JCI The Journal of Clinical Investigation

PROTECTIVE EFFECT OF VACCINATION AGAINST INDUCED INFLUENZA B

Jonas E. Salk, … , Philip N. Brown, Thomas Francis Jr.

J Clin Invest. 1945;24(4):547-553. https://doi.org/10.1172/JCI101634.

Research Article

PROTECTIVE EFFECT OF VACCINATION AGAINST INDUCED INFLUENZA B [1]

By JONAS E. SALK,[2] HAROLD E. PEARSON, PHILIP N. BROWN, AND THOMAS FRANCIS, JR.

(From the Department of Epidemiology and Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor, and the Ypsilanti State Hospital, Ypsilanti, Michigan)

(Received for publication December 19, 1944)

In the previous paper (1), evidence was presented to show that subcutaneous vaccination of human subjects with a vaccine containing inactivated influenza viruses, Types A and B, was followed by an increased resistance to experimentally induced infection with influenza virus, Type A. The present report deals with the clinical and laboratory results of a similar study illustrating the effect of subcutaneous vaccination upon resistance of another group of individuals to induced infection with influenza virus, Type B.

A brief summary of the clinical results has been reported (2).

MATERIALS AND METHODS

Vaccine. The vaccine employed was the same as that used in the preceding study (1) and contained influenza virus of both Types A and B. The immunizing capacity of the vaccine for mice was such that 2 doses of 0.5 ml. each of a 2×10^{-4} dilution of vaccine, given intraperitoneally a week apart, protected mice against at least 1,000 50 per cent mortality doses of mouse passage Lee virus, given intranasally, 1 week after the last intraperitoneal injection.

Virus preparation used for infection. The Lee strain of Type B influenza virus was used. Virus contained in frozen and dried lung tissue of mice infected with this strain was transferred to eggs for preparation of the infected allantoic fluid that was employed for the human infection. The mouse lung tissue consisted of a pool of lungs containing virus which had been passed 1 to 5 times in mice, after 9 to 12 passages in ferrets. Allantoic fluid from the second and third egg transfers was concentrated approximately 10-fold by adsorption onto red blood cells of the embryo and elution into physiological salt solution (3). Fluid containing the virus was placed in a rubber-capped vaccine vial and stored at 4° C. for one week before use. Tests for sterility were made. At the time the fluid was used to infect humans it had the following properties:

(*1*) Infectivity for mice:

10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
3, 4, 4	3, 4, 4	4, 4, 5	5, 6, +++	6, 7, +++	++++, +++, +++

(Figures denote day of death of individual mice. Plus signs refer to degree of pulmonary consolidation in survivors autopsied on the 11th day.)

(*2*) The hemagglutination titer was 10, 240 (4, 5).

Subjects. The subjects were similar to those described in the preceding paper (1) and included 96 men residing in a single ward of the Ypsilanti State Hospital, Ypsilanti, Michigan.

Vaccination. On December 21, 1942, a subcutaneous injection of 1 ml. of the combined Types A and B vaccine was given to each patient in half of the ward population. The others were given physiological salt solution containing formalin and preservative in the quantities present in the vaccine.

During the subsequent 4½ months, all cases of respiratory disease resembling influenza were studied. Although a few sporadic cases of Type B influenza were observed in some of the women's wards in March, 1943, no evidence of Type B infection was found in the male side of the instiution.

On April 13, 1943, approximately 4 months after the initial inoculations, the residents of the ward comprised 46 vaccinated persons and 50 unvaccinated controls. At this time, 19 of the vaccinated group were given a second injection of the same lot of vaccine administered in December; 23 of the control group received an initial injection of vaccine.

Infection. Two weeks before exposure to infection by inhalation of the Lee strain of Type B virus, the subjects involved in the present experiment inhaled for 1 minute allantoic fluid containing the Baum strain of Type

[1] These investigations were aided through the Commission on Influenza, Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army. This study was also aided by a grant from the International Health Division of the Rockefeller Foundation.

[2] Fellow in the Medical Sciences of the National Research Council 1942–1943.

A virus. The experiment was unsatisfactory since only a few febrile reactions were observed after this brief period of inhalation. Accordingly, the Type A experiment (1) was repeated on an adjacent ward and the present group was used in the test with the antigenically unrelated Type B virus.

On May 10, 1943, all subjects were exposed to infection with the Lee strain of influenza virus Type B. At this time, the ward population was comprised of the following groups:

(*1*) A control group of 27 unvaccinated individuals;

(*2*) 27 men who had received vaccine 4½ months previously;

(*3*) 23 individuals who had received vaccine 4 weeks before infection;

(*4*) 19 men who were vaccinated both 4½ months and 4 weeks prior to infection.

The methods of inducing infection and of making observations were essentially the same as described in the previous study. The only difference was in the duration of exposure. In the present experiment, approximately 0.6 ml. of fluid containing virus was inhaled during a period of 5 minutes. Virus was distributed in the atmosphere during the spraying procedure and persisted in the ward for at least 24 hours; virus was detected by exposure of mice in the manner described in the previous report (1).

Throughout the interval of 7 hours during which the virus inhalation was being administered, groups of 10 mice in wire mesh cages were kept in the treatment room and in two other locations on the ward, 25 to 30 feet away. On the following day, another cage of 10 mice was placed on the floor of the corridor just outside the treatment room for a period of 2 hours. Half the mice in each group were given an intranasal inhalation of sterile broth 4 days after exposure of the original groups. All were observed for 11 days, at which time survivors were autopsied for evidence of pulmonary involvement. The results were as follows:

Location	Exposed only	Exposed and inoculated intranasally with sterile broth
Treatment room	7, 7, 7, 8, 9	6, 7, 7, 7, 8
Corridor	9, +++, +++, ++, ++	7, 7, 7, 7, 8
Day room	++, 0, 0, 0, 0	7, 8, ±, 0, 0
Outside treatment room 24 hours later	0, 0, 0, 0, 0	++++, +++, +++, +++, +++

(Numerals denote day of death after exposure. Symbols indicate degree of pulmonary involvement in survivors autopsied on 11th day.)

Apparently active virus persisted in the atmosphere or floor dust in the vicinity of the mice exposed 24 hours after the virus was sprayed.

Clinical observations. Before admission to the group all subjects were examined for signs of respiratory disease. Temperatures were taken sublingually prior to and then twice daily for several days following the inhalation of virus. All subjects were seen twice daily by nurses who were familiar with their usual behavior. Observations were also made by physicians. Observers did not know to which group the subjects belonged.

White blood cell counts were obtained from 21 individuals on the day of exposure to the virus and then daily for 3 days.

Serology. Blood for serological study was obtained from individuals vaccinated on April 13, 1943. On April 27, 1943, samples of blood were taken from all subjects. Titers of antibodies in the sera were determined using the agglutinin-inhibition reaction (4, 5). An interval of 13 days elapsed between the pre-infection bleeding and exposure to the virus. Blood was drawn 2 weeks after inhalation of the active virus.

CLINICAL PICTURE OF EXPERIMENTALLY INDUCED INFLUENZA B

The clinical picture of the experimentally induced Type B infection was similar to that previously described (6). When compared with the illness induced by the Type A virus (1), several differences were evident. In the Type B infection, the incubation period appeared to be shorter, with symptoms and fever beginning less than 20 hours after inhalation of the virus. The illness was milder and of shorter duration than in the infection induced with the Type A virus. In only one subject did a temperature of 100° or greater persist longer than 24 hours after exposure to the virus. The maximal temperature observed was 102.6°. In the Type B infection, respiratory symptoms were usually absent, and in only a few instances was a mild, dry cough observed. Symptoms of chilliness, headache, bodyaches, and malaise generally were parallel to the degree of fever. Recovery was complete in 24 to 48 hours after the inhalation of virus.

Physical signs of infection were few. Flushed skin and some prostration accompanied the fever. Signs of involvement of the respiratory tract were not detected.

DISTRIBUTION OF CLINICAL DISEASE IN CONTROL AND VACCINATED GROUPS

As in the study with influenza A, temperature reactions of 100° or more were considered indicative of distinct clinical response.

The febrile reactions of the vaccinated and control subjects are shown in Table I. In the unvaccinated group, 11, or 41 per cent, of the 27 individuals had temperatures of 100° or more; 6, or 22 per cent, had temperatures of 101° or above; and 2, or 7 per cent, had temperatures of 102° or higher. Of the 69 vaccinated subjects, 7, or 10 per cent, had temperatures between 100° and 100.8°, while none had a higher temperature. There was no significant difference in the responses of the groups vaccinated 4½ months before, 4 weeks before, or vaccinated twice before exposure. In contrast to the results obtained in the study with influenza A, there appears to have been no difference in resistance of the group vaccinated 4½ months before, as compared with the groups inoculated within the shorter interval before exposure to infection.

TABLE I

Effect of subcutaneous vaccination upon febrile response of human subjects to induced infection with influenza virus, Type B

Vaccination record	Number of subjects	Highest temperature									
		<99		99+		100+		101+		102+	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Unvaccinated	27	5	19	22	81	11	41	6	22	2	7
4½ mos. before	27	12	44	15	56	2	7	0	0	0	0
4 weeks before	23	12	52	11	48	3	13	0	0	0	0
4½ mos. and 4 weeks before	19	4	21	15	79	2	11	0	0	0	0

SEROLOGICAL RESPONSES OF VACCINATED AND REVACCINATED SUBJECTS

In this study, the antibody response to the Type B component of the vaccine was determined both in subjects receiving their first injection and in subjects inoculated for the second time. The results shown in Figure 1 were similar to those described for the Type A antigen.

All 21 of the group vaccinated for the first time had a demonstrable increase in serum antibody; 4, or 19 per cent, had a 2-fold change, and 17, or 81 per cent, had a change of 4-fold or more. In the group of 17 who had been vaccinated 4 months earlier, 12, or 70 per cent, had no additional rise in titer following the second inoculation, and the rest showed only a 2-fold change. The distribu-

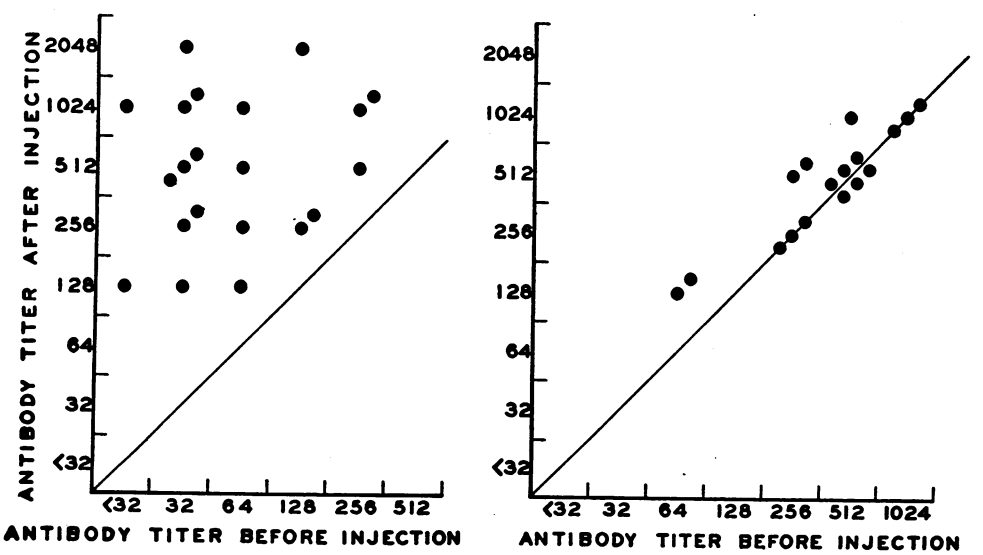

FIG. 1. A. CHANGE IN TYPE B ANTIBODY TITER FOLLOWING FIRST INJECTION OF VACCINE. B. CHANGE IN TYPE B ANTIBODY TITER FOLLOWING SECOND INJECTION OF VACCINE 4 MONTHS AFTER FIRST.

tion of titers before and after vaccination was as follows: In the previously unvaccinated group, 86 per cent had titers of 128 or less before vaccination, while after vaccination only 15 per cent remained within this zone. In the group receiving their second dose of vaccine, 2, or 12 per cent, had titers of 128 or below, both before and after the inoculation. Thus, the effect of the initial inoculation persisted for an interval of 4 months and vaccination at this time exerted little or no effect upon the serological titer. These observations conform with those described in the previous study (1).

RELATION BETWEEN TITER OF SERUM ANTIBODY AND RESISTANCE TO INFECTION

Analysis of the data of this experiment, in order to determine to what extent resistance to induced influenza B may be related to the level of serum antibody, has revealed essentially the same problem that was encountered in the experiment with influenza A. As shown in Figure 2, the distribution of antibody titers in the control group was predominantly in the lower range, while in the vaccinated groups, titers were clearly higher. Since, in any one group, antibody titers were either in the lower or upper zones, the presence of any relationship between antibody level and resistance would not be evident from an examination of the results observed in any one of the 4 groups. However, from the composite chart, it appears that the incidence and degree of febrile reactions were lower in the group of individuals with antibody titers in the higher zone, as compared with those having lower levels of antibody. Of 58 subjects with antibody titers of 256 or above, only 5, or 9 per cent, had temperatures of 100° or more, while 15, or 35 per cent, of 37 individuals having titers of 128 or less developed fever of 100° or more. Furthermore, only 1 of the former, and 9 of the latter, had temperatures exceeding 100.2°.

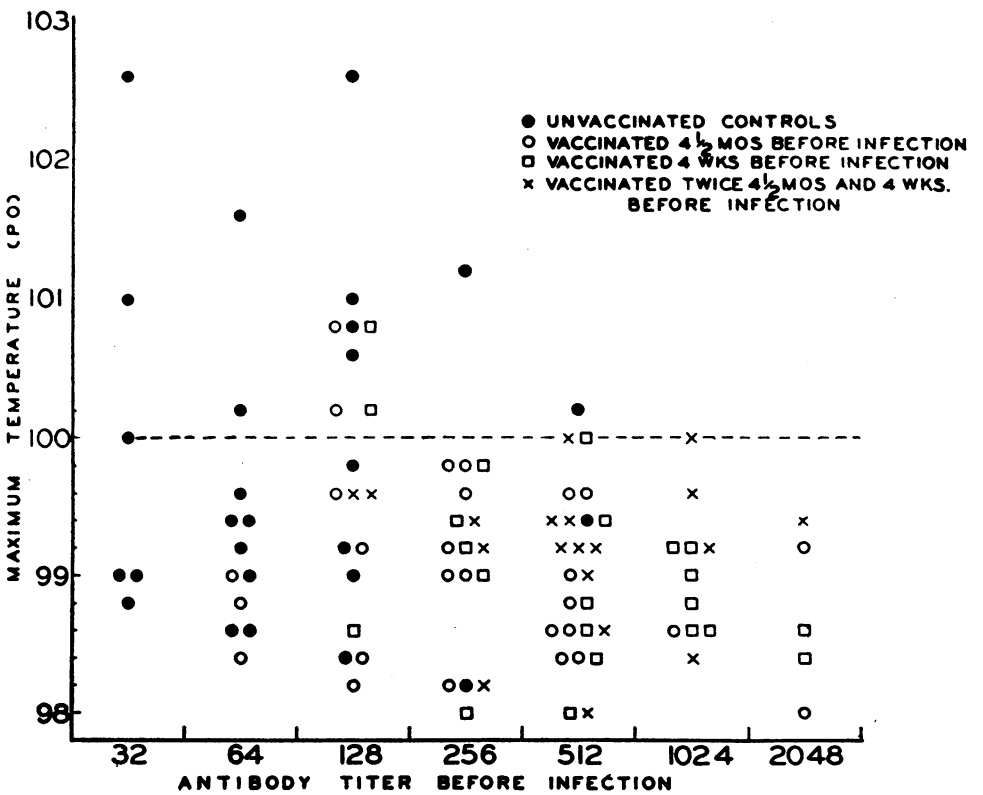

FIG. 2. RELATION OF ANTIBODY TITER AND FEBRILE RESPONSE TO INFLUENZA VIRUS, TYPE B, IN VACCINATED AND CONTROL SUBJECTS

DIAGNOSIS OF INFECTION BY SEROLOGICAL MEANS

In the previous report, it was pointed out that while a fairly high correlation was found between distinct clinical responses and positive serological reactions to inhalation of active influenza virus Type A, in a group of unvaccinated individuals, the correlation between the two was very low in a group of vaccinated subjects.

In the present experiment with a preparation of influenza virus Type B, there were many more instances in which clinical reactions occurred in the absence of serological reactions, both in control and vaccinated subjects. Of the 12 subjects in the control group who had temperatures of 100° or more, 7 had no demonstrable increase in antibody titer; while of the 9 subjects in the vaccinated group who had this degree of fever, 8 had no rise in antibody titer. Among those in the respective groups who did not develop distinct fever, 68 per cent of the controls and 88 per cent of the vaccinated showed no increase in serum antibody.

The reason for the low frequency of serological response in the presence of clear-cut clinical reactions is not apparent. Several pairs of sera were tested by means of the mouse protection test, and the results confirmed the findings of the red cell test.

COMPARISON OF RESISTANCE RESULTING FROM VACCINATION AND INDUCED INFECTION

It is of interest to compare the results of the present study with those reported earlier (6) which were concerned with the response to a second exposure of individuals previously infected with influenza virus, Type B. There were certain differences between the two. In the re-infection study all 23 of the controls reacted with temperatures of 100° or higher and 14 of them had temperatures of 102° or more, while in the control group of the vaccination study, 11 of 27 had temperatures of 100° or more and only 2 of them had temperatures of 102° or higher. Furthermore, in the re-infection experiment, rectal temperatures were recorded, while in the vaccination study temperatures were taken orally.

The results of these two experiments are compared graphically in Figure 3. While temperatures were generally higher in the re-infection experiment as compared with the vaccination study, the relationship between the curves describing the febrile reactions in treated and control groups in both studies was similar. It is probable that the size of the challenge dose in the re-infection experiment had been too great for the forces of resistance in a considerable proportion of individuals who might have resisted a smaller infecting dose. That the previous treatment in each instance had a buffering effect is clearly demonstrated. In fact, it almost seems that the recent infection exerted a greater effect than vaccination, as indicated by the degree of difference between control and treated groups in the respective experiments. While the greater difference between treated and controls in the re-infection study may indicate relatively greater resistance of recently infected subjects as compared with vaccinated individuals, it is equally conceivable that this effect may be related to the degree of multiplication of free virus available for infection, which probably does not vary in simple proportion with the amount of free virus present at the beginning of infection.

DISCUSSION

The results of the present study have clearly demonstrated the beneficial effect of subcutaneous vaccination in preventing infection with influenza virus, Type B, administered intranasally. The effect of the vaccine upon resistance appeared to have persisted for at least 4½ months. It is apparent that, in general, the levels of serum antibody in the vaccinated subjects were higher than in the unvaccinated individuals, and that clinical reactions were, for the most part, fewer and less marked in the former. Nevertheless, it is not possible to conclude from the present data that the beneficial effect of vaccination was due to the heightened concentrations of serum antibody.

Whatever relationship may exist between serum antibody titer and resistance is certainly not an absolute one, as indicated by the observation that many individuals with low levels of serum antibody appear to have resisted infection, whereas some with higher concentrations of serum antibody developed distinct clinical reactions. Because of the superficial site of influenza infection, relatively remote from the direct effect of immune substances in the blood, it is believed that the

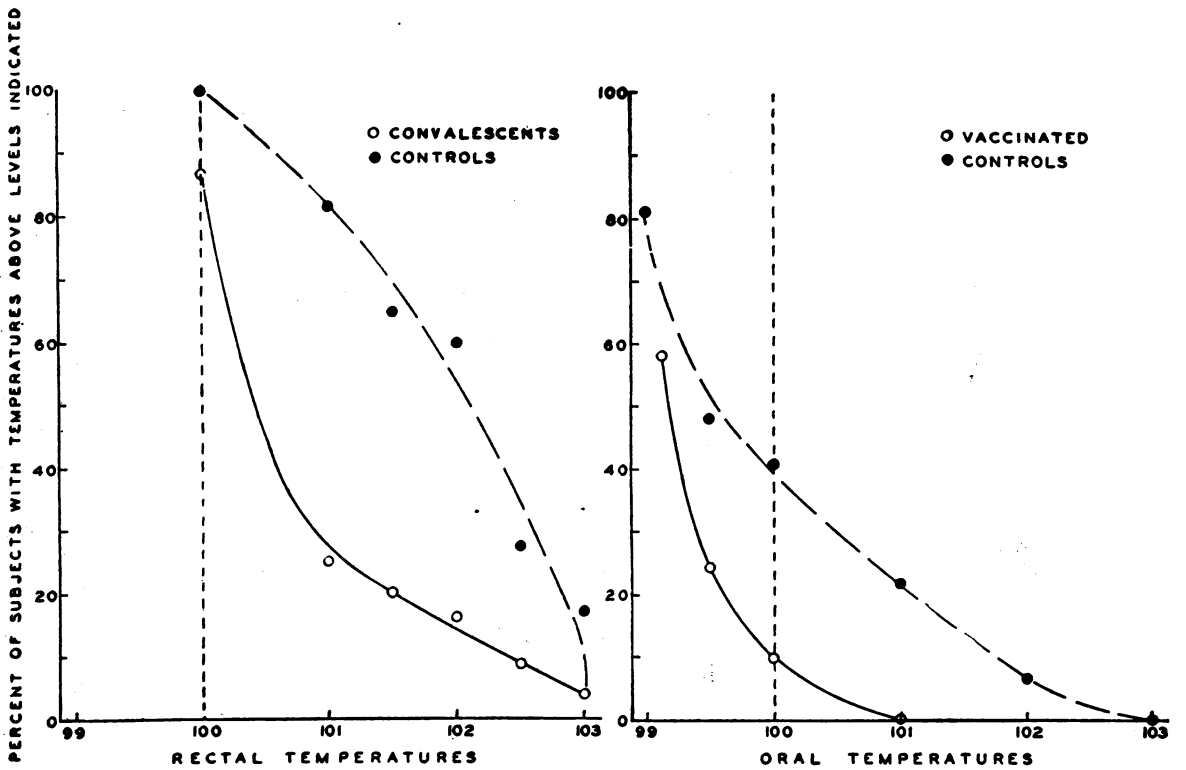

FIG. 3. A. RE-EXPOSURE TO INFLUENZA B, 4 MONTHS AFTER INITIAL INFECTION. B. EXPOSURE TO INFLUENZA B INFECTION FOLLOWING VACCINATION

concentration of antibody in the general circulation cannot be considered more than an indirect index of immunity, and its significance is probably proportional to the effect it may have upon the concentration of antibody in the superficial secretions of the respiratory tract. Physiological features which might affect the interchange of antibody between the two media could under these conditions influence resistance.

The extent to which the mechanism which has been suggested is complemented by cellular modifications, which might result from repeated exposures and increasing age, in contributing to clinical immunity, is not known at the present time. However, recent studies (6) have shown that induced infection with influenza virus, Type B, did not result in uniform resistance to the same strain when tested 4 months later. It is true that the challenge infection was rather severe. Nevertheless, the reaction at the time of the second exposure to virus was not one of complete refractoriness, but rather one which might be interpreted as due to a quantitative reduction in the amount of virus available for infection (Figure 3).

SUMMARY

A study was made of the reactions of previously vaccinated individuals to intranasal administration of influenza virus, Type B.

A group of 96 human subjects was divided into 4 groups. They were exposed to infection by inhalation of a strain of Type B influenza virus in the form of a concentrate of virus from allantoic fluid. Clinically recognizable disease, associated with fever of 100° or more, was observed in 41 per cent of the controls, 7 per cent of the group vaccinated 4½ months before infection, 13 per cent of the group vaccinated 4 weeks before, and 11 per cent of those receiving two inoculations, 4½ months and 4 weeks before infection.

The relationship between antibody level and resistance was analyzed and discussed. While 35

per cent of 37 individuals with pre-infection antibody titers of 128 or less developed fever of 100° or more, 9 per cent of 58 subjects having antibody titers of 256 or above had distinct clinical reactions. Moreover, 9 of the former and only 1 of the latter had temperatures exceeding 100.2°.

The restricted antibody response to revaccination within an interval of 4 months, reported in the preceding studies of experimental influenza A, was again noted.

The duration of the immunizing effect of the vaccine appeared to have persisted for at least 4½ months.

BIBLIOGRAPHY

1. Francis, T., Jr., Salk, J. E., Pearson, H. E., and Brown, P. N., Protective effect of vaccination against induced influenza A. J. Clin. Invest., 1945, **24**, 536.
2. Salk, J. E., Pearson, H. E., Brown, P. N., and Francis, T., Jr., Protective effect of vaccination against induced influenza B. Proc. Soc. Exper. Biol. and Med., 1944, **55**, 106.
3. Francis, T., Jr., and Salk, J. E., A simplified procedure for the concentration and purification of influenza virus. Science, 1942, **96**, 499.
4. Hirst, G. K., The quantitative determination of influenza virus and antibodies by means of red cell agglutination. J. Exper. Med., 1942, **75**, 49.
5. Salk, J. E., A simplified procedure for titrating hemagglutinating capacity of influenza-virus and the corresponding antibody. J. Immunol., 1944, **49**, 87.
6. Francis, T., Jr., Pearson, H. E., Salk, J. E., and Brown, P. N., Immunity in human subjects artificially infected with influenza virus, Type B. Am. J. Pub. Health, 1944, **34**, 317.