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Experiments on Immunization of Human Beings Against Influenza A.*

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Previous investigators have reported stimulation of virucidal antibodies[1, 2] and some evidence of protection against influenza[3] in persons inoculated subcutaneously with active virus of influenza A. Others using formalinized inactive preparations[4, 5] have demonstrated an increase in antibodies, but found no evidence of active immunity in the vaccinated groups.

Recently Horsfall and Lennette[6] prepared a complex formalinized vaccine by infecting chick embryos simultaneously with canine distemper and influenza A virus. In a recent influenzal outbreak in California, this vaccine, and another vaccine made from active influenzal virus were used prophylactically.† The active-virus vaccine was prepared in our laboratory by growing the virus of influenza A strain Melbourne in minced chick embryo suspended in saline. This material was centrifuged at 1000 rpm for 10 minutes and the supernatant fluid was stored at —60°C to be used as vaccine when needed. The intranasal MLD for mice was approximately 0.05 cc of a dilution of 1:1000. In this paper, the active tissue-culture vaccine will be termed V-1 and Horsfall's complex inactive vaccine V-2.

Method. In 2 large institutions in Northern California, which we shall designate X and Y, a group of the personnel was vaccinated

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1 Francis, T., Jr., and Magill, T. P., *J. Exp. Med.*, 1937, **65**, 251.

2 Stokes, J., Chenoweth, A., Waltz, A., Gladen, R., and Shaw, D., *J. Clin. Invest.*, 1937, **16**, 237.

3 Stokes, J., McGuinness, A., Langner, P., and Shaw, D., *Am. J. Med. Sci.*, 1937, **194**, 757.

4 Med. Research Council, Special Report No. 228, 1938, 141.

5 Taylor, R., and Dreguss, M., *Am. J. Hyg.*, 1940, **31**, 31.

6 Horsfall, F. L. Jr., Lennette, E. H., and Rickard, E. R., *Jour. Exp. Med.*, 1941, **73**, 335.

† The authors are indebted to Dr. L. L. Stanley and Dr. P. W. Day for collecting the clinical data reported in these studies. The complex vaccine was supplied by the International Health Division Laboratories in New York.

2 weeks before an outbreak of acute febrile respiratory disease designated by the clinicians as influenza. At institution X about half of the vaccinated group was composed of new entrants and the other half of persons who entered the institution approximately 16 months previously. At institution Y the vaccinated group was selected according to serial number. Between the vaccinated and control groups no differences in chances of exposure to infection within the institution could be found. Approximately equal numbers of persons received V-1 and V-2. Each of the vaccines was given subcutaneously as a single inoculation in doses of 1 cc. All cases of acute febrile respiratory diseases were recorded after the completion of the vaccinations and attempts were made to study a representative group of the cases by serological methods. Bloods were obtained in the acute and convalescent stages of the disease and tested for complement-fixing antibodies against influenza A, by the method previously described[7] which was a modification of that originated by Smith.[8] Blood specimens were also obtained before and after the epidemic from a group of vaccinated individuals and from a group of nonvaccinated persons taken at random.

Results. The cases were recorded from December 1 to December 28 at both institutions. The peak of the epidemic extended from December 1 to 12 at institution X, and from December 17 to 19 at Y. At the close of the period of observation, the records submitted to us revealed 395 clinical cases at institution X and 413 at Y. Serological studies indicated that at both institutions the principal causative agent was the virus of influenza A. Of 50 cases tested by complement-fixation at institution X, 32 were positive for influenza A and all but 6 showed a rise of fourfold or greater. At institution Y, 24 of 26 cases tested showed a rise of fourfold or greater. All acute bloods were obtained during the peak of the epidemic at institution Y, whereas at X the epidemic had no sharp

TABLE I.
Summary of Incidence in Vaccinated and Control Groups.

	Institution X			Institution Y		
	Total in group	Total cases	Incidence %	Total in group	Total cases	Incidence %
Vaccine —1	193	16	8.3	169	10	5.9
Vaccine —2	223	12*	5.4*	244	18	7.4
Total vaccinated	416	28	6.7	413	28	6.8
Controls (not vaccinated)	4560	365	8.0	2,487	385	15.5

*Not counting 2 cases with onsets 6 and 7 days after vaccination.

[7] Eaton, M. D., and Rickard, E. R., *Am. J. Hyg.*, 1941, **33**, 23.

[8] Smith, W., *Lancet*, 1936, **2**, 1256.

peak and a number of the bloods were obtained late from some cases which were probably not influenza.

Table I reveals the morbidity in 2 vaccinated and 2 control groups. At institution X the difference between the incidence of infection in the control and vaccinated groups was much less definite than at institution Y where the incidence in the control group was 15.5% as compared to 6.8% in the vaccinated group. On reviewing the data in Table II these conflicting results may be explained in part by the lapse of time between vaccination and the peak of the epidemic. At institution X most cases that had received V-2 were recorded between 9 and 13 days after vaccination. However, most of those who had received V-1 at this institution became ill 2 weeks or more after vaccination. At institution Y the individuals were all vaccinated on 2 different days and the lapse of time between the completion of vaccinations and the peak of the epidemic was about 2 weeks.

Complement-fixation tests on sera taken before and after the epidemic from a group of 92 unvaccinated individuals selected at random in institution X revealed an increase in complement-fixing antibodies to influenza A in 9.8% with history of illness and in 19.5% with no evidence of clinical infection. There were apparently a great many cases of subclinical infection. These results are in accord with those found by Horsfall, Hahn, and Rickard.[9]

Table III presents serological results in the vaccinated group at institution X. These individuals were bled 4 to 6 months before, and one month after vaccination. Histories revealed no evidence of acute febrile respiratory disease in the interval between bleedings.

TABLE II.
Date of Onset of Cases in Relation to Date of Vaccination.

	Institution X					Institution Y				
		V-1 cases Days after vac.		V-2 cases* Days after vac.			V-1 cases Days after vac.		V-2 cases Days after vac.	
Date of illness December	Non-vac. cases	9-13	14-42	9-13	14-21	Non-vac. cases	9-13	14-25	9-13	14-25
1-4	110		8	6		8				
5-8	99	1	1	2		6	2			
9-12	75	1	1		2	14	1			
13-16	43		2		2	71		2	2	
17-20	22		1			222		3		9
21-24	10		1			53		2		7
25-28	6					11				

*2 cases receiving V-2 with onsets less than 9 days after vaccination occurred at institution X.

[9] Horsfall, F. L., Hahn, R. G., and Rickard, E. R., *J. Clin. Invest.*, 1940, **19**, 379.

TABLE III.
Serological Results in Vaccinated Group at Institution X.

Vaccine	Test*	Pre-vaccination titer	No. showing increase of titer (post-vaccination) of			
			Nil	2-fold	4-fold	8-fold or over
V-1	C.F.—PR8	0 to 4	22	2	0	0
"	C.F.—PR8	8 or over	10	6	1	0
"	Neutr. PR8	0 to 4	1	3	1	0
"	" PR8	8 or over	4	0	1	0
V-2	C.F.—PR8	0 to 4	37	0	19	4
"	C.F.—PR8	8 or over	1	0	0	0
"	Neutr. PR8	0 to 4	0	4	2	2
"	" PR8	8 or over	3	2	2	0

*C.F.—Complement-fixation test with PR8 antigen from infected mouse-lung. Neutr.—Neutralization-test against 1000 intranasal MLD of the strain PR8.

V-1 produced a rise in complement-fixing antibodies in 9 out of 32 of the individuals studied. In 10 cases the pre- and post-vaccination bloods were tested for neutralizing antibodies against the strain PR8. There was a significant rise of fourfold in 2 of the group and a twofold rise in 3 others. A twofold increase in neutralizing antibodies was considered of doubtful significance.

V-2 produced a rise in complement-fixing antibodies in 23 out of 38 individuals tested. In 15 cases the pre- and post-vaccination bloods were studied for neutralizing antibodies against PR8 virus. In 6 individuals there was a significant rise in antibodies after vaccination. The 3 cases which showed no rise at all had a high pre-vaccination titer, which may explain the lack of antibody-response to the vaccine in these individuals.

At institution Y only post-vaccination bloods were obtained from 13 individuals who had received V-1 and 15 individuals who had received V-2. In the complement-fixation test the geometric mean of the titer was 1:11 after V-1 and 1:12.9 after V-2. In the neutralization-test against 100 MLD of PR8 virus, the mean titer was 1:7.5 after V-1 and 1:16.8 after V-2.

Discussion. The protection afforded by either vaccine at institution X is questionable, but at Y the difference in incidence of cases between the vaccinated and control groups is sufficiently great to be of some significance, especially since the incidence of influenza A was high at this institution. The failure of V-2 to protect at institution X could be attributed to the short lapse of time between vaccination and the peak of the epidemic. However, in the groups vaccinated with V-1 at both institutions, the onset of illness in most cases was more than 2 weeks after vaccination, yet this vaccine

apparently gave some immunity in the vaccinated group at institution Y, but none at X.

V-2 produced a slightly, but perhaps not significantly, greater antibody-response than did V-1, as judged by complement-fixation and neutralization-tests. It is quite probable that subclinical infections accounted for some of the increase in antibodies. It also appeared that the response in complement-fixing antibodies produced by the two vaccines was considerably less in degree than the response following infection.

Summary. Studies were conducted at two large institutions during an outbreak of influenza A which was proven by complement-fixation tests. Persons at both institutions received a living virus and complex formalinized vaccine previous to the outbreak. An epidemiological survey revealed some protection at one institution, but none at the other. There was no obvious difference in the prophylactic efficacy of the two vaccines in the relatively small group of 829 individuals studied.

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Reaction of the Rat Omentum to Injections of Particulate Matter.

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It has been known for many years that certain cells widely distributed throughout the body have a special property which enables them to collect and store foreign particulate matter. On the basis of this common property it is now rather generally believed that these cells should be considered as belonging to a single system, the reticulo-endothelium. The critical reviews of Maximow[1] and Jaffe[2] are the most useful of the numerous articles available concerning the distribution, morphology and reactive characteristics of this system.

In spite of the many studies carried out in this field, the special

[1] Maximow, A. A., The macrophages or histiocytes, Section XIX in Cowdry's *Special Cytology*, 1932, p.711.

[2] Jaffé, R. H., The reticulo-endothelial system, Section XV in Downey's *Handbook of Hematology*, 1938, p. 974.