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Received September 7, 1967. P.S.E.B.M., 1967, v126.

Comparison of HI and HAdI Antibody Response of Military Recruits to Monovalent Vaccines.* (32604)

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The development of efficient and reliable methods for the evaluation of vaccine strains has been an area of continued study by the Virus Laboratory, School of Public Health, University of Michigan. Accumulated experience has led to the thesis that the most pertinent assessment of the relation of strain variation to vaccine protection can be obtained by a comparison of the hemagglutination inhibiting (HI) antibody response of humans given monovalent vaccine containing either the new variant or the previously accepted vaccine strain.

It has been shown by others that the hemadsorption inhibition (HAdI) test, which measures neutralizing antibody is more sensitive and specific than is HI for demonstrating antigenic differences between strains of influenza viruses when sera from lightly immunized mice or chickens are employed(1, 2). Moreover, it has been suggested that such findings might be used as a guide for decisions concerning strain substitution in influenza virus vaccines when antigenic variation is recognized(1).

To evaluate this suggestion a comparison was made of the antibody response of humans to vaccination with monovalent Asian influenza vaccines using HI and HAdI procedures. The vaccines given contained the most widely divergent antigenic prototypes available at the time of the study. The findings comprise the subject of this report.

Materials and methods. Subjects, vaccines, and bleeding schedules. In the Spring of 1963 two groups of 22 military recruits received 1 ml of aqueous monovalent influenza virus vaccine containing 200 CCA units of either A_2/Japan/305/57 or A_2/Japan/170/62 virus. The vaccines were made on special order by a commercial pharmaceutical firm. The subjects were bled before and 2 weeks after vaccination. The sera were separated and stored at 4°C until used.

Viruses. The viruses used for testing were from the files of the Strain Study Center, Commission on Influenza, Armed Forces Epidemiological Board at the School of Public Health, Virus Laboratory, Department of Epidemiology, University of Michigan, Ann Arbor, Michigan.

* This investigatiion was conducted under the sponsorship of the Commission on Influenza, Armed Forces Epidemiological Board, and was supported by the U. S. Army Medical Research and Development Command, Department of the Army, under research contract number DA-49-193-MD-2066.

TABLE I. Comparison of HAdI and HI Antibody Response to Monovalent Vaccines.

Test antigen:	A_2/Japan/170/62				A_2/Japan/305/57		A_2/AA/23/57	
Vaccine	HAdI	FR*	HI	FR	HAdI	FR	HI	FR
A_2/Japan/170/62	107/2068† (20/22)‡	19	14/512 (19/22)	37	302/5412 (22/22)	18	30/696 (20/22)	23
A_2/Japan/305/57	96/1198 (21/22)	12	<8/108 (19/22)	27	111/2210 (19/22)	20	12/224 (19/22)	19

* Mean fold rise.
† Pre- and post-geometric mean titers.
‡ Frequency of 2-fold or greater antibody rise.

HAdI test. The tissue culture system used was calf kidney to which A_2/Japan/305/57 and A_2/Japan/170/62 were adapted using the method developed by Cohen and Maassab(3). Prior to use sera were treated by heat at 56°C for 30 minutes. The HAdI procedure was that described by Johnston and Grayston (4). Serum dilutions (0.1 ml) were incubated at room temperature for ½ hour with 4 HAd units of virus (0.1 ml) and then the mixtures were inoculated into calf kidney cell cultures each of which had been washed in 1 ml of BSS. The maintenance media was 2× Eagle's. After 48 hours the medium was removed and 0.2 ml of 0.4% guinea pig erythrocytes was added and the tubes examined for hemadsorption. The end point was taken at the dilution in which the hemadsorption was 2+.

HI tests. These were performed after treatment of the sera with trypsin and periodate according to a standard procedure(5).

Results. The geometric mean pre and post vaccination antibody titers determined by HAdI or HI using serum samples obtained from groups of 22 military recruits given either A_2/Japan/170/62 or A_2/Japan/305/57 monovalent influenza virus vaccines are recorded in Table I. The values were ascertained with homologous and heterologous strains except that to minimize the effect of nonspecific inhibitor the antigenically equivalent, but inhibitor insensitive A_2/AA/23/57 virus was substituted for the A_2/Japan/305/57 strain in the conduct of the HI test. The mean fold antibody rises are also shown as well as the proportion of individuals exhibiting a 2-fold or greater rise in titer to each strain used for measurement.

Note that the HAdI titers are much higher than the related HI values, reflecting the greater sensitivity of the HAdI technique for measuring antibody. Nevertheless, the trends demonstrable by application of either technique and the inferences that may be drawn from the data are the same.

Thus, the corresponding prevaccination antibody titers of both vaccine groups determined by either method with either strain appear comparable, indicating that randomization of vaccines had been successful in equalizing the prior antigenic experience of the subjects. Post vaccination antibody titers measured by both methods with both strains were higher in the group given the A_2/Japan/170/62 preparation. These findings demonstrate that the A_2/Japan/170/62 vaccine was the more potent antigen, even though each preparation contained the same number of CCA units.

The frequency of antibody increase was found to be similar in both vaccine groups when tested by either method with either strain. Likewise, the mean fold antibody rises measured with the 1957 isolates were similar. However, the mean fold rises against A_2/Japan/170/62 measured by both methods were higher in the group receiving the homologous vaccine. Nevertheless, the proportionate difference in the antibody response of the 2 vaccine groups was found to be essentially the same when the data obtained by the use of both methods were used for calculation, *i.e.*, 19/12 yields a quotient of 1.6 and 37/27 yields a quotient of 1.4.

Discussion. The data presented provide an example of some of the factors to be taken into consideration in evaluating the expected efficacy of influenza virus vaccines. Both vaccines induced the same frequency of homologous and heterologous antibody rises. Hence on that basis of comparison either

would be acceptable. The post vaccination antibody levels measured with either strain by HI after administration of either vaccine were in the range previously found to be protective(6). The results of field trials conducted in 1963 and of observations since on the incidence of influenza in the completely vaccinated military demonstrate that polyvalent vaccines containing either strain have yielded a high degree of protection against Asian influenza(7,8,9). Nevertheless, the superior antigenic potency of the A_2/Japan/170/62 vaccine is apparent and vaccines containing that strain have proven to be protective in the military(9). The observation that vaccine of equal CCA content can differ so markedly in antigenic potency points up the fact that determinants of antigenicity in different strains of influenza virus warrant further investigation.

The findings demonstrate that while there are quantitative differences in the titer values found by HAdI and HI there are no differences in the quality of the information obtained in studies in man directed to the question of the relation of strain variation to vaccine protection.

Summary. A comparison of the antibody response of humans vaccinated with either A_2/Japan/305/57 or A_2/Japan/170/62 monovalent vaccine as measured by HAdI and HI techniques has been presented. Although the HAdI antibody titers were higher the information obtained from comparisons of the frequency of antibody response and of mean fold titer increases was the same for both methods. Thus, it would appear from this experience that the HAdI technique offers no great advantage for the selection of vaccine strains when the criterion is the antibody response in man. In addition, it should be pointed out that the HAdI test is a more laborious, expensive and time consuming procedure than the HI method.

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Received September 11, 1967. P.S.E.B.M., 1967, v126.

Incorporation and Fate of Estradiol-17β-6, 7-H^3 in Rabbit Oviducts.* (32605)

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The normal growth and function of certain mammalian tissues are known to be dependent on the continued presence of delicately balanced amounts of steroid hormones. Jensen and Jacobson in their studies with immature rats have shown differences between the incorporation patterns of estradiol-17β in the uterus and vagina, and in those tissues which are not responsive to estrogens (1,2). The affinity of uterine tissue for estradiol, but not estrone, was demonstrated in both *in vivo* and *in vitro* systems. These

* Supported by NIH Grants HD01199 and HD01810.

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