SEROLOGICAL STUDIES ON INFLUENZA DURING A NINE-MONTH PERIOD [1]

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The anticipated influenza B epidemic materialized late in November, 1945 and reached its peak during the month of December. Sporadic cases were recognized in the United States (1) for a period of some months preceding the actual epidemic. The importance of recognizing this pre-epidemic period is unquestionably a necessity in order to avail ourselves of the prepared vaccines. It has been demonstrated (2–12) that immunization against the influenza viruses elicits an antibody increase and offers protection to the experimental inhalation of the virus strains. Francis and coworkers (13) have demonstrated further that vaccination with inactivated influenza viruses Types A and B offers protection against influenza B during an epidemic. That this epidemic was due to Type B has also been shown by Dalldorf and Rice (14).

It is recognized that other agents are responsible for certain cases of bacteria-free pneumonias. Clinically these various types of virus pneumonia often resemble each other. It is essential, therefore, to determine, if possible, the agent responsible for the infection. Their differentiation may be made by isolation of the virus or by serological studies. In many instances, failure to isolate an organism does not eliminate its possible role as causative agent. On the other hand, the development of specific antibodies in convalescent sera is one criterion that is considered to be conclusive evidence of contact with the specific antigen. Many agents have been isolated and serological technics for their identification have been developed.

These various viral technics available for laboratory diagnosis are now routinely employed in many laboratories. The examination of serum samples for the presence of antibodies to viruses, as well as those producing a clinical syndrome similar to that produced by virus agents, may be made by the Hirst test (15) for influenza; cold agglutination (16) or its modification (17), and the indifferent Streptococcus MG (18) for atypical pneumonia; and the commercial product Lygranum CF [2] (19 to 23) for members of the psittacosis group.

The experiments were designed in order (*a*) to ascertain the approximate time of the epidemics' appearance or an indication of the epidemics' approach by pre-epidemic increases in serum antibodies, (*b*) to determine the nature of the epidemic in this area, (*c*) to determine the effect of vaccination, and (*d*) to determine what antibody titers may be expected in a group of presumably normal individuals.

MATERIALS AND METHODS

Sera were derived from 2 sources. One group was obtained from 52 medical students who were bled each month for a period of 9 months from October, 1945 to June, 1946, inclusive. In this group 3 were females and the remainder were males. Of these, 15 students were given the concentrated A and B influenza vaccine prepared from chick embryos. Except for 1 member of this group, all received their vaccinations after their second blood specimen had been obtained. The lone student was vaccinated after his third blood sample had been taken. The 37 other members of this group were either members of the naval unit or civilians and were not required to be vaccinated.

A careful record of each student's health was maintained with histories obtained before the program was established and at each monthly bleeding. By coincidence, the vaccination program and the influenza epidemic were concurrent.

Another group of sera were obtained from the university student infirmary and consisted of 66 acute phase sera and 25 pairs of acute and convalescent phase sera. The donors of these sera were all university students admitted with upper respiratory infections during the

[1] Aided by a grant from the Hendricks Research Fund.

[2] (E. R. Squibb & Sons.) This antigen is used only because of its convenience, rather than its specificity. The use of a psittacosis antigen would be preferable, but more difficult to obtain.

epidemic period. In all cases, attempts were made to obtain a convalescent phase serum sample. Wherever cooperation was lacking, the acute phase sample was placed in the "single serum group" (these are discussed more fully under results). All acute phase sera were taken upon admission to the infirmary and in most cases were obtained during the first 2 days of illness. The convalescent sera were obtained 2 to 6 weeks following infection. The clinical diagnosis of this group was varied, but all were considered to be respiratory infections. The number of males and females in this group was approximately equal.

All sera were handled in an identical manner. The blood samples were obtained under sterile conditions and maintained in the sterile state. Upon receiving the blood sample, it was allowed to stand at room temperature for several hours, then centrifugated, and the serum removed. All sera were frozen and stored at −70° C. until ready to be tested. Prior to the titration of the serum, it was thawed and sufficient amounts removed and inactivated at 56° C. These were to be used in the agglutination-inhibition and complement-fixation tests. The cold agglutination and the streptococcus agglutination tests were made immediately following the thawing of the serum sample. All sera from the same individual were treated at the same time using the same test preparations.

The sera were stored in sterile vials. All titrations were completed within 24 hours after removal of the sera from the frozen state and the sera were kept at 4° C. when not in actual use.

Cold agglutination: The procedure was essentially that recommended by the Commission on Acute Respiratory Diseases (17). It consisted of adding 0.5 ml. of a 0.2 per cent suspension of fresh type O cells, obtained from the same donor throughout, to 0.5 ml. 2-fold serial serum dilutions starting at 1 : 5. The tubes were shaken and kept overnight at 4° C. Readings were made on the following day before the tubes had any opportunity to become warm. The end point consisted of that dilution giving definite agglutination of the cells. All titers expressed are reciprocals of the *final* dilutions of the serum.

Streptococcus MG No. 9: [3] From a culture of this organism bacterial suspensions were made and tested in the manner described by Thomas, *et al* (18). The organisms were washed 3 times in saline and a suspension corresponding approximately to a No. 3 McFarland standard was made. The streptococci were killed by heating at 60° C. for 1 hour. To 0.5 ml. serum dilution starting at 1 : 5, an equal volume of streptococcus suspensions was added. This mixture was placed in a 37° C. water bath for 2 hours, then overnight at 4° C., and then again at 37° C. for 2 hours. Following this, the tubes were shaken and read. The degree of agglutination used as a standard was that of the original workers. The dilution of serum giving a 1 plus reaction was used as the titer and is expressed as a *final* dilution.

[3] A culture of this organism was received from Dr. Frank L. Horsfall, Jr.

Agglutination-inhibition test: The procedure used here is the same as is described by Hirst (15) except that we employed a 1 per cent chicken red cell suspension as used by Henle, *et al* (12). The virus was obtained from the allantoic fluid of 10- to 11-day-old embryos after numerous passages. The fluid from the embryos was obtained in the usual manner and then pooled. This pool was then titrated and separated into smaller amounts for storage. All virus pools were stored at −70° C. until needed. When fresh virus was required, the tube was removed at least 1 day prior to its use, thawed, and allowed to stand at 4° C. A hemagglutination titration was again made and exactly 4 units antigen were used. The pools maintained the same titer for the entire test period.

Sera were tested for inhibitory substances to the influenza viruses A, B,[4] and Swine.[5] The PR 8 and Lee strains of influenza A and B were used respectively. Readings were made after 75 minutes at room temperature and the titers expressed are those giving a 2 plus or better inhibition of agglutination. A standard red cell suspension as well as the degree of clumping was used for comparison. All sera from the same individual were titrated against the influenza strains at the same time. The saline and the red cells were dispensed with an automatic pipetting machine. To avoid possible mixing of influenza strains, the same person worked with the same strains throughout the experiment. Each virus strain had its own glassware. The expressed titers are *final* dilutions.

Henle and coworkers (12) discuss the possibility of crossing the strains, which, on the basis of Ziegler's and Horsfall's work on interference (24), appears to preclude this possibility. However, titrations in immunized mice demonstrated that our virus strains were pure.

Complement-fixation: All sera were tested for antibodies to members of the psittacosis group of viruses with Lygranum CF. To 0.2 ml. inactivated serum dilutions, 0.2 ml. complement (2 units) was added, followed by 0.2 ml. antigen (1 unit). These were placed in a 37° C. water bath for 1¼ hours and then 0.4 ml. sensitized cells were added to all tubes. These were then further incubated for ½ hour and reading made. The usual anticomplementary and non-specific controls were included in all tests. The titers of sera giving 50 per cent or better fixation were considered positive and are expressed as *initial* dilutions.

Included with all tests, *i.e.* cold agglutination, streptococcus-agglutination, agglutination-inhibition, and the Lygranum complement fixation tests, were positive serum controls.

RESULTS

Patients

An increase in admissions to the student infirmary became apparent late in November.

[4] These two strains were supplied by Dr. Werner Henle.

[5] Dr. Gilbert Dalldorf supplied the Swine strain.

TABLE I

Incidence of influenza B among students admitted to the student infirmary with upper respiratory infections, November 1945 to February 1946

Month	Total number of admissions	Total number of acute and convalescent sera tested	Influenza B	Incidence
				per cent
Nov.	4	1	1	4.0
Dec.	48	18	17	68.0
Jan.	8	5	2	8.0
Feb.	6	1	0	0.0
Total	66	25	20	80.0

These admissions increased rapidly during the month of December, gradually receding during the months of January and February. Table I shows the total number of students admitted into the student infirmary during this 4-month period [6] for acute upper respiratory infections. Of the 66 admissions, 25 pairs of acute and convalescent sera were obtained. The greatest number of admissions occurred in December with 48 cases. There were 4 cases late in November, 8 in January, and 6 in February. Of the paired sera, we obtained 1 in November, 18 in December, 5 in January, and 1 in February. Upon titration for influenza B, these were shown to have an incidence of 4.0 per cent, 68.0 per cent, 8.0 per cent, and 0.0 per cent for those months respectively. The total of 80.0 per cent shows a 4-fold or better increase in antibodies to influenza B.

The sera were divided into 2 groups for convenience, one group consisting of the paired sera, the other group composed of a single serum sample. The results obtained from the cold agglutination, indifferent streptococcus No. 9 agglutination, complement-fixation (for members of the psittacosis group) and agglutination-inhibition (for influenza A, B, and Swine) tests on the first group are shown in Table II.

Of the 25 paired sera, 3 showed increases in cold agglutinins, whereas 3 showed cold agglutinins but no increases or a drop in titer during convalescence. Of the 3 with titer increases, patients Nos. 58 and 108 were diagnosed clinically as atypical pneumonia with characteristic pulmonary involvement. Case 96, although showing a rise in cold agglutinins, demonstrated a more significant increase in influenza B antibodies. Clinically, this patient did not have any extensive pulmonary involvement and was considered to be influenza B. Patients 79, 85, and 86 all showed the presence of cold agglutinins. These titers, because of their failure to increase, were considered to be of little significance, and therefore, non-specific in nature.

[6] Because of facilities at the infirmary, this represents only a small part of the university students. Many were entered in other hospitals.

TABLE II

Titers of acute and convalescent sera obtained from patients during epidemic; winter 1945–1946

Serum no.	Cold agglutination	Streptococcus MG no. 9	Psittacosis group	Influenza		
				A	B	Swine
56 A	0	10	0	80	320	60
B	0	15	0	80	640	60
58 A	20	0	0	320	80	20
B	40	0	0	80	10	40
59 A	0	0	0	160	0	0
B	0	0	0	160	160	0
60 A	0	0	0	160	1,920	80
B	0	0	0	160	1,920	60
66 A	0	20	0	80	320	40
B	0	10	0	80	480	40
67 A	0	0	0	80	40	20
B	0	0	0	80	2,560	20
74 A	0	0	0	40	80	20
B	0	0	0	40	5,120	20
75 A	0	10	0	160	160	40
B	0	10	0	320	2,560	40
72 A	0	20	0	320	160	40
B	0	40	0	320	10,240	40
76 A	0	0	0	80	320	160
B	0	0	0	80	2,560	320
79 A	20	20	0	80	640	640
B	10	20	0	160	2,560	640
83 A	0	10	0	40	320	40
B	0	10	0	60	5,120	40
84 A	0	10	0	160	320	320
B	0	20	0	320	10,240	320
85 A	15	15	0	160	320	40
B	0	10	0	320	2,560	40
86 A	15	15	0	80	640	320
B	15	20	0	80	10,240	640
87 A	0	40	0	640	640	320
B	0	40	0	640	10,240	320
94 A	0	10	0	160	1,280	160
B	0	10	0	160	2,560	320
96 A	0	20	0	640	320	80
B	20	40	0	1,280	5,120	80
100 A	0	20	0	320	1,280	80
B	0	20	0	640	2,560	80
108 A	0	20	0	320	460	960
B	30	40	0	640	320	1,280
111 A	0	0	0	320	160	1,280
B	0	0	0	320	160	1,280
110 A	0	0	0	320	40	640
B	0	0	0	640	160	320
113 A	0	10	0	80	80	320
B	0	20	0	80	10,240	60
114 A	0	20	0	80	320	320
B	0	15	0	80	320	320
121 A	0	0	0	320	640	160
B	0	0	0	640	640	320

Sixteen patients demonstrated antibodies to the indifferent streptococcus MG No. 9. Their titers ranged from 10 to 40 without any showing significant increases. Of the patients showing increases in cold agglutinins, patients 96 and 108 gave a 2-fold rise in streptococcus antibodies. Patient 58, although diagnosed as atypical pneumonia, did not have any agglutinins for this indifferent streptococcus. Many of the patients with influenza B had low titers for this organism.

In this group, there were no complement fixing antibodies to the members of the psittacosis group. Several, however, were reactive with the normal chick embryo control.

The titers for influenza A and Swine influenza showed no significant increase although several had high antibody levels. When tested against the Lee strain of influenza B, 15 patients showed 4-fold or better increases in their convalescent sera. Five patients showed only 2-fold or even no increase over their acute phase titers. However, inasmuch as the acute phase sera were obtained after several days of illness and the titers were quite high, we have considered them to be indicative of influenza B.

Of the single serum group, 7 patients demonstrated cold agglutinins in titers ranging from 10 to 60. A large number showed agglutinins for the streptococcus; and, as in the other group, they were mainly of low titer. One serum however, had a titer of 160. This titer along with a cold agglutinin titer of 60 and low influenza titers makes one strongly suspect a possible atypical pneumonia. There seemed to be little correlation between the cold agglutinin and the streptococcus titer, as in the other group.

Titrating these sera for antibodies to the psittacosis group gave 3 positives. Two of these were quite low, a 1 : 5 serum dilution in each case giving better than 50 per cent fixation, while one serum titered to 20.

The influenza results are the usual titers obtained when single serum samples are titrated. A wide individual variation was apparent. Several of the sera did show high titers, one giving a titer of 10,240.

These sera that had high influenza B antibodies were from students who had been ill several days before entering the infirmary. This would account for the high titers due to the rapid production of influenzal antibodies.

Many of the patients from whom only single serum samples were obtained perhaps would have demonstrated antibody increases had convalescent serum been received. In some of these, as stated above, where the acute phase specimen was taken after a few days of illness, significant titers may be noted.

Students: The sera obtained from these students were handled in the same manner as the hospitalized patients. All 9 serum samples from each student were titrated at the same time using the same test materials. Of the 52 students, 3 were unable to continue after their fourth bleeding. Their 4 samples, however, were tested.

The majority of the student serum samples had titers of less than 10 for cold agglutinins. There were a few that showed a titer of 10 and some a titer of 20. There was only 1 student with a titer of 30 and again only one had a titer of 40. No greater titers were obtained. Although there was a greater number of students with titers for the indifferent streptococcus, here, again, relatively low titers were obtained throughout. There seemed to be little correlation between the presence of cold agglutinins and streptococcal antibodies as was noted in the hospitalized group. There may have been a slight correlation between streptococcal antibodies and the presence of upper respiratory infections such as colds and sore throats, but the evidence is insufficient to warrant any definite conclusion.

With regard to the psittacosis group, there were a few sera that gave positive reactions for the group antigen in dilutions of 1 : 5. In 1 case, all 9 sera, *i.e.*, each monthly sample, were positive at this dilution. The other sera were positives usually for 1 or 2 months at most. These positives did not react with the normal control antigen. In a few other instances, there were some students who had sera that reacted both with the test antigen and the control antigen. These apparently were non-specific, and so were not included with our positive results.

Table III contains the results of titrating sera for antibodies to influenza A, B, and Swine. The results have been summarized on this table and show the titers of the students for each month.

Each number represents the number of students having that titer for the designated antigen in each represented month. Fifteen students in this group were vaccinated with the army vaccine against influenza A and B following their November bleeding. The first two serum samples were quite low when tested for influenza A and in the main showed very little variation during the course of the winter. It may be seen in Table III that the majority of students did not demonstrate titers greater than 320. The unvaccinated group maintained a constant titer with some students showing a 1-tube variation which was considered insignificant. Two members had no demonstrable titers for the entire period, while 5 students, although initially having no titer, developed antibodies during the course of the winter. The vaccinated group demonstrated, in most instances, at least a 4-fold antibody rise. However, 3 members of the group gave only a 2-fold rise and 2 members showed no antibody increase at all. Of those producing increased antibody levels, the levels resulting were variable with relation to the increase and duration. Several maintained constant titers for the 7 tested months following their vaccination, whereas others demonstrated antibody decreases for 1 or more months previous to their last sample.

The titers for influenza B were considerably higher in the majority of cases. Table III shows that the majority of students had antibody titers greater than 320. This was evident before the start of the vaccination program and was considered to be indicative of subclinical infection prior to the beginning of this study. Several unvaccinated students demonstrated 4-fold or better antibody rises to this virus in their December sample. None of these had reported infection or any illness during the preceding month. This would appear to indicate that they had come in contact with the virus during the epidemic without any apparent effect other than the stimulation of influenza B antibodies. As in influenza A, there are individual variations in the duration of the antibody level. Interesting is the fact that the titers of those receiving the vaccine became higher for this virus than for the Type A strain. The high initial titer, *i.e.*, the antibody level of October's serum sample, would seem to indicate that contact

TABLE III

The monthly titers of the student group, to influenza A, B, and Swine

Following the November bleeding, 15 students received the influenza A and B vaccine.

Influenza antigen	Antibody titer (final dilution)	Month (1945–1946)								
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
A	0	8	7	2	3	3	3	3	3	3
	40	9	9	8	6	5	5	5	5	16
	80	14	15	12	10	9	6	9	9	14
	160	13	13	17	16	18	6	19	19	14
	320	4	3	5	9	4	21	4	4	3
	640	3	4	6	5	5	5	6	6	6
	1,280	1	1	1	1	1	5	2	3	3
	2,560	0	0	1	2	2	3	1	0	0
	5,120	0	0	0	0	0	0	0	0	0
	10,240	0	0	0	0	0	0	0	0	0
B	0	2	2	0	0	0	0	0	0	0
	40	2	3	1	1	1	1	2	1	1
	80	4	2	1	2	2	2	1	2	2
	160	4	5	4	1	2	3	4	3	3
	320	8	6	4	7	8	4	4	7	9
	640	12	17	14	14	11	16	15	14	12
	1,280	10	7	9	8	8	6	6	6	6
	2,560	4	5	8	8	9	9	9	9	9
	5,120	6	5	8	7	3	4	5	5	6
	10,240	0	0	3	4	5	4	3	2	1
Swine	0	6	5	4	3	3	3	3	3	3
	40	5	5	3	1	2	2	2	2	3
	80	6	7	7	8	8	7	9	10	9
	160	15	17	17	19	17	19	17	15	17
	320	10	9	12	12	10	9	9	9	9
	640	6	5	4	3	3	3	3	5	3
	1,280	3	4	4	4	3	5	6	5	4
	2,560	1	0	1	2	3	1	0	0	1
	5,120	0	0	0	0	0	0	0	0	0
	10,240	0	0	0	0	0	0	0	0	0

to the virus had been made, and makes us feel that the virus was present in the community several months prior to the actual outbreak. We were interested in observing that the general titers for Swine influenza, although not comparable to those for influenza B, were usually higher than those for influenza A. There appeared to be little effect resulting from the vaccination; only 3 of the 15 had more than a 2-fold antibody increase. Also, the titers of this group appeared to have very little relationship to the influenza A titers. Three members of this group demonstrated no antibody level during the entire series.

We have averaged the titers of the vaccinated and unvaccinated groups for each month. The averaged titers for each group are represented in Figure 1. It is evident that the titers for influenza

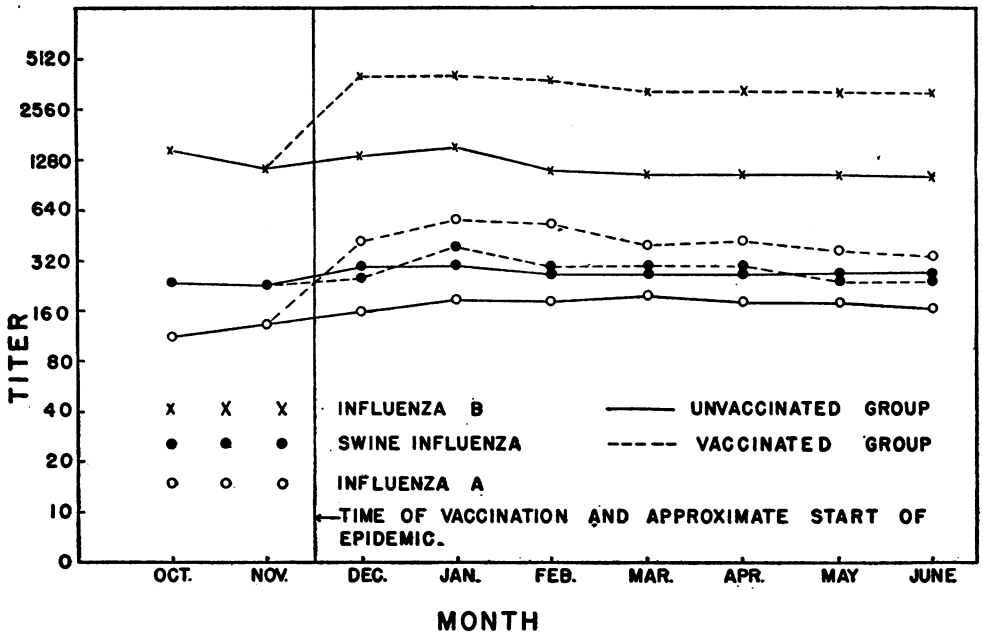

FIG. 1. THE MONTHLY TITERS OF THE VACCINATED AND UNVACCINATED STUDENTS
The points represent the average of the groups

B were higher than the other viruses, even among the unvaccinated group. The vaccinated group (represented by the broken line) subsequent to their vaccination had immediate increases in their titers. Of the 15 students receiving the vaccine, none was hospitalized, although several did report "colds." There was a slight antibody level increase among the unvaccinated group coincident with the time of vaccination. This was probably due to contacts or to cases in this group during the epidemic.

A few of the students were ill with influenza B, as is evident by the increased antibody titers seen in the serum samples following their illness. In some cases, as mentioned above, antibody increases were obtained, but without any history of illness prior to the antibody increase reported.

The individual variation to vaccination is quite apparent in several cases. For example, student 10 (Figure 2) maintained a constant titer for influenza A and Swine, of 160 throughout the test period. On the other hand, his titer for influenza B remained unchanged following his vaccination but jumped from a relatively high titer to a much higher titer 2 months after vaccination. This higher titer was maintained for a period of 5 months before dropping to its original level. Another student, No. 17, also showed no reaction to the vaccination, maintaining the same level for all 3 viruses for some time.

These titers with several other representative titrations may be seen in Figure 2. It is quite apparent that individual variation with response to vaccination and infection may be noted in immunological studies of this type.

DISCUSSION

The evidence presented indicates that the epidemic in the civilian population of this area was due to influenza B. Indications for the presence of this virus in the population previous to the actual outbreak have been mentioned (1, 13, 25), and further evidence is now reported.

The importance of recognition of the virus in advance of the epidemic cannot be stressed sufficiently. The value of vaccination during this epidemic and the importance of the agent's recognition have been stressed by Francis and coworkers (13). We can only speculate, but the use of influenza vaccines at the proper time may well have reduced our number of cases considerably. That there were many more cases among the population

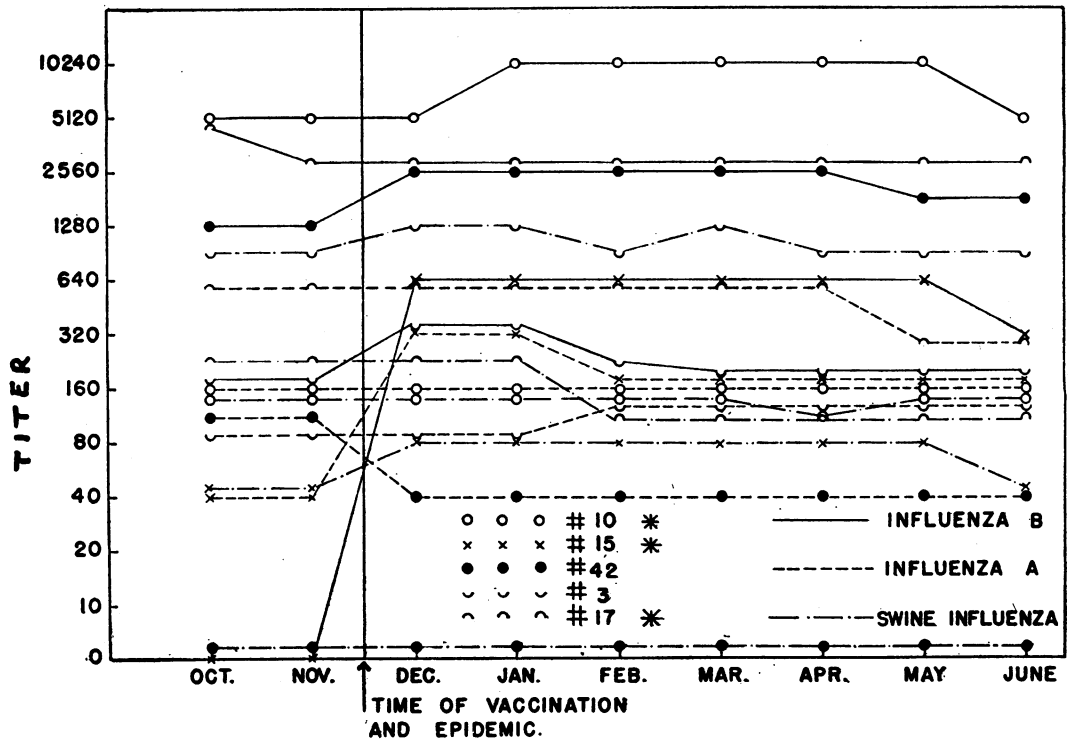

FIG. 2. INDIVIDUAL TITERS OF FIVE STUDENTS OVER THE NINE MONTH PERIOD FOR INFLUENZA A, B AND SWINE

*Vaccinated following their November bleeding.

was recognized, since the disease varies among individuals in its severity and need for hospitalization. The need for studies along these lines is imperative for the early recognition of the etiologic agent in order to administer vaccines at the proper time. The time element for administration of the vaccine is of importance as has been shown by several workers (8 to 11). Reinfection, although in a milder form, may occur after several months have elapsed between vaccination and experimental infection. This is apparent in the antibody decline following vaccination subsequent to its initial increase. The results indicate that the prophylactic administration of the influenza vaccine should be given during the month of September in order to offer the desired protection before the outbreak of the epidemic.

The significance of antibody levels is still questionable in resistance to influenza. What may be considered a high level apparently is relative to the individual. Several individuals in our groups had no initial level. This was evident among the patients and the students. There were several who had no initial titers to one or more of the influenza strains. Then, only because of vaccination or possibly infection, were titers obtained. We are still unable to determine what may be considered as a protective antibody titer to each individual.

Usually, the antibody response to infection with influenza B resulted in the production of antibodies specific for that agent. There were some individuals who developed a 2-fold antibody increase to our PR 8 strains and some to our Swine strain. We feel confident that these slight increases are not due to antibody destruction because all materials were handled in such a manner as to avoid bacterial growth. The possibility of an anamnestic reaction as postulated by Bodily and Eaton (26) must be considered. These authors also suggest that their high titers to the Swine strains may be due to the strain responsible for their cases which have a greater antigenic fraction in common with the Swine strain than

other strains of A. Another consideration may be that some individuals respond with greater antibody levels than others to the antigenic components present in the infecting strains. Henle, *et al* (12), although using sterile sera, also found similar reactions, *i.e.*, the failure to respond to vaccination among certain individuals as well as the increased antibody to the heterologous virus. These factors may be applicable to our cases, except for the usual consideration that there are no related antigenic components between influenza B and Swine. However, there is the possibility that the strain of influenza B causing the epidemic in this locality may have contained some Swine component. The failure to find significant increases to influenza A makes us feel that we are not dealing with a dual infection.

The presence of antibodies to Swine influenza in human sera has been a debatable point for some time. Whether these antibodies are resultant from previous infection with a virus of the pandemic type, as had been postulated by Shope (28), or due to repeated exposure to the human influenza virus of the pandemic type, as proposed by Francis and Magill (29), cannot be answered yet.

Our results would seem to indicate that the titers obtained for Swine influenza could be due only to infection with an agent containing Swine antigenic components. This would be possible only if infection occurred with an A strain composed of some Swine components, a Swine strain which is a variant of influenza A, or a B strain with Swine components. This last possibility is unlikely unless there is a B strain as yet unisolated or untested for the presence of common antigenic fractions with the Swine strain. If the Swine titers are due to infection with an A type, what happened to the A antibodies? Several of our group had no measurable amount of A antibody. Does the A antibody disappear before the Swine antibody? It appears that the probable explanation is that the Swine strain is a type A strain differing from the PR 8 strain in its antigenic structure.

From the results it is evident that the individuals' immunological response to these 3 influenza strains are in many instances completely independent.

With our results, we were able to ascertain many of the answers to our questions. We were somewhat disappointed in the results of the titrations inasmuch as we had anticipated a much sharper antibody increase during the epidemic. However, this was compensated by the fact that the evidence indicated that the virus for the epidemic was present far before the actual outbreak. Also, factors other than the presence of the virus are probably necessary for the outbreak. That the epidemic was due to influenza B has been discussed. There was little need for any differential diagnosis of this epidemic from other syndromes since infections by other agents did not occur to any great extent. That the method we used was satisfactory remains as yet to be determined under conditions other than those present during this study. Perhaps other agents, for example, that isolated by Meicklejohn and coworkers (30), should be tested for at the same time. At any rate, it is felt that studies along these lines would strongly indicate the etiological agent of this type of respiratory infection.

Although the vaccination occurred incidental to our original experiments and the members vaccinated are relatively few, the results of the vaccination confirm the findings of other workers in that usually strong results were obtained. The somewhat ambiguous immunological response demonstrated by a few does not appear to belittle the actual efficacy of vaccination. This seems to be in accord with the findings of other workers (12, 26). As would be expected, the antibody level of such a group would be varied. In all cases, the antibodies present were indicative of the patients' past contacts. In most cases, antibodies were present for the influenza viruses. One must also consider the possibility of other respiratory infections which are clinically similar to influenza. These may be differentiated only by the failure to produce a specific influenza antibody response and the production of an antibody to the particular etiological agent. Such agents are members of the psittacosis group (22, 31, 32, 33) and the virus of atypical pneumonia (30). Florman (23), employing Lygranum CF for studies on these agents as possible causes of virus pneumonias, suggests employment of this type of antigen for the diagnosis of agents of this group. As we have demonstrated, the presence of antibodies to the

psittacosis group were few and the titers obtained could easily have been non-specific. Several workers have reported that a low percentage of normal sera will give positive reactions with antigens of this nature when employed in low dilutions (21, 23, 34, 35). None of the students gave histories to indicate contact with members of this group. The patient who titered to 20, was Frei negative and unable to give a history of contact with birds. The interpretation of this titer is, therefore, difficult.

The value of cold agglutinins and streptococcal agglutination is still questionable. On the basis of our results, their value still remains an enigma. However, in agreement with Favour (36) and Finland, *et al* (37), we feel that our results demonstrate that normal individuals as well as many conditions cause type O cells to agglutinate in the cold with titers of less than 40. And still more individuals cause the agglutination of the indifferent streptococcus in low dilutions, as also observed by Finland, *et al* (38). We believe that these diagnostic procedures are of aid in atypical pneumonia only when viewed with other procedures such as x-ray and clinical findings.

It is felt that this group of tests may be of value to the laboratory in establishing the etiology of respiratory infections. Also, because of their simplicity, diagnostic laboratories should be able to employ them as routine group tests in the laboratory diagnosis.

CONCLUSIONS

1. The epidemic in this area was due to influenza B.

2. This virus was present for several months before the actual outbreak.

3. Vaccination results in an antibody increase lasting in most cases for at least 5 months. Vaccines should then be administered in the fall of the year in order to prevent infection.

4. Serological studies of this nature are of value in: (*a*) determining the etiologic agent, (*b*) ascertaining the duration of antibody titers, and (*c*) estimating the approximate time for administration of vaccines.

5. These tests may be used as a battery of tests by the laboratory, in order to ascertain the etiology of this type of respiratory infection.

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