though this might have been caused by growth in the chick embryo, since similar characteristics were not observed in the TC isolates, it is also reasonable to assume that preferential growth of Asian virus in the egg allowed retention of certain natural characteristics longer than TC. Probably the same mechanism accounted for lack of reactivity with inhibitors and antibody and might be of consequence in explaining spread of the virus if it occurred during infection of man. One might speculate that virus propagated in TC was antigenically closer to the natural agent, but that grown in the egg was reactively more similar.

Summary. The amniotic sac of the chick embryo was much more effective than monkey kidney cultures for isolation of the Asian strain of influenza A, and virus was obtained from at least 60% of serologically diagnosed illnesses. Both monkey kidney culture and chick embryo propagated virus agglutinated human, fowl, sheep and monkey erythrocytes at 4° or 24°C, but titers were lower with sheep red blood cells. Asian virus multiplied readily in monkey kidney cultures, but several passages were required for adaptation to the allantoic sac. Some strains during early egg passages were not inhibited by non-specific substances in normal sera, and some were initially non-reactive with antibody, especially in rde treated sera. Increased sensitivity to inhibitors and antibody occurred with additional chick embryo passages, but remained lesser than the tissue culture line. Asian virus propagated in monkey kidney cultures was preferable to the egg line for determinations of hemagglutination-inhibition and neutralizing antibody by virtue of the higher titers, comparability of response and reproducibility.

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1. Meyer, H. M., Jr., Hilleman, M. R., Miesse, M. S., Crawford, I. P., Bankhead, A. S., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 609.

2. Jensen, K. E., *J. Am. Med. Assn.,* 1957, v164, 2025.

3. Mogabgab, W. J., Green, I. J., Dierkhising, O. C., Phillips, I. A., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 654.

4. Mogabgab, W. J., unpublished data.

5. Isaacs, A., Andrewes, C. H., *Brit. Med. J.,* 1951, v2, 921.

6. Vogel, J., Shelokov, A., *Science,* 1957, v126, 358.

7. Green, I. J., Lieberman, M., Mogabgab, W. J., *J. Immunol.,* 1957, v78, 233.

8. Simpson, G. I., Mogabgab, W. J., *ibid.,* 1957, v78, 456.

9. Henry, C., Youngner, J. S., *ibid.,* 1957, v78, 273.

10. Mogabgab, W. J., Pelon, W., *Clin. Research,* 1958, v6, 151.

11. Pelon, W., Mogabgab, W. J., Dietlein, L. F., Burch, G. E., Holmes, B., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 120.

12. Mogabgab, W. J., Simpson, G. I., Green, I. J., *J. Immunol.,* 1956, v76, 314.

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Antibody Response to Asian Influenza Vaccination in Man.* (24267)

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Although much information has been available on the immune response and protective effects resulting from influenza vaccines, the novelty of the Asian strain of influenza A provided an opportunity for pre-epidemic investigations in populations that were essentially

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free of antibody(1). Such data were desirable in order to evaluate the immunogenicity of a new antigen and its behavior in the inexperienced host(2). Variations in antibody titers dependent upon amount of vaccine and route of administration, upon methods employed for determination of antibody, including preparation of the antigen used for this purpose, are here described.

Materials and methods. Strains of Asian virus used, technics for determination of hemagglutination-inhibition (HaI) and neutralizing antibody and methods of preparation and maintenance of monkey kidney tissue cultures (TC) have been described(3). Monovalent vaccines for experimental use, obtained from commercial sources, had been prepared from Asian virus grown in the allantoic sac of embryonated eggs and purified and concentrated by adsorption-elution with fowl erythrocytes or by protamine precipitation followed by centrifugation. Determinations of chick cell agglutinating (CCA) units were made in a Klett photoelectric colorimeter according to procedures described by Hirst as modified by Stanley(4,5). Equivalent results that were also reproducible were obtained with a Beckman, Jr. spectrophotometer ($\lambda = 545$). Titers that were greater, but that also showed the same relative differences between various lots of vaccine were also obtained by replicate determinations by the hemagglutination pattern technic with pooled fowl erythrocytes. An October 1957 lot of vaccine containing 400 CCA units/ml and previously checked with standard vaccine from the Biological Control Division, Nat. Inst. of Health was accepted as a baseline. Adjustment of CCA unit determinations was made accordingly in order that results would be equivalent to those of a standardized vaccine. Individuals, 20 to 35 years of age, were vaccinated during the period from July to Oct. 1957. Excluded from analysis of results were all persons who developed a clinical illness resembling influenza or all those who had pre-vaccination hemagglutination-inhibition antibody titers greater than 1-8.

Effect of route of administration on antibody response. Comparison of the effect of intradermal, subcutaneous or intramuscular administration of various amounts of vaccine can be made from the titers of HaI antibody that resulted (Table I). Although differences ascribable to route of administration were not produced with small amounts of antigen, a slight advantage of the intradermal route was shown following inoculation of larger quantities of vaccine. This did not appear sufficient to compensate for the higher antibody titers obtained after the larger doses that were feasible by the intramuscular or subcutaneous routes.

Effect of dosage and spacing of inoculations. In general the total amount of vaccine in terms of CCA units injected determined the titer of antibody that resulted. Whether the inoculation was given as a single injection or divided into 2 doses did not appear to affect the final outcome. Possibly, an interval of more than 3 weeks would have allowed a recall response. Nevertheless, there were fewer and milder reactions when the vaccine was given in divided doses, and this was a consideration when 400 CCA unit material was used.

Evaluation of hemagglutination-inhibition antibody titers after vaccination. To compare the magnitude of the post-vaccinal antibody response to that following natural infection the geometric mean hemagglutination-inhibition titer of a large group of convalescent sera was obtained as shown at the bottom of Table I. Since determinations were done with monkey kidney culture propagated Asian virus and sera were treated with receptor destroying enzyme of *Vibrio cholerae* for titrations of antibody in post-vaccinal as well as post-influenzal sera a basis of evaluation was obtained(3). Differences in titer could accordingly be used in comparing results of studies in other laboratories even when dissimilar virus strains or methods were employed. Because there were low titers of inhibition in some acute sera, results were analyzed in terms of fold increase as well as final titers. It can be seen that few of the vaccinated groups had antibody increments as great as those with natural illness. In addition to comparison of results with those ob-

TABLE I. Hemagglutination-Inhibition Antibody Titers Two Weeks after Various Dosages and Routes of Administration of Monovalent Asian Influenza Vaccine in Adult Humans.*

Vaccine	Route and dose in ml†	CCA units	No. tested	% with fold increase of 8 or more	% with titer of 8 or more	Geometric mean fold increase	Geometric mean titers
A	I.D. .1	7	38	11	24	2	3
A	S.C. .1	7	34	3	18	1	2
A	I.M. .5	35	71	13	30	2	3
C	I.D. .1	10	61	28	77	4	11
C	I.M. .2	20	12	33	83	6	15
D	I.M. .5	20	37	5	27	2	2
D	I.M. 1.0	40	54	26	48	3	6
C + C	I.D. .1	10	35	37	87	5	14
	+ I.D. .1	+ 10					
C + C	I.M. .2	20	5	20	80	4	14
	+ I.M. .2	+ 20					
D + D	I.M. .5	20	25	24	56	3	6
	+ I.M. .5	+ 20					
A + B	I.D. .1	7	16	50	88	7	14
	+ I.D. .1	+ 40					
A + B	S.C. .1	7	9	22	50	3	6
	+ I.D. .1	+ 40					
A + B	I.M. .5	35	14	64	93	8	23
	+ I.D. .1	+ 40					
A + B	I.D. .1	7	5	80	80	5	9
	+ I.M. .5	+ 200					
A + B	S.C. .1	7	8	63	88	8	16
	+ I.M. .5	+ 200					
A + B	I.M. .5	35	13	69	92	11	22
	+ I.M. .5	+ 200					
A + B	I.M. .5	35	11	100	100	16	41
	+ I.M. 1.0	+ 400					
Natural infection			177	73	100	11	29

* HaI antibody titers are expressed in terms of initial dilution of serum. Monkey kidney propagated Asian strain influenza A was used in all cases. Serum was treated with RDE.

† Injections were separated by an interval of 3 wk. Convalescent sera were obtained 2 wk after each inj. I.D.—intradermal, S.C.—subcut., I.M.—intramusc.

tained from influenza cases, consideration of the passage history of the virus used appeared relevant to interpretation. As described in an accompanying report(3), antibody titers in convalescent sera from natural illness determined with the TC line of Asian virus were greater than those obtained with the egg (E) or egg- ferret- mouse-egg (EFME) line. In post-vaccinal sera, however, titers with the EFME line were equivalent to those with TC virus (Table II). Accordingly, if one chose the mean antibody response produced by natural illness as the desirable goal for satisfactory response to vaccination, it would be preferable to utilize results obtained by methods that yielded maximum titers. On the other hand, employment of the E virus line to determine mean antibody titer of a group of convalescent sera would make it necessary to obtain antibody response to vaccine 2- to 4-fold greater than this mean when both are determined with the same E line(6).

TABLE II. Mean Hemagglutination-Inhibition Antibody Titers Determined with TC and E Lines of Asian Virus.*

	Virus			
	TC_{2-4}	E_1	E_7	EFME
Treatment of serum	RDE	△ 56°C	RDE	Periodate
Pre-vaccination serum titer	<4	<4	<4	<4
Post-vaccination serum titer	50	13	17	50

* Geometric mean titers determined from 14 paired sera.

Comparison of HaI and neutralizing antibody titers determined with TC and E lines of

TABLE III. Mean Neutralization Titers Determined with TC and E Lines of Asian Virus.*

	Virus			
	TC_{2-4}	E_4	E_8	EFME
Host system	TC	Egg	Egg	Egg
Pre-vaccination serum titer	<4	<4	<4	<4
Post-vaccination serum titer	68	8	15	16

* 10^2 ID_{50} of virus was added to dilutions of heat inactivated sera. Results are geometric mean titers determined from 6 paired sera.

Asian virus. Variations in HaI antibody titers dependent upon the line of virus used are shown in Table II. In each case the optimum method of removal of non-specific inhibitor was used(3). In contrast to the results with post-influenzal sera, titers with the EFME line were equal to those obtained with the TC line. Although a lesser degree of reactivity of the E lines as compared to the TC line occurred with neutralizing antibody (Table III), this was not nearly as pronounced as that with post-influenzal sera. The difference was especially striking when the titers obtained with the E_8 virus in post-influenzal and post-vaccinal sera were compared. Thus, it appears that specificity of antigen or antibody as well as avidity or reactivity must all be considered in explaining variations in antibody titers.

Discussion. Although the degree of protection against infection or illness is the test of a vaccine in the final analysis, correlative data can be obtained by other means of antibody measurement. With viruses such as the influenza group natural alterations in antigenic composition necessitate a corresponding change in vaccine. Accordingly, rapid means of evaluating efficacy of vaccine prior to epidemic spread are essential. Differences in antibody titers dependent upon the passage history of the virus as described herein indicate some of the problems that might be encountered in such an endeavor. Even determinations of the amount of virus in the vaccine has limitations due to inaccuracies of the quantitative aspects as well as variations in response due to immunogenicity and antigenic specificity of the strain. The results of these studies suggest that a vaccine prepared with monkey kidney propagated virus might have produced a more specific antibody to the natural virus. Although this problem was overcome by larger egg vaccine doses, there were advantages to smaller amounts of virus such as a reduction in toxic reactions. Specificity of the antigen is probably not always as noticeable unless a major antigenic change has occurred in the virus. Thus, it was not possible to show antigenic differences between the 1953 influenza A human tissue culture and E lines by HaI with human convalescent sera from natural illnesses(7).

Summary. The route of administration, either intradermal, subcutaneous, or intramuscular, did not significantly affect the antibody response following Asian influenza vaccination. Antibody titers produced were proportional to the total amount of vaccine inoculated whether given as a single dose or divided into 2 injections separated by an interval of 3 weeks. Hemagglutination-inhibition antibody titers in post-vaccinal sera were greater when determined with the TC and EFME lines of Asian virus than with early or later passages of the straight E line. Neutralization titers with the TC line in monkey cultures were considerably greater than those determined with the E lines in the allantoic sac. The geometric mean hemagglutination-inhibition antibody titer of a large group of post-influenzal sera was used as the baseline for evaluation of antibody response to vaccine by using the same virus line and method of inactivation of non-specific inhibitor in sera in both cases.

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1. Francis, T., Jr., *Ann. Int. Med.*, 1955, v43, 534.
2. Davenport, F. M., Hennessy, A. V., *J. Exp. Med.*, 1957, v106, 835.
3. Mogabgab, W. J., Pelon, W., Burch, G. E., Holmes, B., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 116.
4. Hirst, G. K., Pickels, E. G., *J. Immunol.*, 1942, v45, 273.
5. Miller, G. L., Stanley, W. M., *J. Exp. Med.*, 1944, v79, 185.

6. Hilleman, M. R., Flatley, F. J., Anderson, S. A., Leuking, M. L., Levinson, D. J., *J. Am. Med. Assn.*, 1958, v166, 1134.

7. Mogabgab, W. J., Simpson, G. I., Green, I. J., *J. Immunol.*, 1956, v76, 314.

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Animal Responses to 2-Deoxy-D-Glucose Administration. (24268)

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Wick(1) has demonstrated that following 2 deoxy-D-glucose (2-DG) administration there is a decreased requirement for glucose to maintain a constant blood glucose concentration in eviscerated-nephrectomized rabbits. 2-DG has been reported to inhibit growth of experimental animal tumors(2) and to inhibit glycolysis in leukemic cells(3), slices of Walker 256 carcinoma(4) and Krebs carcinoma ascites cells(5). These observations are consistent with the hypothesis that 2-DG is a metabolic blocking agent for glucose. A survey of the response of several animal species to 2-DG was undertaken prior to clinical studies to be reported elsewhere(6).

Materials and methods. 2-DG was obtained from the Aldrich Chemical Co., Milwaukee, Wis. Blood 2-DG concentrations were determined by the Quinaldine method(7) and blood glucose concentrations by a modification of the Nelson method(8).

a) In acute toxicity studies 2-DG in doses of 1-3 g/kg body weight as a 10-40% aqueous solution was administered(1) rapidly by tail vein to 20 Swiss mice, each weighing 20 g, and (2) by ear vein over a 2-minute period to white New Zealand rabbits weighing 1 kg. Blood glucose concentrations before and blood glucose and 2-DG concentrations after infusion were determined in the rabbits.

b) In chronic studies 20 male rats of the Sprague-Dawley strain of initial weight 50 g and on a standard purina diet, were divided into 5 groups of 4 rats each. Group I received 0.5 cc of distilled water s.c. twice daily (8 a.m. and 4 p.m.); Group II received 200 mg of 2-DG/kg initial body weight in 0.5 cc of distilled water s.c. twice daily for a total dose of 400 mg/kg; Group III received 400 mg/kg twice daily; Group IV 600 mg/kg twice daily; Group V 800 mg/kg twice daily. Body weight was checked frequently throughout the study. Administration was discontinued after 19 days, and 2 hours after the last injection 2-DG and glucose concentrations were determined on tail vein blood. One week later fasting blood glucose concentrations were determined.

c) On each of 3 white New Zealand rabbits, fasted 24 hours and weighing 3-4 kg, an intravenous glucose tolerance test (500 mg/kg) and an intravenous 2-DG tolerance test (200 mg/kg), and an oral 2-DG tolerance test (400 mg/kg administered by stomach tube) was performed. Also a glucose tolerance test and glucose-insulin tolerance test (0.5 units/kg)* was performed on each rabbit following intravenous administration of 2-DG (200 mg/kg). There was a time interval of several days between tests.

Results. Mice. The LD_{50} for mice receiving 2-DG acutely by tail vein was between 2 and 3 gm/kg *body* weight. Within 5 minutes after administration the mice became ataxic and then were unable to right themselves. Respirations became rapid and shallow and convulsions occurred occasionally. The mice then became unresponsive to painful stimuli. At a dose of 1 g/kg after about 45 minutes, recovery was noted. It was only after several hours that recovery was observed in mice receiving the higher doses.

Rats. The results of administration to rats of 2-DG subcutaneously over a 19-day period

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