Effect of Neuraminidase on Antibody Combining Unit (ACU) Titers of Human Sera Determined by Drescher's Photometric Procedure[1] (36846)

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Drescher-photometric technique, which differentiates between inhibition of hemagglutination strain specific antibody from that caused by nonspecific inhibition or cross reacting antibodies, has been used in our laboratory to validate the specificity of antibody patterns found in human serum by conventional HI tests (1). On the basis of the age distribution of antibodies to prototype strains, the data have been interpreted to indicate that mankind has experienced in succession past periods of prevalence of swine, Hong Kong, Asian and Equine-2 like viruses, respectively, during the outbreaks of 1918–19, 1900 and 1889–90 and on at least 2 occasions during the period 1874–1888 (2–6).

At the time that these studies were being carried out, it had not been recognized that in special circumstances antibody mediated inhibition of hemagglutination could be effected solely by antineuraminidase antibodies presumably by causing steric hindrance with viral hemagglutinin (7). That finding raised the question whether the antibody patterns heretofore described might not be related to similarities of enzymes rather than to similarities of hemagglutinins present in the strains of former periods of prevalence. This alternate explanation seemed unlikely because in our experience HI titers of human serum caused by antineuraminidase antibodies were demonstratable only by use of erythrocytes of selected chickens or by addition of anti-human IgG to the reaction mixture, conditions that were not required for the demonstration of the antibody patterns (8). Moreover, the frequency and the height of titers observed at the peak of the antibody patterns were considerably greater than was found when measuring antineuraminidase antibody by HI under the best of conditions (12). However, to obtain direct evidence on the question whether the antibody patterns of human sera are oriented to viral hemagglutinin rather than to viral enzyme, a set of convalescent specimens were tested by Drescher's technique using a homologous natural virus and a recombinant containing the homologous hemagglutinin but a heterologous enzyme. The results obtained are the subject of this report.

Materials and Methods. Virus. The virus strains A/Hong Kong/16/68(H_3)-NWS(N_1) and A/Hong Kong/16/68(H_3N_2) were supplied by Dr. Edwin Kilbourne. Infected allantoic fluid suspensions were used as antigens.

Sera. Sera were stored at 4° until used and were treated with 0.011 *M* potassium periodate before testing.

Hemagglutination-inhibition antibody measurement. Drescher's photometric procedure has been fully described elsewhere. It has been found that when an excess of virus is reacted with a series of dilution of antiserum, the ratio of bound and free virus to the concentration of antibody can be expressed in terms of an equation developed by Freundlich for the description of adsorption data. As applied, $\log c_{bv}/c_{ab}) = \log a + 1/N \log c_{fv}$ in which c_{bv} represents the concentration of bound virus, c_{fv} the concentration of free virus, and c_{ab} the concentration of antibody present, whereas a equals the intercept and $1/N$ the slope of the line established by the appropriate experimental data to express the

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TABLE I. Comparison of Titers Measured Photometrically in Human Sera with Either A/HK(H_3)-NWS(N_1) or A/HK/16/68(H_3N_2).

Sera	ACU titer	
	A/HK(H_3)-NWS(N_1)	A/HK/16/68
1 FD	267	208
2 AG	93	88
3 CD	339	256
4 FP	442	344
5 HS-17	200	193
6 HS-18	189	146

relation between c_{fv} and c_{bv}/c_{ab} (8, 9). The titer of a serum is expressed as the number of antibody combining units (ACU) present in undiluted serum. For Hong Kong virus log a is 2.37107 and $1/N$ is 0.21107 as determined by Drescher. Optical density determinations were made with a model B Beckman spectrophotometer.

Results. A number of sera were available that had been obtained from patients who contracted Hong Kong influenza in 1968. The diagnosis was established either by virus isolation and/or conventional HI tests using paired specimens (10). Six convalescent specimens were selected on the basis of excellent antibody response by HI to homologous virus and of antineuraminidase antibody response measured either by neuraminidase inhibition (range 190–710) or by a modified HI technique (range 16– 512) (11, 12). These were then tested photometrically using A/HK/16/68 (H_3N_2) and the recombinant A/HK(H_3)-NWS(N_1) as antigens. It was reasoned that if viral enzyme participated significantly in the determination of ACU titers measured photometrically, the recombinant would not score as a specific homologous antigen–antibody reaction since it contains a false enzyme, *i.e.*, that of the heterologous strain NWS.

The results are shown in Table I. The average titers, as measured with either strain, were of similar magnitude although the ones found with A/HK/16/68 (H_3N_2) were consistently slightly lower than those determined with A/HK(H_3)-NWS(N_1). In each instance, the data fit the isotherm characteristic of Hong Kong virus. The criteria for fit is that in a series of dilutions a zone is reached which yields relatively constant titer values and in this range the reaction appears to be governed solely by the Hong Kong isotherm. If such a range is not reached, this is an index that the kinetics of the virus antibody reaction do not fit the isotherm used and the reaction observed is, therefore, heterologous (8). From these findings it is clear that the specificity of antibody measured by Drescher's photometric technique is not affected by the presence or absence of the corresponding neuraminidase antigen.

The findings that the titers measured with A/HK/16/68(H_3N_2) were consistently slightly lower may reflect steric hinderance by neuraminidase antibody bound at the enzyme sites of A/HK/16/68(H_3N_2). This could be possible since the neuraminidase antibody oriented to Hong Kong strains shows no cross reaction with the enzyme of the recombinants (7).

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