STUDIES IN HUMAN IMMUNIZATION AGAINST INFLUENZA

Duration of Immunity Induced by Inactive Virus

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Recent evidence on the effect of subcutaneous vaccination with influenza virus showed that the incidence of epidemic influenza was consistently reduced by about 75 per cent when the period between vaccination and epidemic was short (1). The present communication presents briefly a study on a similar vaccine in which the period between vaccination and epidemic was about 1 year. Since there is reason to think that the vaccines used in the two studies were in many ways comparable, the present results may be considered as an extension of the previous observations.

Methods

Preparation of the Vaccine.—Strains employed: The PR8 strain of influenza A and the Lee strain of influenza B were used for the production of most of the vaccine. In one small experiment (done in institution 7) the vaccine used was made with the PR8 and W.S. strains of influenza A only.

Concentration of virus: The virus was grown in the allantoic sac of the developing chick embryo and was concentrated from allantoic fluid by adsorption on the precipitate which forms when frozen allantoic fluid is thawed. The details of the technique have been previously described (2). After concentration, if sterility tests were negative, formalin was added to a final concentration of 0.1 per cent. Concentrates of PR8 and Lee were combined in equal proportions and the mixture was frozen and dried (3). Infectivity tests (in mice and chick embryos) following drying were uniformly negative for active virus. Seven different lots of such vaccine were prepared and each lot was tested in human beings before use in the field to insure that its antigenic potency was of the right order. The vaccine was stored in the dried state for an average of 2 to 3 months before use.[1]

[1] By this method over 90 per cent of the PR8 and 75 to 85 per cent of the Lee virus was removed from the allantoic fluid. The main disadvantage of this technique is the large amount of insoluble precipitate left in the vaccine. The adsorption-elution phenomenon of influenza virus with avian red blood cells (4) provided an obvious method of virus concentration which would eliminate much non-virus material and which we immediately attempted to use. While the concentration of influenza B virus was satisfactory by this technique the results with PR8 were disappointing. At 37°C. for 2 hours it was not possible to elute more than 50 per cent of the adsorbed PR8 virus (see (4) fig. 3) and the prolonged incubation at this temperature greatly favored the multiplication of bacterial contaminants. For these reasons the method was not used. Francis and Salk subsequently published a similar method (5) for the use of the adsorption-elution principle but made no mention of experiencing these difficulties.

Institutions Used for Vaccination.—Penal institutions in five widely separated states of midwestern, southern, and eastern United States were chosen for study. These institutions offered obvious advantages for this type of work since in each of the groups the medical care was good, there was adequate, readily available hospital space, and there were full-time physicians in attendance. Seven such institutions were studied and in the description below they will be referred to by number. The vaccinations were done on volunteers only, and usually because the number of volunteers was less than half the total population, they were all vaccinated and the remainder of the population was listed as controls. The list of vaccinated and controls was made up at the time of inoculation and special groups such as those in isolation quarters, hospital attendants, and detached workers were excluded. After completion of the vaccination no new men were admitted to either group.

Administration of Vaccine.—The vaccine was rehydrated in distilled water just before administration and aliquot portions of the seven vaccine lots were mixed in the fluid state so that each individual in the seven institutions received equivalent material. The vaccine was injected subcutaneously in a volume of 1 cc. This inoculum contained the concentrate from 5 cc. of PR8 and 5 cc. of Lee allantoic fluid. At institution 7 the inoculum contained the concentrate of 5 cc. of PR8 and 5 cc. of W.S. allantoic fluid. Special attention was devoted to insuring equal amounts of insoluble precipitate in each dose.

Reactions to Vaccination.—The injection of this material was not followed by any untoward immediate reactions. Between 12 and 24 hours after inoculation there were a few individuals (approximately 1 in 200) who developed a mild febrile response (100.0 to 100.5°F. by mouth) and some had slight chills and malaise but these reactions were of brief duration and were not serious. Slight redness and swelling at the site of injection with moderate sore arm for 24 hours was common.

RESULTS

Serological Response Following Vaccination.—A number of individuals at each institution were bled before vaccination and again 2 weeks, and 11 to 14 months, after vaccination. A few additional subjects had only two blood specimens taken, the second being either at the 2 week or 11 to 14 month interval. These sera were tested for agglutination inhibition titer against influenza A strains PR8 and NY-43[2] and for complement-fixing antibodies against NY-43 (6, 7). In every case all the sera from one individual were run in the same test and all runs were corrected to the same level through the use of standard ferret sera. Table I gives in summary form the geometric mean antibody levels at these various time intervals.

The mean antibody increase 2 weeks after vaccination was approximately sixfold against the strains used in vaccination (Lee and PR8) and fourfold against the current strain NY-43, whether tested by agglutination inhibition or complement fixation. This is the same order of rise as was described previously when amounts of virus of this order of size were given to human beings (8, 2). Although the mean level dropped over a year long interval, nevertheless the titers of PR8 and Lee antibodies were still about three times the pre-

[2] NY-43 is an influenza A strain isolated from a patient at the height of the 1943–44 epidemic in New York City. It was isolated by inoculation of throat washing filtrate into the amniotic sac of chick embryos.

vaccination level, while the NY-43 titers were twice the original by both methods of testing.

While the 11 to 14 month specimens were taken during an epidemic of influenza A it seems unlikely that infection unduly raised the mean titer of the tested group since: (*a*) Individuals giving a history of infection were excluded. (*b*) The over-all attack rate of influenza was low among the populations concerned (5 per cent), and the number of unapparent infections was probably correspondingly small. (*c*) Individuals showed a great uniformity of response with a 1 year level consistently below their 2 week level. (*d*) The results with influenza B, in the absence of a B epidemic, corresponded well to those with

TABLE I

Change in Antibody Levels Following Vaccination of Human Subjects with PR8 and Lee Virus

Testing strains	Mean serum antibody titers			No. times mean increase in titer over previous level		Per cent of individuals 30 per cent or more above prevaccination titer at 11–14 mos.
	prevaccination	2 wks. postvaccination	11–14 mos. postvaccination	2 wks.	11–14 mos.	
PR8 agglutination	197	1209	704	6.1	3.6	84
Lee agglutination	121	709	340	5.9	2.8	90
NY-43 agglutination	44	163	96	3.7	2.2	76
NY-43 complement fixation	14	58	30	4.1	2.1	58
No. of subjects	85	64	61	64	61	61–64

influenza A. (*e*) Eighty-five and 90 per cent of vaccinated persons (in respect to PR8 and Lee antibody titer) were, after 1 year, significantly (30 per cent) above their prevaccination level. In view of these facts it is felt that the elevated titer after 1 year was a result of vaccination.

Collection of Clinical Data.—The vaccinations were done in the last 2 months of 1942 and early in 1943 and no influenza epidemic was reported in any of the groups until the nation-wide epidemic started in November, 1943, when institutional epidemics lasting 4 to 6 weeks were reported in all seven groups, concurrent with influenza in the general population.

At each institution the diagnosis of influenza was made by the attending physicians. All cases included in the study were admitted to the institution hospitals for observation. The disease was very similar clinically to cases seen outside the institutions and was usually very mild. The temperature was

not high nor was it usually elevated for more than 2 days. Coryza and cough were common but less than half had typically severe constitutional symptoms. Few individuals were seriously ill, convalescence was short, and there were few complications and no deaths. Because of the relatively mild symptoms the differential diagnosis from other respiratory diseases was not always easy, and in selecting the group considered to have influenza error was made in the direction of including rather than excluding suspicious cases. This would, of course, tend to decrease any specific effect of vaccination. As in previous studies the main criteria for diagnosis were fever (100.0°F. or more by mouth) and respiratory symptoms. Those making the diagnosis were, with one possible exception, unaware of the patient's status in the study.

Etiology of Epidemics.—In four institutions (1, 2, 3, and 7) paired acute and convalescent sera were obtained from a few patients with influenza and the number of these showing a fourfold or greater rise to the PR8 and NY-43 strains was sufficient to indicate that the epidemic was in all probability due to influenza A. Two influenza A strains were isolated from patients in institutions 2 and 3, on primary inoculation of throat washings into the amniotic sac of chick embryos. In four institutions (3, 4, 5, and 6) a number of convalescents were bled 1 to 2 months following the disease. These convalescent sera were tested against the PR8 strain and part of them against the Lee strain. At the bottom of Table II is shown the distribution of PR8 titers in these groups together with a group of normal pre-epidemic titers from the same institutions. Among the convalescent sera only 10 per cent had a titer of 512 or less, while 89 per cent of the normal group fell below this level. The mean titers were also considerably elevated above that of the general population in respect to influenza A but not influenza B. This is reasonably clear evidence not only that the epidemics in these institutions were influenza A but also that the great majority of cases selected for testing had been infected.

Evaluation of Clinical Results.—For purposes of analysis the population of each institution was divided into three groups: (*a*) Those who were vaccinated, (*b*) those listed as controls at the time of vaccination and who were still resident at the time of the epidemic, and (*c*) inmates admitted since vaccinations were performed (called recent group). The first two groups were greatly reduced from their original size due to discharges during the 1 year interval. All three groups excluded special prisoners as mentioned above.

In Table III are given the data regarding the number of cases of influenza observed in the different groups, together with calculated attack rates and the percentage reduction of attack rate in the vaccinated group compared with the controls. Owing to the loss of subjects and the low general attack rate the number of cases for consideration in any institution is small. Nevertheless the attack rate in the vaccinated was consistently lower than that of the control group in each institution and the over-all reduction was 35 per cent. None of

the results of individual institutions varied from this average figure by a statistically significant amount, so that the differences in reduction from 23 to 58 per cent could be due entirely to sampling. Although the result in many of the groups is not significant statistically, the total result, since all the groups

TABLE II

Summary of Serological Tests Done in Different Institutions to Establish the Etiology of Epidemics

Analysis of Paired Sera

Institution No.	No. of paired sera examined	No. of pairs positive* for influenza A	No. of pairs negative for influenza A
1	15	12	3
2	6	4	2
3	2	2	0
7	4	3	1

Analysis of Convalescent Serum Titer against Influenza A‡

Institution No.	No. of conval. sera	Individuals in titer group								Geometric mean titer	
		Less than 64	64–128	128–256	256–512	512–1024	1024–2048	2048–4096	More than 4096	Influenza A	Influenza B
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
3	17	0	11	0	12	18	35	12	12	1002	—
4	30	0	0	0	6	37	37	10	10	2520	158
5	36	0	0	3	8	39	22	14	14	1270	158
6	30	0	0	3	3	17	28	46	3	1620	130
Control population.....	85	6	20	33	29	11	1	0	0	205	187

* The criteria for a positive result were that there be at least a fourfold rise in titer in two of the following three serological tests: Agglutination inhibition titer with PR8 and NY-43, and complement fixation titer with NY-43.

‡ Sera from individuals included as cases in the study, taken 4 to 8 weeks after the acute illness.

are consistent, is highly significant. It should also be noted that the recent inmates had attack rates very similar to those of the control groups.

Because the vaccinated subjects in these institutions were a group of volunteers and not chosen at random, it was desirable to eliminate as far as possible any linked variables which might have influenced the result in either direction. From three of the institutions (1, 2, and 3) data were obtained concerning the age, length of residence, location of cell block, occupation during the day, and

color of the total population. The attack rates of unvaccinated controls in these various categories were determined and from them were calculated the number of cases expected among the vaccinated in each category, assuming that the vaccine had no effect. The same order of differences was found between the calculated expected rate and the actual rate as was found between the crude rates in the entire group. In other words, the distribution of vaccinated and control groups among these categories had no significant effect on the crude rates given in Table III.

One possible linked variable could not be ruled out by these calculations, namely, that some unknown factor having an effect on incidence might be

TABLE III

Summary of Clinical Data on the Incidence of Influenza among Control and Vaccinated Populations and among Those Recently Admitted to the Seven Institutions under Study

Institution No.	Total population	No. in			Total cases	No. of cases of influenza in			Attack rate in			Reduction between control and vaccinated groups
		Recent group	Control group	Vaccinated group		Recent group	Control group	Vaccinated group	Recent group	Control group	Vaccinated group	
												per cent
1	1796	337	852	607	122	29	63	30	8.6	7.4	4.9	34
2	1356	301	647	408	107	30	53	24	10.0	8.2	5.9	28
3	1561	704	512	345	87	48	29	10	6.8	5.7	2.9	49
4	1047	415	353	279	40	18	15	7	4.3	4.2	2.5	40
5	1298	396	458	444	52	17	20	15	4.3	4.4	3.4	23
6	3116	949	1480	687	107	40	56	11	4.2	3.8	1.6	58
7	658	334	149	175	56	38	10	8	11.4	6.7	4.6	31
Total.....	10,832	3436	4451	2945	571	220	246	105	6.4	5.5	3.6	35

linked with the fact of volunteering.[3] Three considerations make the operation of such a factor in the present study unlikely: (*a*) The means of making a diagnosis always included an objective factor, namely fever. (*b*) The lapse of a year's time between inoculation and infection decreased the degree of consciousness of the subjects regarding their part in an experiment, in fact many saw no connection. (*c*) The group of recent admissions formed a sort of added control group which had not been separated into volunteers and controls and yet had nearly the same attack rate (a little higher) as the regular controls.

[3] Such a factor was demonstrated in a common cold study by Diehl and coworkers (9) in which they obtained a reduction of attack rate following the injection of saline in volunteers. Their experiment was very different from the present one however, in that (*a*) the subjects chosen were all highly susceptible to colds, (*b*) they were treated every 2 weeks, and (*c*) the comparison of attack rates was made between the years of treatment and the year prior to treatment (by history). These differences may have enhanced the apparent curative effects of "treatment," but were not operative in the present study.

DISCUSSION

A number of vaccination experiments were carried out during the 1940–41 epidemic of influenza A in this country using a ground chick embryo vaccine described by Horsfall and collaborators (10). Martin and Eaton (11) and Brown, Eaton, *et al.* (12) were the only groups that reported experiments with this preparation in which a significant reduction in attack rate followed vaccination. They reported attack rate reductions of 32 and 52 per cent in two institutions and of roughly 50 and 60 per cent in two other hospital groups that were less well controlled. It seems significant that all these results were obtained in epidemics which occurred at the time or shortly after vaccination was performed. Following this, Dalldorf *et al.* (13) and Siegel *et al.* (14) in two independent experiments with the same type of vaccine obtained no evidence of reduction of the attack rate, with an interval between vaccination and epidemic of 6 and 8 weeks. Horsfall *et al.* (15) reported a much larger series with the same preparation, in which the vaccination-epidemic interval was 4 months. They obtained an attack rate reduction of 25 per cent on a clinical basis. This would seem to be a significant reduction were it not for the fact that the results in individual institutions showed such enormous variation, well beyond the sampling error. This large variation precludes any definite conclusions being drawn from these results.

Following the 1940–41 experience an investigation was carried on in this laboratory (8) in which some of the factors affecting the human antibody response to parenteral vaccination were evaluated. Among other things it was shown that 1 cc. of a 20 per cent chick embryo vaccine or 0.5 cc. of infected allantoic fluid raised the mean antibody level of a population about twofold while the inoculation of the virus from 5 cc. of allantoic fluid raised the mean level sixfold. The same enhanced antibody response was obtained with large amounts of virus concentrated by two different methods (8, 2) and this observation was confirmed by Hare *et al.* (16).

During the epidemic of 1943–44 the members of the Commission on Influenza (1) tested a vaccine which consisted of virus concentrated from allantoic fluid by adsorption on and elution from embryonic red cells (4, 5). While no human antigenic studies using virus prepared in this way have as yet been published, there is no reason to expect that the human response would differ in any important way from that obtained with similar amounts of virus concentrated by other methods. Using this vaccine the reduction in incidence of epidemic influenza was uniformly good (75 per cent) for cases whose onset was within 6 weeks of the time of vaccination (1). The enhanced protective effect of this vaccine over that used in 1940–41 might have been anticipated in view of the better antibody response produced by concentrated preparations.

Most of the evidence in the report of members of the Commission on Influenza (1) covers cases infected up to 7 weeks after vaccination. When the

total of all reported cases is considered in terms of the length of time between vaccination and onset, it is found that the effect of vaccination was greatest in the 2nd week following inoculation (approximately 85 per cent reduction) and was poorest in the 6th and 7th weeks when the reduction (in 94 cases) due to vaccination fell to about 40 per cent. The results of Eaton and Meiklejohn stand out in the report as an exception since they obtained only a 30 per cent reduction in the vaccinated group. The low order of vaccine effect obtained by these workers is significantly different from the results of all the other investigators and it may be pertinent that their observations covered only cases in which there was a period of 6 to 12 weeks between vaccination and infection while all the results showing a high order of vaccine effect were in cases occurring in the first 6 weeks after inoculation.

The present report covers a group of individuals vaccinated 11 to 14 months before the epidemic with material as high in virus content and at least as antigenically potent as that used by the group mentioned above. Serological evidence indicates that vaccinated individuals had of the order of three times as much circulating antibody at the time of the epidemic as they had before vaccination. Those individuals vaccinated in seven different institutions showed a consistently lower attack rate than did the corresponding unvaccinated controls. The reduction in attack rate (35 per cent) was not significantly different from the effect obtained by Eaton and Meiklejohn 6 to 12 weeks after vaccination with a preparation of similar potency.

This evidence indicates that both in 1940–41 and in 1943–44 a definite reduction in influenza attack rate was obtained with formalin-killed vaccine, when given in the face of or shortly before the outbreak. In both instances there was evidence that the peak of the immune effect declined rapidly, so that with the weak preparation used in 1940–41 no clear cut immunity was evident at 6 and 8 weeks, while with the more potent, concentrated preparations the maximum effect in the 2nd week declined markedly by the 6th and 7th weeks but then persisted at a low level for a year. Such a precipitous drop in immunity from a high peak with ultimate levelling off and with a lesser order of decline at a low level is a well established immunologic phenomenon.

If this view of the facts is relevant, it would obviously mean that there would be much greater economy in the administration of vaccines of this type if they were given in the face of an outbreak, after its identification, rather than if the vaccine were administered in advance of each epidemic season, since the exact seasonal onset and yearly periodicity of influenza epidemics is too capricious to permit accurate prediction. The protection obtained after 2 months' lapse of time is insufficient for practical purposes, and furthermore the results of Hale at the University of Iowa (1) and results from the laboratory at the City College of New York (1) have shown that immunity may be expected within 8 days following vaccination.

SUMMARY

The administration to human beings of formalin-killed influenza virus, concentrated from allantoic fluid, resulted in a high order of antibody response within 2 weeks after injection. Even after 1 year the great majority of individuals vaccinated had antibody levels considerably above their prevaccination titer for the PR8, Lee, and a current 1943 strain. An investigation of the occurrence of epidemic influenza A in seven widely separated populations, 1 year after vaccination of part of these groups, showed that the attack rate among vaccinated persons was consistently lower than that of control individuals. The average reduction in attack rate was of the order of 35 per cent.

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