THE ANTIBODY RESPONSE OF HUMAN SUBJECTS VACCINATED WITH THE VIRUS OF HUMAN INFLUENZA

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(Received for publication, October 28, 1936)

Previous studies (1, 2) have revealed that the virus of human influenza is capable of infecting mice and ferrets only when introduced into the respiratory tract. When mice are inoculated subcutaneously or intraperitoneally infection does not occur, but with repeated inoculations in this manner the animals develop an active resistance to infection by the respiratory route. Similarly, though to a somewhat less extent, ferrets, while exhibiting no evidence of infection from subcutaneous injections of active virus, develop circulating antibodies and increased resistance to the virus introduced by way of the nasal passages.

Because of the significance of these observations as a possible guide toward preventive measures, it was important to determine the effects of the introduction of untreated active virus in human individuals. The use of animal tissues as the source of virus for human study is accompanied by certain undesirable features, such as the possibility of protein sensitization, or the introduction of bacterial or extraneous virus contaminants. In order to eliminate these difficulties, the virus derived from infected mouse lungs was introduced into tissue culture medium. The medium was that described by Li and Rivers (3), which consists of minced chick embryo suspended in Tyrode's solution.

Under these conditions the virus multiplied readily (4, 5) and has been carried through 160 subcultures in 11 months. The culture virus maintains its capacity to infect mice and ferrets by intranasal inoculation and also to induce immunity in these animals when administered by subcutaneous and intraperitoneal routes. It was employed for the work described in the present paper. This embodies the results of

titrations of the capacity of the serum of 22 human individuals, before and at intervals up to 5 months after vaccination, to neutralize approximately 1000 lethal doses of the mouse passage human influenza virus as measured by mouse protection tests.

EXPERIMENTAL

The results of studies of Andrewes, Laidlaw, and Smith (6) in England and those of Francis and Magill (7) in the United States have shown by mouse protection tests that the serum of a high percentage of individuals of all ages possesses the capacity to neutralize the human influenza virus. In order to test the effect of vaccination, it was necessary to select so far as possible subjects whose serum beforehand possessed the least neutralizing capacity.

From 60 available volunteers, 23 were chosen for the test. All but 5 were medical students in the third decade of life. The others ranged from 35 to 64 years of age. The supernatant fluid of cultures made as above described from which the cells had been removed by centrifugation at low speed, was used for vaccination. To 11 of the subjects doses of 0.5 cc., 1.0 cc., and 1.0 cc., respectively, were given subcutaneously at weekly intervals, and after a further interval of 2 to 3 weeks an additional dose of 2.0 cc. of the virus-containing fluid was given by the same route. To 5 individuals doses of 1.0 cc., 1.0 cc., and 2.0 cc., respectively, were given subcutaneously at weekly intervals. The remaining 7 subjects were given three successive doses of 0.5 cc. of culture virus intradermally at weekly intervals.

A sample of serum was obtained from each subject before vaccination, before each subsequent injection, and 10 days after the final injection (8). Where possible, serum was again obtained from each volunteer 2 and 5 months after the final vaccinating dose of virus.

The virus used in the protection tests was the mouse passage Puerto Rico 8 strain. The virus was obtained from the lungs of infected mice, which were ground and suspended in 10 per cent normal horse serum in physiological salt solution. After centrifugation at 2000 revolutions per minute for 15 minutes, the supernatant fluid was diluted to a 2 per cent virus concentration. Each serum was tested undiluted and in dilutions of 1:5, 1:10, 1:20, 1:40, 1:80, and 1:160. Serum dilutions were made with physiological saline. To each serum dilution was added an equal quantity of 2 per cent virus suspension; the mixture was incubated at 37°C. for 30 minutes; and 3 mice were then inoculated intranasally with 0.03 cc. of the mixture. The 4 specimens of serum taken at various times from the same individual were subjected to test at the same time, and usually those from 4 individuals were tested together. As a control, the serum of one individual taken 5 months after recovery from influenza was titrated in each experiment.

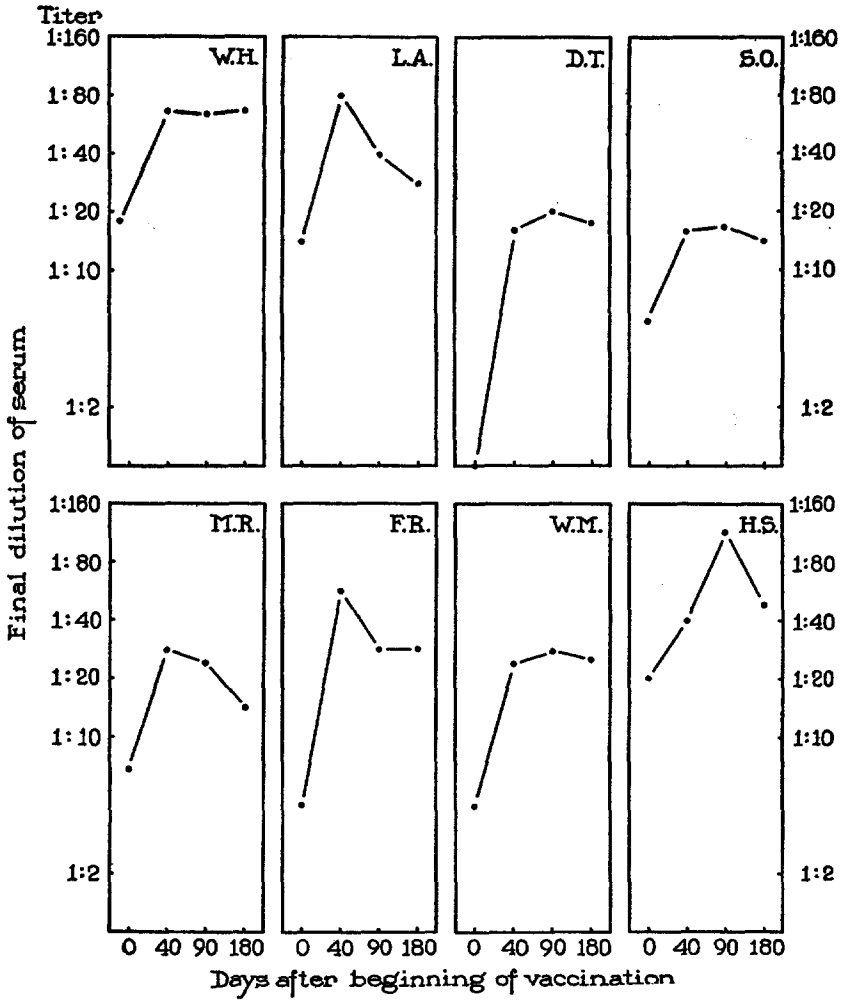
Each was terminated on the 7th day after infection: all surviving mice were autopsied then and the extent of the pulmonary involvement was recorded. The titer of the serum was estimated as the greatest dilution which resulted in a 50 per cent survival of the mice throughout this period. Mice which presented extensive lung lesions at autopsy on the 7th day were considered, however, not to have been protected.

RESULTS

The results were uniform in that the serum of each individual developed an increased capacity to neutralize human influenza virus as a result of vaccination. This increase is not a slow, gradual rise throughout the period of vaccination. On the contrary, the most significant rise in antibodies occurs rather abruptly in the 2nd week. The serum titrations reveal the facts that the antibody levels are highest immediately following the course of vaccination, that in general the same approximate concentration is maintained for 2 months, but that at 5 months a decline in titer is observed (Text-figs. 1, 2, 3). In spite of the fact that a decline in titer occurs, the residual titer of neutralizing antibodies in all but one instance remained at a level well above that of the original.

While the same general trend prevailed in the entire series, considerable variation occurred in the antibody response of different individuals. The height of the titer attained and the rate of decline in titer with the passage of time seem to be functions of the individual subject. Nevertheless, the results suggest that those persons whose serum possessed the most antibody prior to vaccination responded to vaccination with the formation of less additional antibody than subjects whose original titer was quite low. For comparison the sera of three patients who actually suffered from influenza (2) and their sera 3 weeks after recovery and again 5 months after recovery were titrated (Text-fig. 4). In these three cases a sharp antibody rise is evident after 3 weeks' convalescence. The height of antibody in these cases is not strikingly dissimilar to that of the vaccinated group (Text-fig. 5). Moreover, the same tendency to decline after 5 months is observed, though perhaps to a less extent.

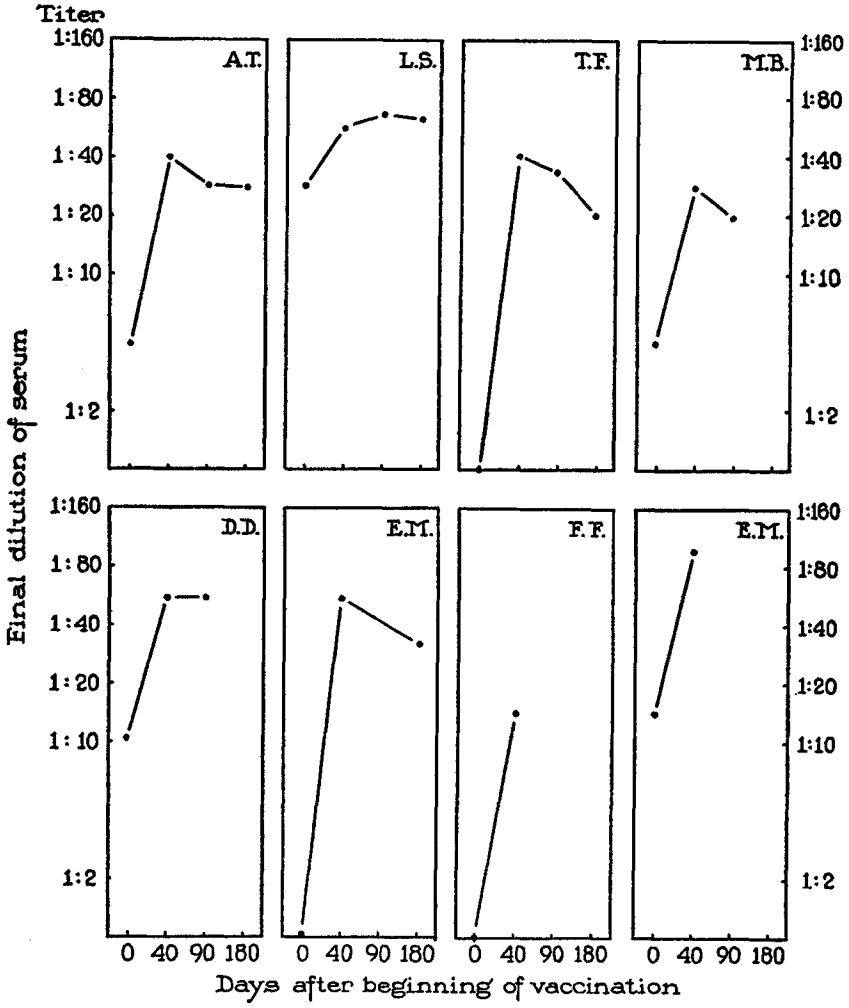
The results in the group of individuals receiving vaccination by the intradermal route appear to follow the same general course as in those inoculated subcutaneously. The mean titer of the serum of the former



TEXT-FIG. 1

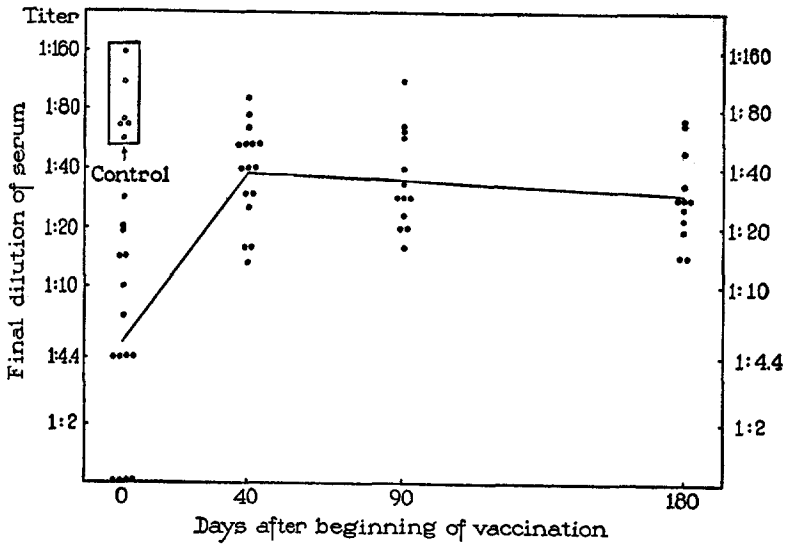
TEXT-FIGS. 1 and 2. Antibody response to subcutaneous vaccination. Each block represents the findings in a single individual vaccinated by the subcutaneous route. The time interval between the first vaccinating dose of virus and the date on which the serum was obtained, representing the end of the period of vaccination, was 40 days. The time intervals are thus measured from the date on which vaccination was begun. The titer of the serum is recorded in terms of final effective dilution.

group before vaccination was approximately 1:10. It increased to about 1:40, a fourfold increase. The mean titer of the subcutaneously vaccinated group was about 1:4 before and 1:40 after, a tenfold

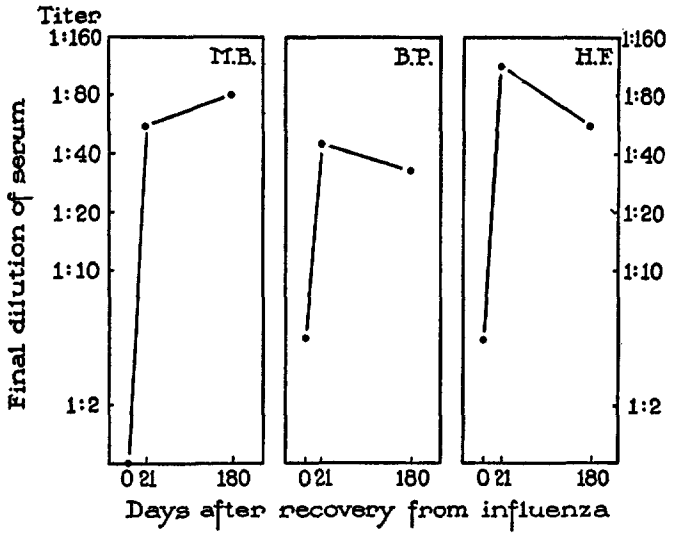


TEXT-FIG. 2

increase (Text-fig. 6). Two modifying factors may play some rôle in this result: first, the total amount of virus administered is smaller; second, these subjects had comparatively high original antibody titers.



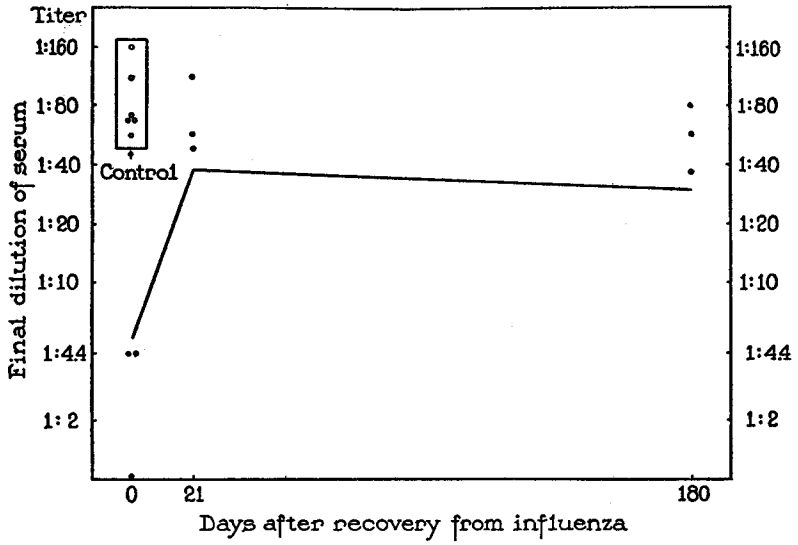
TEXT-FIG. 3. Titers after subcutaneous vaccinations. (Line connects means of groups.) The solid line represents the geometrical mean of all tests with the sera of the subjects vaccinated by the subcutaneous route. The black circles represent the results of individual tests. The titers of the control serum, as measured in different tests, are enclosed in the left upper corner.



TEXT-FIG. 4. Antibody response to natural infection. The titer of the serum of 3 human individuals tested during the acute phase of influenza, 21 days and 180 days later, respectively.

Absence of Unfavorable Reactions Following Vaccination

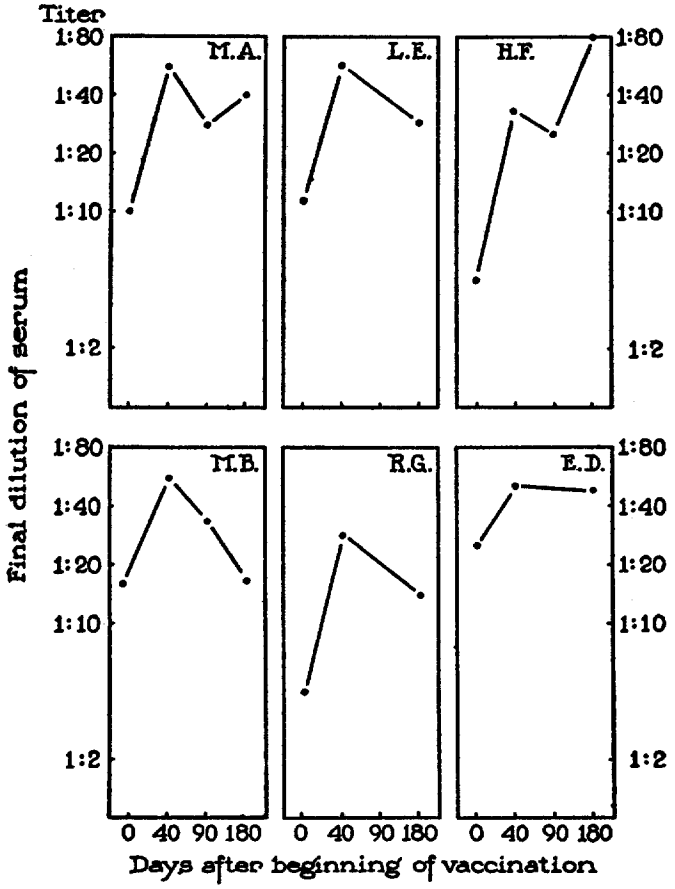
In addition to the knowledge obtained regarding the development and persistence of antibodies following vaccination, it was important to determine whether any ill effects were contributed by the introduction of untreated, active virus. To this end each subject was admitted to the hospital isolation ward for 48 hours at the time of the first injection, and again at the time of the largest (2 cc.) dose. Care-



TEXT-FIG. 5. Titers after natural infections. (Line connects means of *subcutaneous* groups.) The titers of the serum of 3 patients during the disease and up to 180 days after recovery are shown as black circles. For comparison, a solid line is superimposed, representing the mean titers of the sera taken within a similar time period after the subcutaneous vaccination (see Text-fig. 3).

ful isolation precautions were observed, repeated temperatures were taken, all symptoms were recorded, and examinations of the site of inoculation were made. In those who received the virus subcutaneously no significant elevation of temperature occurred, and the local reactions were inconstant and extremely mild—frequently unnoticed by the subject himself. In those who received virus intradermally more immediate erythematous reaction was observed at the site of injection, but no unpleasant features occurred. Furthermore, two

subjects were inoculated with influenza virus while suffering from common colds. No aggravation of symptoms was noted. One subject was given but one dose of 3 cc. of tissue culture virus subcutaneously. After an asymptomatic interval of 48 hours, nasal and



TEXT-FIG. 6. Antibody response to intradermal vaccination. The titer of serum of individuals, taken at intervals up to 180 days after beginning of vaccination by the intradermal route.

pharyngeal washings were obtained to ascertain whether virus could be recovered from the respiratory tract. The concentrated mucus was inoculated into the nose of a normal ferret, which exhibited no evidence of infection, nor were specific antibodies subsequently

demonstrable in the ferret's serum. This indicates, of course, that virus introduced subcutaneously does not readily find its way to the respiratory tract. Furthermore, Chenoweth *et al.* (9) have vaccinated a large group of human subjects with influenza virus obtained from mouse lung. In no instance was evidence of infection observed.

SUMMARY

Human influenza virus cultivated in tissue culture medium may be administered subcutaneously or intradermally to human individuals without causing evidence of infection. Subjects so treated develop a good titer of circulating antibodies effective against mouse passage virus and, if antibodies were previously present, vaccination stimulates the production of more antibody. The antibodies so induced persist for at least 5 months, although in this period of time some decline in titer may have begun. The antibody response to vaccination parallels both in extent and persistence that occurring as a result of the naturally acquired disease.

The available data do not enable one to evaluate the effect of vaccination in preventing human infection with influenza. It seems not unlikely that the increase in circulating antibody will be accompanied by an increased ability to combat the natural infection.

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