before and after the epidemic, 52 showed 4-fold increase or greater, and 4 showed twofold increase in antibody titer with the Weiss antigen, indicating that 41 per cent had experienced infection with the type A virus. There were no instances of change in titer to type B influenza virus.

The records of attendance at sick call for each individual were reviewed and the findings correlated with the results

, was reduced from 48 per cent. Vaccination, however, the proportion of individuals with the lowest antibody titers, cent, and in the course of

CONTROL GROUP

DISTRIBUTION OF ANTIBODY TITERS.

INCIDENCE OF INFLUENZA AT EACH ANTIBODY LEVEL.

48 4096 8192

VACCINATED GROUP

48 4096 8192

STRAIN)

fluza patients in relation to and the curves describing the dy in control and vaccinated tted with different degrees of

of serological tests. These are shown in table 10.

It would appear from these data that asymptomatic infection had occurred in 21 of 138 (16 per cent) of individuals in the control sample group. An addi-

TABLE 10
Correlation of serological evidence of infection in control sample group with records of attendance at sick call

Diagnosis	No. of subjects	Serological reaction			
		Positive *		Negative	
		No.	Per cent	No.	Per cent
No illness †	82	21	25.6	61	74.4
Influenza (hosp. and disp.)	24	20	83.3	4	16.7
L. R. I.	21	11	52.4	10	47.6
Common cold	11	4	36.4	7	63.6
Totals	138	56	40.5	82	59.5

* Twofold or greater change in antibody titer for Weiss strain of type A influenza virus by agglutinin-inhibition test.

† Did not report to sick call.

tional 35 (25 per cent) had experienced infection associated with clinical manifestations. Of the group of 82 who did not report to sick call, 25.6 per cent showed serological evidence of infection; while of the group of 56 who reported to sick call, 60 per cent had reacted serologically. It is of interest that the highest proportion of positive serological reactions occurred in the group in which a clinical diagnosis of influenza had been made.

The incidence of subclinical infections in the vaccinated population could not be evaluated because of the influence of the previous inoculation and the unreliability of serological diagnosis of infection in vaccinated individuals with high antibody titers.

DISCUSSION

Since 1936, several groups of investigators (20-33) in this country and abroad have attempted to determine, by clinical trial, the effectiveness of subcutaneous vaccination for the prevention of influenza. While many of these studies were inconclusive, others were suggestive and indicated the desirability of further investigation. Some of the reasons suggested for the failure to obtain conclusive evidence as to the value of vaccines against influenza are: (a) uncertainty that influenza virus was the cause of the disease observed in the groups under study; (b) difficulty in distinguishing influenza from respiratory disease of other etiology, on the basis of clinical findings; (c) low incidence of infections of proven etiology; (d) use of vaccines of low potency; (e) the possibility that the strain of virus in the vaccine may have been distantly related, antigenically, to the strain prevalent during the outbreak; and (f) administration of vaccine either too long before onset of the epidemic or too late after its inception.

The results of the study by the Commission on Influenza (1, 34-38) during the winter of 1943-1944, of which this is a part, have demonstrated conclusively the distinct effect of subcutaneous vaccination with concentrated, inactive virus in reducing the incidence of influenza. The probable advantage of employing a concentrated virus preparation for vaccination was suggested by earlier studies in animals and human beings. The quantitative relationship between the immunizing dose of influenza virus and the resultant immunity in ferrets and mice was demonstrated by Francis (39). Hirst, Rickard, Whitman and Horsfall (40) have shown that, within limits, proportionately higher

DISCUSSION

several groups of investigators in this country and attempted to determine, by the effectiveness of subcutaneous vaccination for the prevention of influenza. While many of these studies were inconclusive, others were indicated the desirability of further investigation. Some of the reasons cited for the failure to obtain evidence as to the value of vaccination against influenza are: (a) that influenza virus was the cause of the disease observed in the field study; (b) difficulty in isolating influenza from respiratory specimens of other etiology, on the basis of serological findings; (c) low incidences of proven etiology; (d) vaccines of low potency; (e) that the strain of virus used may have been distantly related, antigenically, to the strain causing the outbreak; and (f) that the dose of vaccine either too small or too large for the onset of the epidemic or too late for its inception.

The results of the study by the Commission on Influenza (1, 34-38) during the epidemic of 1943-1944, of which this is the first, demonstrated conclusively the effect of subcutaneous vaccination with concentrated, inactivated virus in preventing the incidence of influenza. The probable advantage of concentrated virus preparation was suggested by studies in animals and human beings. A quantitative relationship between the immunizing dose of influenza virus and the resultant immunity in mice was demonstrated by

Hirst, Rickard, Whitfall (40) have shown that, proportionately higher

mean antibody levels are obtained when increasing amounts of influenza virus are injected subcutaneously in human beings. The latter was confirmed (41, 42) by the use of influenza virus vaccines concentrated by a method described independently by Hirst, Rickard and Whitman (41) and Hare, McClelland and Morgan (43).

Preliminary studies with the vaccine employed in the present studies had been carried out during the winter of 1942-1943 (12), but the absence of an outbreak did not permit observation of any effect against the natural disease until the following winter. Nevertheless, studies were made of (a) the antibody response to vaccination, (b) the persistence of this effect over a period of 1 year, (c) degree of reactions following the administration of the vaccine, and (d) the stability of the immunizing potency. Moreover, (e) the protective effect of vaccination employing the dosage used in the present field study had been demonstrated against experimentally induced infection (18, 19).

Apart from the greater concentration of virus, the composition of the vaccine employed in the present study differed in one other respect from those used heretofore in that it contained a strain of virus (Weiss) isolated from a case occurring less than 6 months prior to the onset of the epidemic. The strain of type A virus in circulation during the preceding spring appears to have been closely related, antigenically, to the one in operation during the epidemic in this area.

By virtue of the basic plan of study it was possible to obviate certain variations that might have obscured the effect of vaccination. The study was conducted in rather closed units in respect to housing, activities, and relative segregation. All of the individuals partici-

ated and each unit was equally divided into vaccinated and control subjects. In this manner, the control individuals were in all respects identical with the vaccinated subjects except for the factor of vaccination.

The epidemic of influenza A reached its peak, in this area, about 4 weeks after vaccination was completed. The incidence of clinically recognizable disease, etiologically identified, was of sufficient magnitude for evaluation of the effect of vaccination. The results which have been described have revealed that the incidence of typical influenza, as indicated by hospitalized cases, was 8.58 per cent in the control group of 875 men and 2.27 per cent in the 878 vaccinated individuals. Seventy-nine per cent of the cases occurred in the controls. Hence, 3.7 times as many controls as vaccinated persons were admitted to hospital with influenza. The diagnosis was confirmed serologically in 90 per cent of the control and 75 per cent of the vaccinated cases. Among the milder cases of influenza studied in the dispensary an incidence of 8.04 per cent was observed in the control group and 4.78 per cent in the vaccinated. The diagnosis was confirmed serologically in 65 per cent of the controls and 18 per cent of the vaccinated cases in the dispensary series. Even if the attack rates of mild influenza are corrected, on the basis of the serological findings, as suggested in the text, the proportion of mild disease in the vaccinated group is appreciably greater than in the control group. Thus, while vaccination prevented, to a considerable degree, the development of clinical infection, it had also reduced the severity of disease.

It is of interest that no significant differences in incidence were observed between control and vaccinated subjects when the diagnosis of common cold or

local respiratory infection were analyzed, even though a small proportion of the cases was due to the virus of influenza. Moreover, the incidence of local respiratory infections in either group did not vary significantly from the pre-epidemic level. These facts suggest the specificity of action of the vaccine.

In view of the impression obtained in previous studies that the effect of vaccination was obscured by the difficulty in distinguishing influenza from other respiratory disease, it is noteworthy, in the present instance, that even when the results are charted in terms of the overall attack rate of all forms of respiratory disease, the incidence was considerably lower in the vaccinated half of the population, due, obviously, to the reduced incidence of influenza.

As has been pointed out above, the control and vaccinated subjects were equally divided within each company, thus obviating any selection on the basis of residence, activity or opportunity for exposure. A comparison of the incidence of illness in control and vaccinated groups, set up in this manner, does not necessarily give a true picture of the effect of vaccination since the observed incidence in the control half of the population is assumed to be the expected incidence in an entirely untreated population. That the latter may not be the case is suggested by the observation of a 20 per cent incidence of hospitalized illness in a company of unvaccinated men. Thus, the incidence of illness in the control half of the population may well be influenced by mixing the controls with an equal number of vaccinated persons. It is not unlikely that a comparison of attack rates in completely vaccinated populations with those in unvaccinated groups, if all other conditions were equal, would dem-

onstrate an even greater effect of vaccination than that revealed in this study. It may be that vaccination of an entire population would not permit the virus to gain a foothold; or that vaccination of a sufficient proportion would prevent epidemics, as has been demonstrated in smallpox, diphtheria and other epidemic diseases.

In a recent paper, Hirst, Rickard and Friedewald (33) have discussed the question of duration of immunity following vaccination. In their analyses they express the effect of vaccination as percentage reduction in attack rate in the vaccinated group at intervals following inoculation, assuming the weekly attack rate in the control group as the expected incidence if the vaccine had had no effect. In commenting upon the results in the preliminary report by members of the Commission on Influenza (1), they point out that "when the total of all reported cases is considered in terms of the length of time between vaccination and onset, it is found that the effect of vaccination was greatest in the second week following inoculation (approximately 85 per cent reduction) and was poorest in the sixth and seventh weeks when the reduction (in 94 cases) due to vaccination fell to about 40 per cent." They conclude that this evidence indicates a maximum effect in the second week with marked decline by the sixth and seventh weeks. Upon examination of the curves describing the weekly incidence of influenza in control and vaccinated groups in the present study (figure 3) it is seen that after the epidemic peak had passed, the incidence of influenza in the control group descended to the level of the incidence in the vaccinated group. This suggests that the approximation of weekly attack rates in control and vaccinated groups at the end of the influenza

FRANCIS, JR.

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In commenting upon the preliminary report by the Commission on Influenza they point out that "when all reported cases is compared of the length of time of incubation and onset, it is the effect of vaccination was the second week following approximately 85 per cent and was poorest in the sixth weeks when the reduction due to vaccination fell to 10 per cent." They conclude the evidence indicates a maximum reduction in the second week with marked reduction in the sixth and seventh weeks. The coincidence of the curves describing the incidence of influenza in the vaccinated groups in the control group (figure 3) it is seen that the epidemic peak had passed, the influenza in the control group had declined to the level of the unvaccinated group. This is the approximation of the attack rates in control and vaccinated groups at the end of the influenza

epidemic is due to an increase in degree of resistance of the control group, rather than to diminishing resistance of the vaccinated group, as inferred by Hirst

Serological evidence indicates that 41 per cent of the controls, predominantly those with low antibody titers, experienced, in the course of the outbreak, symptomatic or asymptomatic infections. Assuming that such infections result in immunity, then, by virtue

of widespread exposure of both vaccinated and control subjects to this natural immunizing procedure, it seems probable as a result of the epidemic experience that the concentration of susceptibles in the two groups would tend to approximate the same level as the epidemic progressed. Thus, after the height of the epidemic was passed, the controls arrived by natural means at a state of immunity approximating that present in the vaccinated group prior to the onset of the epidemic. To attribute the decreased difference in incidence of infection in vaccinated and control individuals at the end of the outbreak to waning immunity, resulting from the increased interval between vaccination and infection, without regard for the epidemiological dynamics involved, seems to ignore important factors.

Contrary to the conclusions reached by Hirst et al (33), evidence obtained in the present studies suggests the likelihood that the degree of persistence of immunity to influenza, following vaccination, is rather prolonged. If, as has been indicated, there is an inverse relationship between antibody level and frequency of infection, one would expect the degree of persistence of antibody to furnish some index of the degree of persistence of immunity. A comparison of serum antibody titers, in 70 vaccinated individuals in the present series, 2 weeks and 3 months after

inoculation has revealed a fall in titer for the PR8 strain in 14, for the Weiss strain in 18, and for the Lee strain in 19; except for two instances, in which a 4-fold change in titer was observed in the tests with PR8 and Weiss strains, the change was limited in extent to a twofold decrease. Moreover, since the changes in titer occurred almost entirely among individuals with the higher levels of antibody, the proportion of individuals with antibody titers in the zone of presumably highest susceptibility was not altered appreciably in the course of 3 months. Although the intercurrent epidemic could have been instrumental in maintaining the type A antibody titers during the interval, this would not apply in the case of the type B antibody titers. Furthermore, in 1942-1943 (12), at which time there was no epidemic of influenza A to influence the results, antibody titers persisted at a relatively constant level over a period of 3 months following the use of a similar vaccine.

That the level of antibody furnishes some index of the degree of immunity, even long after the original antigenic stimulus, is indicated by the progressive reduction in attack rate among unvaccinated subjects with increasing concentrations of antibody for the Weiss strain. In view of the likelihood that the majority of these individuals had not been in contact with type A influenza virus more recently than 3 years prior to the present outbreak, it appears that the antibody titers exhibited by them and the resistance represent a persistence of effect over that period of time at least. Since the frequency of infections severe enough to necessitate hospitalization of the patient was the same in vaccinated and unvaccinated individuals having the same concentrations of antibody for the Weiss strain,

it is probable that the degree of immunity resulting from vaccination persists in proportion to the level of antibody maintained. A study of antibody titers for 1 year following vaccination (12) has revealed that while antibody levels gradually decline in the course of a year, this tendency is most marked in those with the highest titers. Although, as might be expected, the decrease in mean antibody titer of a group over a period of time would be sharply influenced by the titers falling from the highest levels, it is perhaps of greater significance that even 1 year after vaccination the majority of individuals still possess antibody titers above the levels that appear to be associated with the highest degree of susceptibility.

It was of interest that the relationship between serum antibody level and the frequency of hospitalized influenza was prominent when antibody titers for the Weiss strain of type A virus were measured but the correlation was not more than suggestive when the clinical results were analyzed in terms of titer for the PR8 strain. This is in accord with the observation that serological reactions of convalescent sera were more marked with the Weiss antigen than with the PR8 in the agglutination-inhibition test. This indicates a closer antigenic relationship between the Weiss strain and the strain of virus prevalent during the epidemic than between the latter and the PR8 strain. These findings are in accord with the suggestion that strain specificity within the type A group of influenza viruses is a factor of practical importance in immunity to the natural disease.

It should be pointed out that the correlation observed between antibody levels to the Weiss strain and incidence of infection applies only to infections of the degree of severity observed among

the hospitalized cases. The results of serological tests made in the sample of the control population after the epidemic had subsided gave evidence that half of those with titers of 64 or less and one-fourth of those with titers of 128 or more had been infected during the epidemic period. These figures represent the incidence of infections diagnosed serologically, without reference to clinical manifestations. When this evidence is considered in the light of the observed relationship between level of serum antibody and frequency of hospitalization, it becomes apparent that the frequency of infections accompanied by the more severe and typical manifestations of disease was proportionately much less among those with high antibody titers, even though a considerable proportion of the latter exhibited serological evidence of infection. It has been observed previously, both in the field (8, 15, 44-47) and in studies on experimentally induced infections (16-19, 48, 49) that the relationship between serum antibody titer and susceptibility to infection is far from absolute; this is confirmed in the present study in which it has been observed that a proportion of those with the lowest titers escaped infection, while others with higher titers became ill. Nevertheless, the present data, as a whole, point to the existence of an antibody zone above which antibody titers may have to be raised to produce the desired effect. Since the range of numerical values expressing antibody titers vary in different laboratories, the data should be interpreted only as indicating that a zone of serum antibody concentration exists above which there appears to be a sharp reduction in the probability of developing illness, and that this zone is well within the range of titers readily attained by vaccination and includes a

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On the basis of results obtained with the Weiss strain, it is seen in table 8 that 82 per cent of the hospitalized cases among controls occurred in the 48 per cent of the population with antibody titers of 64 or less. Moreover, a greater frequency of more severe infections, as indicated by febrile reaction and duration, was observed among patients with the lowest antibody titers. A comparison of the incidence of infection at the various levels has revealed that corresponding antibody titers in control and vaccinated individuals appear to have the same significance, as regards probability of contracting influenza comparable in severity to that observed in the hospitalized cases, even though antibody titers in vaccinated subjects were raised artificially and in unvaccinated individuals represent the residual reaction to previous natural exposure.

A further implication of the observed relationship between antibody titer and frequency of infection was suggested by the data shown in figure 7 and table 8 which has indicated that just prior to the outbreak of influenza 48 per cent of the general population, as indicated by serological study of unvaccinated individuals, had antibody titers within the zone of presumably highest susceptibility, and that at the end of the outbreak in the study group an estimated 14 per cent of this population had antibody titers in this lower zone. If the serological status of the group at the termination of the epidemic indicates the reduction in concentration of susceptibles required to limit the spread of influenza, it would be necessary, in the absence of some simple method of selection, to vaccinate at least 70 per cent of the population in order to reduce the proportion of such individuals from

48 per cent to 14 per cent. Although a complexity of factors is responsible for the periodic recurrence of epidemics of influenza, these observations suggest that one factor may be the accumulation of a sufficient concentration of individuals whose antibody titers have fallen into the lower range. If these thoughts gain further confirmation, it would seem possible to control the epidemic recurrence of influenza by revaccination at intervals shorter than the epidemic periodicity, or at intervals determined by immunological surveys. This would appear to be a more practicable method of administering vaccine for prophylaxis than to vaccinate in the face of an outbreak, after its identification, particularly since influenza spreads with such rapidity that the epidemic might be well under way before it would be possible to vaccinate a significant proportion of the population.

Certain epidemiological observations deserve emphasis. It is of interest that evidence was obtained of the presence of influenza virus in the population about 2 weeks before any clinical cases were recognized and about 4 weeks before the epidemic peak was reached. Together with the isolation and identification of type A influenza virus from a case occurring in this area less than 6 months prior to onset of the epidemic, the evidence points to the circulation of influenza virus in the population in the interepidemic intervals. A further point of interest is the fact that the single instance of influenza infection 2 weeks before onset of the epidemic appears to have been asymptomatic, suggesting that such individuals may serve as interepidemic carriers.

The effect of dispersal of a population upon the course of localized outbreaks is suggested by the observation in Company G in which no further ill-

ness occurred after the company went on furlough the week following a sharp increase in the number of cases of influenza. The fact that the groups under observation by Eaton and Meiklejohn (1, 38), at the University of California were dispersed shortly after appearance of the first few cases and that the epidemic peak was less marked may be one reason for their results, which appear to be at variance with the results of other investigators participating in the study by the Commission on Influenza.

This discussion has attempted to emphasize the interrelationship of the numerous variables that operate in immunity to influenza and to emphasize the importance, in interpreting the results, of considering the various factors as parts of a complex dynamic system. Moreover, with regard to the quantitative immunological principles that appear to apply in immunity to influenza, there are striking similarities when compared to those that prevail in other immunological and epidemiological phenomena. By virtue of these considerations, analysis of the observations gathered has indicated some of the areas for further application and experimentation toward achieving effective control of influenza.

SUMMARY

1. During the epidemic of influenza A that occurred in November and December, 1943, a controlled study was made of the effectiveness of subcutaneous vaccination with a concentrated, formalinized preparation of influenza viruses, types A and B. The study was carried out in the A.S.T.P. unit at the University of Michigan, as part of the more extensive program conducted by the Commission on Influenza, Board for the Control of Influenza and other Epidemic Diseases in the Army.

2. Vaccinated and control individuals were equally divided within each unit and throughout the observation period. The mean strengths of the vaccinated and control populations were 878 and 875, respectively. Among the controls, 75 persons suffering from typical influenza with temperatures of 100 F or more were hospitalized, an incidence of 8.58 per cent; among the vaccinated, 20 such cases occurred, an incidence of 2.27 per cent. Thus, 79 per cent of the cases developed in the control group, or 3.7 times as many cases among controls as among vaccinated individuals. Diagnosis was confirmed serologically in 90 per cent of the controls and 75 per cent of the vaccinated cases.

3. Mild influenza generally unaccompanied by fever, diagnosed in patients seen in the dispensary but not admitted to hospital, occurred in 8.04 per cent of the control and 4.78 per cent of the vaccinated subjects. Diagnosis was confirmed serologically in 65 per cent of control cases and 18 per cent of vaccinated cases in the dispensary series.

4. No significant difference between control and vaccinated subjects was observed when the incidence of cases diagnosed as local respiratory infection or common cold was considered, although, as indicated by serological tests, a small proportion was due to influenza virus infection.

5. Antibody studies on sera obtained from a sampling of the control group before and after the epidemic revealed serological evidence of influenza A infection in 41 per cent. There was no record of attendance at sick call in 16 per cent, suggesting the extent to which subclinical infections occurred. No evidence for the presence of influenza B infection was obtained.

6. Certain epidemiological factors that bear on the interpretation of the clinical

Results have been considered and discussed.

a. It is believed that dilution of the controls with an equal number of vaccinated individuals in the study population may have reduced the incidence of disease among the controls, and that a comparison of attack rates in completely vaccinated populations with those in unvaccinated groups, if all other conditions were equal, would demonstrate a greater effect of vaccination than was apparent in this study.

b. The observation that the differences in incidence between control and vaccinated subjects were greatest at the height of the epidemic and diminished as the epidemic progressed is interpreted to mean that the difference in concentration of susceptibles was most marked at the onset and peak of the influenza prevalence and as the epidemic advanced, as a result of the natural immunizing procedure, the concentration

of susceptibles in both control and vaccinated groups tended to approach the same level.

7. A striking relationship was observed between level of serum antibody and frequency and severity of illness, in both control and vaccinated groups, when titers for the Weiss strain of type A virus were analyzed, while the trend was only suggestive in terms of the titers for the PR8 strain. The Weiss strain, isolated 6 months prior to onset of the epidemic, appears to have been closely related, antigenically, to the strain prevalent during the outbreak in this area. The data indicate the existence of an antibody zone above which titers may have to be raised to produce the desired effect. Certain practical implications of these observations, with respect to the use of antibody level as an index of degree of immunity, as well as an index of persistence of immunity following vaccination, have been discussed.

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