

# A CONSIDERATION OF VACCINATION AGAINST INFLUENZA<sup>1</sup>

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**W**ITH your tolerance, I shall just talk rather than give a prepared report. First, let me give a brief background of the studies which have led to the present status of vaccination against influenza.

The problem of vaccination against influenza has had a continuous history of intense activity since 1933, when the British workers, Smith, Andrewes, and Laidlaw, first reported the isolation of the virus from persons apparently suffering from influenza. This was preceded a few years by the studies of Shope and Lewis which identified the disease called swine influenza as being caused by a virus in association with a bacterium of the *Haemophilus influenzae* variety. Investigations have been constantly in progress since that time.

Our work began in 1934, when we were able in this country to confirm the observations of the British workers in the isolation of a virus from epidemic influenza.

At the present time there are two types of influenza which have been identified: the original one, isolated in 1933 and first confirmed in 1934, which is now called influenza A. Influenza A was identified subsequently every two years up until 1940. It skipped 1942 but appeared in 1943, so that up until 1940 there were five epidemics in alternate years, one in 1943, and if chronology means anything, the betting odds would be rather long on the probabilities of having influenza A this year.

In 1936 we encountered an epidemic, widespread throughout the United States, which also seemed to be influenza, but we were not able to recover the previously identified virus nor were we able to get any serological information that the influenza which we knew had been in circulation. In 1940 we

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had the same experience but with a little more good luck; we were able to isolate a virus which had many of the characteristics of the previously known virus except one: it gave no immunity against the previous one. By a brilliant piece of thinking, we called that influenza B.

Clinically, it is very much the same as influenza A; epidemiologically, it has certain differences. One of the major epidemiological differences is that it has been recognized at intervals of four to five years. Secondly, in the course of an epidemic such as that of 1945, it was possible to demonstrate that influenza B had been in circulation in the United States and other parts of the world for a full year, a course quite contrary to the epidemiological behavior of influenza A, which has occurred in short epidemic outbreaks which are ordinarily over in two months.

Another important difference between the two is that immunity to one does not give immunity to the other. This can be shown by repeated inoculations of experimental animals such as the white mouse, which is readily immunized, and testing them with the heterologous virus without demonstrating immunity. Furthermore, the individual who recovers from influenza A does not develop antibodies to influenza B, or vice versa; so that in the sense of prevention and epidemiology and problems of immunity, we are actually talking of two diseases which nevertheless are caused by influenza viruses.

Since 1933 it has been possible to identify the recurrent peaks of the respiratory disease as influenza A or B. The bi-annual epidemics of influenza A from 1934 to 1940 and the epidemic in 1943 after a three-year interval have been mentioned. After isolation of influenza B in 1940, we were able to show that the epidemic in the early months of 1936 was influenza B by testing the virus isolated in 1940 against serum which we had saved since 1936. Again in 1945, influenza B was identified. We know of no epidemics in this period that would be called influenza C. For instance, in 1942-1943 there was decided evidence that the prevalent respiratory disease was

atypical pneumonia. No virus was isolated and there was no evidence that it was another form of influenza by any of the serological tests or by any of the attempts to isolate viruses of a similar nature.

The next point in the basic data that I should like to emphasize relates to the manner in which influenza virus produces its injury, because I think that this has certainly colored our approach to immunization and our thinking on the outlook for control of the disease.

First of all, influenza virus infection is a very rapid one. The sequence of events as observed in the ferret's nose illustrates the injury done by the virus. At the point that influenza virus is introduced into the animal's nose, a very rapid and selective injury takes place. In twenty-four hours after inoculation, exudate is beginning to accumulate in the respiratory turbinate, and at forty-eight hours there is a great deal of exudate. The normal ciliated columnar epithelium is completely destroyed, wiped off by the virus; and the adjacent olfactory epithelium ordinarily is left untouched. The primary action of the virus is on the ciliated epithelium of the respiratory tract. It is selective. It is my belief that this is the primary injury and the primary site of localization of influenza virus, while the pneumonia which occurs in experimental animals is secondary to this damage to the epithelium of the larger air passages.

On this basis it seemed probable that the most effective manner of getting protection would be to apply measures which would be protective at the site at which the virus localizes, the site at which the virus enters the system. In other words, if one could supply immunity to the area where virus alights and would produce its primary injury, that would be the desirable, the ideal circumstance.

It had been shown very clearly, and it can be shown in experimental animals, that one may have antibodies in the blood without having complete immunity. An animal which has antibodies may still have this damage to the respiratory epi-

thelium. We were a little interested in how the antibodies in the blood could have an effect upon this local injury.

In the course of our studies, antibodies were found in the nasal secretions. As an individual recovered from the disease, the amount of antibody in the nasal secretion increased, presumably by exudation or secretion through the membranes from the blood. It is our interpretation that antibodies in the blood have their effect primarily by being exuded into the nasal secretion, where they would protect the tissues at the site where virus would enter and produce its primary injury. On the basis of these observations, for a considerable period our belief was that prophylactic methods, whether active or passive, employed by way of the respiratory route, would appear more likely to produce the desired effect.

Certain other studies had been carried out by various workers in mice. Mice were allowed to inhale a spray of immune serum, and it was shown that one could get actual protection of the mice under those circumstances.

In 1943 and earlier, we had attempted to immunize by spraying the virus into the respiratory tract of individuals but were unable to get consistent effects. In 1943, through the Influenza Commission,<sup>3</sup> a group of studies were carried out in which individuals were sprayed with immune serum and then sprayed with virus. We found no protection whatever, although physiologically and logically, this approach offered many advantages over the pararespiratory route for immunization.

In considering the other mode of vaccination, which I should like to do from here on, one can go back to studies which were undertaken in 1935. At that time it was shown that the virus of influenza ordinarily produced infection only when given by way of the respiratory tract. Essentially that is true. If one gave the virus, even in its fully active form, subcutaneously or intraperitoneally, one did not get infection, but resistance and the development of antibodies were observed. It seemed, there-

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fore, that if one were to carry out this procedure in the human individual and obtained an increase in antibody formation, this might also be associated with protection.

Groups of individuals were inoculated with active virus grown in tissue culture to find out what happened. They did not take sick, even though some of them were inoculated when they had common colds. There was no illness produced by the inoculation of active virus and antibodies did develop. From that time on, the thought concerning the development of subcutaneous vaccination against influenza maintained this concept: that if virus could be given subcutaneously by other than the natural route, without producing infection, this might have a beneficial influence on resistance, as it did in experimental animals.

A number of studies were carried out up to 1941. Hoyle and Fairbrother carried out some in England with centrifuged material. The group in London at the National Institute of Medical Research had used formalized ferret and mouse lung; Stokes and his associates used mouse lung and tissue culture; Muckenfuss and Siegel at the New York City Board of Health had carried out some studies with tissue culture virus; and in 1940 Horsfall and his associates at the Rockefeller Foundation carried out studies with a preparation which presumably was a mixture of distemper virus and influenza virus.

None of these studies gave evidence that the vaccination had any significant effect against the natural disease. There were several reasons for this. In some instances there probably was not enough virus used; in others, when vaccination had been done against influenza A, the epidemic was influenza B. In others, after the vaccination was carried out, there was no epidemic; so that by a series of misfortunes, evidence that was significant was not obtained.

In 1941, with the beginning of the war activities, the Influenza Commission was formed under the Army Epidemiological Board through the Surgeon General's office of the Army. Since influenza, because of the epidemic of 1918, was considered by

many people to be a war disease, it was thought one might anticipate another calamitous epidemic of that variety. The Influenza Commission was given as an assignment the question of possibly finding out whether immunity could be induced against the disease by vaccination with inactive virus.

At about this time, work with the embryonated egg had become more and more prominent. It had been shown that the influenza virus grew very rapidly in the chick embryo, that the virus also reached a relatively high concentration in the extra-embryonic fluids, such as the allantoic fluid surrounding the egg. In 1941 we obtained from a number of the commercial firms material suggested by them and prepared from the allantoic fluid. This was allantoic fluid in which influenza virus A was grown and simply inactivated with formalin and then a bacteriostatic agent added. I do not know what happened at that time, but of the five preparations which we were given for testing, three had no immunizing potency in experimental animals, and two of them had very little.

One thing which had become quite obvious was that as one inactivated virus, one lost potency. We then turned to the idea that by concentration of virus one might be able to compensate for the loss of potency which occurred during inactivation.

The other observation, which at least to me was impressive, was that if one used a vaccine subcutaneously which gave rise to a good level of antibodies in the blood, one also increased the amount of antibody in the nasal secretion. Prior to this time it had been rather difficult for me, at least, to see how the simple raising of antibody titers in the blood was going to protect the respiratory area where the virus might get in and produce tissue damage before circulating antibodies would have a chance to come into play.

The question of concentration of vaccine then came up. Hirst, and Hare and McClelland at about this time had reported that virus could be concentrated by a precipitating method. If allantoic fluid in which the virus was present were frozen and then allowed to thaw at about zero degrees, a pre-

precipitate formed and on the precipitate most of the virus became adsorbed. One could then remove the supernatant fluid and, in the precipitate, would get the great bulk of the virus.

Through the Influenza Commission, efforts were made to have some of this prepared for study but we were unable to obtain sufficient material promptly because of the difficulties in maintaining a sterile product. It was at that time that in our laboratory we took advantage of other important observations of Hirst and Hare. They had shown independently that the virus of influenza in the egg fluid became adsorbed to the red blood cells of the chick. If, as the egg was being opened and the virus in the fluid was being harvested, one ruptured the blood vessels and allowed the red blood cells to come out of the blood vessel into the fluid and then collected that fluid and the red blood cells in a cold flask, the virus became adsorbed to the red cells which settled to the bottom. One could remove the supernatant fluid and still retain most of the virus on the red cells. Then all that was needed was to add about one-tenth that volume of a simple solution, such as sodium chloride, bring it up to incubator temperature or room temperature, and the virus would come off the red blood cells. Under those circumstances one could get in 1 cc. of fluid the approximate amount of virus that could be obtained from the 10 cc. of the original fluid. The virus was readily inactivated with formalin, 1:2000, and still retained a high degree of antigenicity.

In 1942, anticipating an epidemic of influenza A on the two-year cycle, through the Influenza Commission we undertook studies of vaccination using material prepared by one of the commercial firms for the Commission in the manner described. In the vaccine were included the viruses of both influenza A and influenza B in equal amounts, so that 1 cc. would contain the equivalent of the virus from 5 cc. of influenza A fluid and from 5 cc. of influenza B fluid.

We vaccinated approximately 8,000 individuals in two institutions in Michigan and Doctors Magill, Plummer, and Smillie also carried out studies at Cornell. In the 8,000 that we vac-

cinated, we maintained alternate controls so that each group within these institutions was divided horizontally, each ward or room being divided so that one person would get the vaccine and the other would get an inoculation of control material. We then sat down to await the epidemic. Well, it did not occur. That was the year it skipped.

However, we were able to do certain things. We were able to study the amount of antibody produced by the vaccine and to find, contrary to certain statements about antibodies and their persistence after vaccination, that at the end of three to four months the fall in antibody titer was slight. At this time, hoping to salvage information, we proceeded to test the resistance of some of the people by actual infection. Individuals who had been vaccinated four and a half months earlier and others who had not were sprayed with influenza virus, type A. Another group was revaccinated two weeks before exposure. Of the ones not vaccinated, 50 per cent came down with temperatures of 100° or more, temperatures up as high as 102° or 103°, with parallel clinical symptoms. Among those who had been vaccinated two weeks before, the incidence of illness was approximately 15 per cent, and none of these had temperatures higher than 100°. Of the ones vaccinated four and a half months before, approximately 30 per cent showed signs of disease.

We also took other groups that had been vaccinated under the same circumstances and tested them by spraying them with influenza B. Forty per cent of the controls came down and 10 per cent of the vaccinated, irrespective of whether they had been vaccinated four and a half months before or two weeks before, had illness but none of the latter had fever as high as 101°. This was a clear demonstration that in a test sufficiently severe to bring down 40 to 50 per cent of the controls, vaccination definitely had exerted an influence in limiting the amount of the disease that showed up.

This result was in contrast to what we had found when we sprayed people with active virus and then resprayed them four



months later with the same virus in the active form. Under those circumstances we did not get as much protection as in the ones that had been vaccinated subcutaneously with inactive virus. It did not seem entirely hopeful because if the natural disease does not give a prolonged immunity, and if the experimental disease produced by spraying active virus does not, it would appear that one could not expect too high a degree of resistance; but the results with the subcutaneous vaccination were better than those following the artificial infection.

On this basis, the next year we proceeded to carry on a more extensive study. This was set up in the ASTP units in universities throughout the country. Six groups of workers from the Influenza Commission participated. The vaccine used in all instances was the same as I have mentioned, and it was given in the same alternate manner—1 cc. inoculation of the vaccine, while alternate individuals received 1 cc. inoculations of salt solution. Each company was divided half and half, so that each unit could be compared within itself rather than with another.

This time we were a little more fortunate because two weeks after the vaccination had been carried out there was an epidemic, and it was influenza A. There were approximately 6,250 men vaccinated, and 6,250 controls in these different units throughout the country. The character of observation was rather uniform, and the investigations for the detection of virus and serological tests made by the different groups within the Commission were essentially the same.

At the University of Michigan there were approximately 1,800 men involved. The epidemic was of the explosive type with rapid development and decline. It started in mid-November, 1943, and lasted about six weeks. The incidence of hospitalized cases diagnosed as influenza, those with temperatures of 100° or more, was 2.3 per cent in the vaccinated, and 8.6 per cent in the control group, nearly 4 to 1. For the noninfluenzal local respiratory disease, or dispensary cases with less than 100° temperature, there was no significant difference between

the vaccinated and control groups. This tends to show that the effect of vaccination is a relatively specific one against influenza.

For all the institutions in which studies were carried out, the total incidence of the disease in the 6,200 vaccinated individuals was 2.2 per cent for the total period, and in the controls it was 7.11. The one place in which the results were out of line was in California where there was no significant difference between the vaccinated and the controls. In the other institutions, however, it was quite uniform and quite similar. In some groups there was a difference of 6 to 1 in favor of the vaccinated individuals. It does show that there was not complete elimination of what was diagnosed as influenza from the vaccinated group. Nevertheless, it was the first clear-cut demonstration that subcutaneous vaccination had actually created a significant difference between vaccinated and control groups in the course of a natural epidemic of the disease.

There is an interesting point for discussion that comes out in these studies. There are reasons to doubt that the incidence in the controls of 7.1 per cent represents the true incidence of the disease in the general unvaccinated population. Both at the University of Michigan and at the University of Minnesota, there happened to be companies of men who for certain reasons were not included in the study. In the one instance the incidence of the disease was 20 per cent, and in the other the incidence was 30 per cent, as opposed to the incidences of 9 and 8 per cent, respectively, in the control group of the vaccinated population, suggesting that vaccination of 50 per cent of the population, as has been indicated frequently, had a definite influence upon the incidence of the disease in the controls as well as in the vaccinated individuals.

There was another interesting observation that came up in the studies at City College of New York and at Iowa. The epidemic began just about the time vaccination was completed, and in the tabulation of the results it was extremely interesting to note that there was no difference in incidence between the

vaccinated and the controls during the first week, while after that the curves diverged very sharply so as to indicate that the effect of vaccination became evident at about five to seven days after inoculation, which would coincide with what we know about the time at which antibodies develop. It was a rather fortunate set of observations which were not planned but nevertheless would suggest that approximately five to seven days after the inoculation, the influence of the vaccine on the incidence of the disease can be detected.

On the basis of these results the Influenza Commission recommended to the Board that widespread vaccination be carried out in the Army during 1945. Provisions were made for obtaining the material, and in October, 1945, practically all the Army was vaccinated.

It looked as if it would be impossible to get any data as to the effect of vaccination, but in November, 1945, an epidemic of influenza B became very prominent in the civilian population, although we had for eight months prior to this been able to identify spotty epidemics throughout the United States, throughout the Pacific area, the West Indies, and to some extent in Europe. But this was a rather sharp upsurge of the disease in the form of an epidemic. At the University of Michigan there were 600 men in the Army group who had been vaccinated on October 16th; there were 1,100 men in another service unit, not Army, who had not been vaccinated. The epidemic began about the 1st of November and all of these men were being taken care of through the student health service under the same conditions, so it was possible actually to add up the score by the number of admissions from these units.

From the first week in November until December 22nd, when the Christmas recess occurred and furloughs, transfers, and all those things broke up the entire study, this is what happened: There were 109 admissions from the 1,100 unvaccinated, and 7 admissions from the 600 vaccinated men. Influenza B virus was isolated from throat washings of sampled patients at intervals throughout the study. Serological studies of 45 cases

were done, and 85 per cent of them were positive for influenza B, pointing again to the fact that the disease was essentially a clear epidemic of influenza B.

These results were paralleled by observations at Yale by Hirst and the staff of the student health service there. The numbers were almost the same. I think there were 550 vaccinated Army men and 1,100 unvaccinated in the other service. In that instance the epidemic occurred in a similar manner and the incidence in the vaccinated unit was 0.5 per cent; in the unvaccinated unit it was 12.5 per cent; so there is a ratio of more than 10 to 1 in favor of the vaccinated individuals.

There are two reasons why the influenza B results might be sharper than the influenza A results which we previously discussed. One is that influenza B may be a better immunizing agent than influenza A. There are reasons to think that is so. Secondly, it may be that one is comparing a totally vaccinated with a totally unvaccinated population.

The incidence of respiratory disease in the vaccinated Army forces in the same area as the University of Michigan at the time the epidemic was prevalent, and the incidence in another branch of the service in the same geographical area which was not vaccinated, showed strongly confirmatory evidence of the results obtained in the smaller unit.

One other point of interest is that the strains of influenza B that were encountered during the last year were in some respects quite different serologically from those that were used in the vaccine. Nevertheless, the effect of the vaccination seemed to be sufficiently great to protect against these different strains.

These are the data upon which we have been moving. The duration of effect, frankly, is not known. In other words, in a given individual, I do not think we can say how long immunity will last. There are certain data which are suggestive. I mentioned the studies we carried out in the institutions in 1942. When the epidemic of influenza A occurred in 1943, this institution was still under observation and we were able to deter-

mine the incidence of the disease in the groups one year after vaccination had been carried out. There were a number of wards in which about 40 per cent had been vaccinated a year earlier, and other wards of a similar character in which no vaccination had been carried out. The incidence of recognized illness in those groups was tabulated.

In the unvaccinated female wards the incidence in all but three was from 6 per cent up to 29 per cent—quite a wide variation. In the vaccinated female wards the highest incidence was 6.5 per cent. In the vaccinated male wards, the highest incidence was 4.4 per cent and many of them had little or no disease, whereas in the unvaccinated wards the incidence was from 2 to 18 per cent, suggesting that as long as one year after vaccination there was an effect. For all groups on the unvaccinated wards the incidence of the disease was 12.4 per cent. In the wards where approximately 40 per cent had been vaccinated, the incidence was 1.9.

These are presumptive data and are subject to obvious criticisms and objections. Nevertheless, they are indications. Hirst and his associates with similar studies in one of the prisons of New York State, where they had vaccinated with a different preparation, also felt that at the end of one year the incidence was reduced one-third in those that had been vaccinated the year before.

Additional data concerning the duration of immunity may be derived from information as to the persistence of antibodies. There is a general trend indicating that in either unvaccinated individuals of the general population or in vaccinated groups the incidence of the disease declines as the level of antibodies rises. When a general population is vaccinated the median level of antibodies is pushed to a considerably higher level. At the end of two weeks it may be ten times the height observed before vaccination.

After three to four months it will still be at two-thirds of the peak level and at the end of the year at one-half peak level, but still considerably higher than the pre-vaccination median.

This persistence of antibodies can certainly be taken to indicate a persistence of the effect of vaccination. If the increased level of antibodies after this time reflects increased resistance, then the observations indicate that increased antibody levels and resistance are demonstrable at the end of a year.

Another point is that by vaccination one can influence the antibody level of a population more effectively than by allowing persons to go through an epidemic, because you can actually pick your population, whereas in an epidemic perhaps not more than 25 to 30 per cent of the population is involved.

Another question that has been very widely discussed is that of reactions. Why do we get reactions and what are the reactions? There are two types. The one with which we have been most commonly confronted is of the type observed with many vaccines, similar to that seen with typhoid vaccination: a local redness, swelling, tenderness at the site of inoculation and a certain proportion of individuals who may have fever and aches and pain.

Some people felt that these reactions were due to egg protein that was in the vaccine, but we have been able to demonstrate that that was not the case, because one could inoculate the individuals with the whole allantoic fluid and not get the reactions. Furthermore, it was possible to show that the number of reactions increased as the amount of virus in the preparation was increased. I think the evidence is quite clear that the amount of reaction is largely related to the virus content. In the preparations that have been employed, there has been considerable variation in the number of reactions and also perhaps in the amount of virus present in the preparation. The suggestion has been made that one can reduce the amount of virus below the level at which reactions are common and get sufficient immunization. That is something for further study.

It is probable that with further technical development the number of reactions will be definitely reduced. In a group of approximately 1,000 persons who were vaccinated last week under our observation, about 1 per cent had febrile responses.

A second type of reaction is related to the matter of sensitization. It has been constantly urged that one must be careful with individuals who are naturally sensitive to eggs. The only anaphylactic reactions of which I am aware occurred in individuals who had known sensitivity to egg. The amount of sensitizing material in allantoic fluid is extremely small and in individuals who have had repeated inoculations we have not seen the development of sensitivity. It may develop, but it certainly is not a common effect.

We have heard comments upon the development of jaundice following influenza vaccine. That is not the case, and I think that belief is drawn from the analogy with yellow fever vaccine with which jaundice occurred at one time. The only instance of which I know when jaundice developed was in the controls of a given study not made by the Influenza Commission who, by a rather unfortunate choice, were given plasma for the control inoculation. In them there was a relatively high incidence of jaundice. But in the ones who received the vaccine, jaundice was not encountered.

We have also heard comments that neurological manifestations had followed influenza vaccination. Among the 7,000,000 men who were vaccinated in the Army, this was not reported.

With respect to the materials being offered for civilian use at present, the Influenza Commission has had nothing to do with their licensing. All I can say is that they are licensed by the National Institute of Health; the original standards they presume to employ are those which were set down for the acceptance of vaccine for Army use. The Washington Letter of the Journal of the American Medical Association, dated October 29, 1945, stated that: "The Public Health Service reported that commercial houses have applied for the right to manufacture the new serum—meaning vaccine—but that the Government had declined to license it for public use." On December 8th, it said that the National Institute of Health had sent a memorandum to producing laboratories saying the favorable consideration would be given to qualified manufacturers, and the

letter of December 17th stated that the material "is now on the market for civilian use."

There is a variety of preparations. There are those prepared by centrifugation, which may have certain advantages. Those are largely matters of production. There is material which is being prepared by calcium phosphate precipitation. There is the material which I think is probably being prepared, although I am not sure, by precipitation with other materials. The comparative advantages of these are not known. The essential problem is a matter of production of effective material. If they are stable, if their potency is high, there is no reason why the one vaccine might not be as effective as the other. However, there are at present no data other than serological to afford a comparison between them.

In this review I am sure there are many things I have not said. I would like only to repeat that the data are the evidence which was obtained in experimental studies, and the results are a demonstration, through close epidemiological and laboratory observation, that subcutaneous vaccination has a definite influence upon epidemics of influenza A and influenza B. If there were a pandemic such as that of 1918—and this is the question that is often asked—what would it do? I think there, again, one would have to say that the results would depend upon the antigenic character of the strain of influenza virus that would be present. My hunch is that it would be a strain similar in its basic characteristics to strains with which we are familiar. However, it might be antigenically different or it might be antigenically the same. The results of vaccination would certainly depend upon that. Another factor would be its virulence and the effect of present therapeutic agents such as the sulfonamides and penicillin and others upon secondary invaders.