NEUTRALIZATION OF INFLUENZA A VIRUS BY HUMAN SERUM

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It has been shown previously (1) that a linear relationship exists between the quantity of serum and the quantity of influenza A virus (2) neutralized. This relationship was established on the basis of experiments performed with the serum of ferrets convalescent from experimental influenza and the serum of rabbits immunized with the virus. It was shown that by means of this relationship it was possible to calculate the greatest quantity of virus which either rabbit or ferret antisera could neutralize in the undiluted state. It was suggested that this fixed value, the neutralizing capacity, which was independent of the quantity of virus used in the neutralization test, had an entirely different significance than the more commonly used serum dilution end point or titer, which was a function of the quantity of virus used.

In order to assess accurately the significance of the antibody levels of the serum of normal human beings against influenza A virus, or the antibody increases which follow an attack of influenza A (2) in man, it was of importance to study the neutralization of influenza A virus by human serum in an identical manner and to determine whether a similar linear relationship between serum and virus neutralized existed under these conditions.

It is the purpose of this paper to report the results of quantitative neutralization tests carried out with two strains of influenza A virus and an acute phase and convalescent serum obtained from one individual who had influenza A.

Material and Methods

Virus.—The PR8 strain (3) and the W.S. strain (4) of influenza A virus were used. Stock suspensions of infected mouse lungs were prepared and stored in a manner identical with that previously described (1).

Serum.—Acute phase serum was obtained from a patient with influenza A 4 days after the clinical onset of the disease. This patient was one of many who suffered from influenza A during an epidemic which occurred in 1939, studies on which have been

reported previously (5). A convalescent serum was obtained from the same patient 26 days after onset.

Neutralization Tests.—Quantitative neutralization tests were performed exactly as described previously (1). Decimal dilutions of virus were each tested against a number of serial serum dilutions. Each test with either strain of virus and either serum was repeated on three separate occasions. Each mixture of serum and virus was inoculated intranasally into a group of six lightly anesthetized mice.

Virus Titrations.—Titrations of the infectiousness of the virus suspensions were performed in a manner identical with that described previously (1). Each individual neutralization test was controlled by a titration of the virus done at the same time. Groups of six lightly anesthetized mice were inoculated intranasally with each dilution of virus.

Calculation of End Points.—Both the virus titration end points and the serum dilution end points were calculated by the 50 per cent end point method of Reed and Muench (6). Since the 50 per cent mortality end point has been found to be somewhat more accurate than any of the other end points which can be calculated in such tests with influenza A virus, this end point has been used throughout the present investigation.

EXPERIMENTAL

Decimal dilutions of the PR8 strain of influenza A virus were each tested against a number of serial dilutions of both the acute phase and the convalescent sera. Each of these two sera was used in three separate tests. The results obtained in one test with the convalescent serum are shown in Table I. It will be noted that the survival or death of mice in groups inoculated with the various mixtures of virus and serum show quite clearly that as the quantity of virus was decreased decimally, the serum dilution end points did not increase proportionately but instead increased by approximately fivefold.

The mean 50 per cent mortality dilution end points obtained in the six neutralization tests with the acute phase and the convalescent sera against varying amounts of the PR8 strain are presented in Table II. It will be observed that the neutralizing titers of both the acute phase and the convalescent sera were increased by approximately 5 times as the quantity of virus against which they were tested was decreased by 10 times. It will also be noted that the increased neutralizing titer of the convalescent serum as compared with the titer of the acute phase serum was practically independent of the quantity of virus used and remained constant at each virus dilution.

The results obtained in all of the neutralization tests with these two sera and the PR8 strain are presented graphically in Fig. 1. The logarithm of each serum dilution end point has been plotted against the logarithm of the quantity of virus neutralized. It will be observed that when the quantity of virus used in each test was taken into

account, the three sets of end points obtained with each serum fell reasonably close together. Straight lines which appeared to fit these end points well have been drawn through them. These lines were purposely drawn with a slope identical to that previously determined in experiments on the linear relationship between serum and virus with ferret and rabbit antisera (1). It is apparent that there does not appear to be any

TABLE I

Results of Neutralization Tests with Decreasing Quantities of the PR8 Strain of Influenza A Virus and Convalescent Human Serum

Virus dilution	No serum	Serum dilution							Virus neutralized
		1:5	1:25	1:125	1:625	1:3,125	1:15,625	1:78,125	
10^{-1}	—	5S 1D	6D	6D	6D	—	—	—	$10^{5.6}$
10^{-2}	—	6S	6S	6D	6D	6D	—	—	$10^{4.6}$
10^{-3}	—	—	6S	6S	6D	6D	6D	—	$10^{3.6}$
10^{-4}	6D	—	—	6S	6S	6D	6D	6D	$10^{2.6}$
10^{-5}	6D	—	—	—	6S	6S	6D	6D	$10^{1.6}$
10^{-6}	6D	—	—	—	—	—	—	—	
10^{-7}	5S 1D								
10^{-8}	6S								

S = mouse survived observation period of 11 days.
D = mouse died with ++++ pulmonary consolidation.

TABLE II

Mean 50 Per Cent Mortality Dilution End Points of Acute Phase and Convalescent Human Sera against the PR8 Strain of Influenza A Virus

Virus		Mean 50 per cent mortality serum dilution end point		Increase in titer
Dilution	Approximate 50 per cent mortality doses	Acute phase	Convalescent	
	log			
10^{-1}	$10^{5.5}$	—	1:7	—
10^{-2}	$10^{4.5}$	1:4	1:35	8.7×
10^{-3}	$10^{3.5}$	1:21	1:186	8.8×
10^{-4}	$10^{2.5}$	1:129	1:742	5.8×
10^{-5}	$10^{1.5}$	1:602	1:5120	8.5×

systematic deviation of the experimental end points from either of the two straight lines. Twelve end points found with the acute phase serum and the PR8 strain are shown in Fig. 1. These end points had, on the X axis, a mean deviation from neutralization line (II) of log ±0.19. The two maximum deviations from this line were log ±0.35. Fifteen end points determined with the convalescent serum and the PR8 strain are also shown. These end points had, on the same axis, a mean deviation from neutralization line (I) of log ±0.11. The two maximum deviations from this line were log ±0.22. The line X_1 has been inserted in Fig. 1 to show graphically the difference between the so called standard neutralizing titers (7) of the acute phase and the convalescent sera.

The length of this line, which corresponds to log −2.37 minus log −1.47 or log −0.90, indicates that the convalescent serum had a standard titer 7.9 times greater than that of the acute phase serum. The line Y_1 also has been inserted in Fig. 1 to demonstrate graphically the difference between the neutralizing capacities of these two sera. The length of this line, which corresponds to log 6.91 minus log 5.61 or log 1.30, indicates that the convalescent serum has a virus-neutralizing capacity 20 times greater than that of the acute phase serum.

Decimal dilutions of the W.S. strain were each tested against a number of serial dilutions of both the acute phase and the convalescent sera. Three separate neutralization tests were run with this strain of virus and each

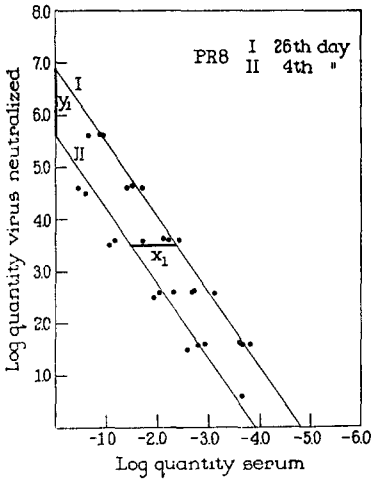

FIG. 1

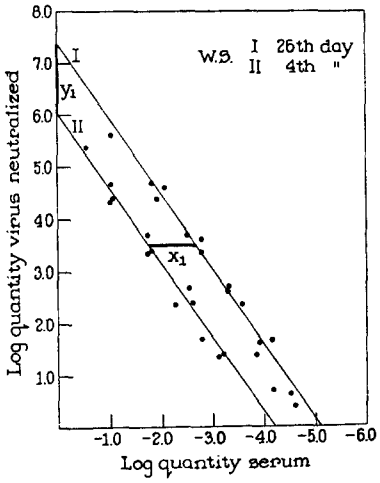

FIG. 2

serum. The mean 50 per cent mortality dilution end points obtained with these two sera and the W.S. strain are shown in Table III. It will be seen again that the titers of both sera were increased by approximately 5 times as the quantity of virus against which they were tested was decreased by 10 times. Moreover, it will be noted that the increase in titer of the convalescent serum as compared with that of the acute phase serum was relatively constant and was independent of the quantity of virus used.

The results of all the tests with the acute phase and convalescent serum and the W.S. strain are presented in Fig. 2. The logarithm of each serum dilution end point has been plotted against the logarithm of the quantity of virus neutralized. It will be observed that the three sets of end points for each serum fell reasonably close together. Straight lines which appear to fit these experimental points well have been drawn through them. These lines were drawn purposely with slopes identical to those shown in Fig. 1. It seems evident that there is no systematic deviation of the end points from these

lines except in the three instances in which less than five 50 per cent mortality doses of virus were neutralized. Thirteen end points obtained with the acute phase serum and the W.S. strain are shown in Fig. 2. These end points had, on the X axis, a mean deviation from neutralization line (II) of log ± 0.11. The two maximum deviations observed were log ± 0.20. Sixteen end points determined with the convalescent serum and the W.S. strain are also shown. These end points had, on the same axis, a mean deviation from the neutralization line (I) of log ± 0.12. The two maximum deviations found were log ± 0.32. The line X_1 has been drawn in Fig. 2 to demonstrate graphically the increase in standard neutralizing titer of the convalescent serum. It will be observed that this increase corresponded to log 0.91, which represented an arithmetic increase in titer of 8.1 times. The line Y_1 has been drawn in Fig. 2 to show graphically the increase in virus-neutralizing capacity of the convalescent serum. This increase corresponded to log 1.31, which represented an arithmetic increase in neutralizing capacity of 20 times.

TABLE III

Mean 50 Per Cent Mortality Dilution End Points of Acute Phase and Convalescent Human Sera against the W.S. Strain of Influenza A Virus

Virus		Mean 50 per cent mortality serum dilution end point		Increase in titer
Dilution	Approximate 50 per cent mortality doses	Acute phase	Convalescent	
	log			
10^{-1}	$10^{5.5}$	—	1:11	—
10^{-2}	$10^{4.5}$	1:11	1:87	7.9×
10^{-3}	$10^{3.5}$	1:59	1:490	8.3×
10^{-4}	$10^{2.5}$	1:302	1:2510	8.3×
10^{-5}	$10^{1.5}$	1:1120	1:9550	8.5×

DISCUSSION

It is quite obvious that serum dilution end points or titers are strictly dependent upon the amount of virus used in a test. If the antibody levels of multiple serum specimens from one individual or from different persons are to be evaluated accurately, it is important that the sera be tested against equal amounts of virus. Frequently it is difficult to accomplish this since it is manifestly impractical to include more than a limited number of sera in one test. In certain previous investigations (5, 7) with human sera this difficulty was circumvented by the determination of so called standard neutralization titers. The quantity of virus, $10^{3.5}$ fifty per cent mortality doses, used in determining the standard titers was so large, however, that approximately 40 per cent of human sera failed to neutralize it (7). On the other hand, when $10^{1.5}$ fifty per cent mortality doses of virus were used, it was found that at least 95 per cent of normal human sera possessed

a definite capacity to neutralize influenza A virus, whereas the sera of normal experimental animals did not.

Not only did the neutralization titer of a given human serum depend upon the amount of virus used in a test, but also, as has been shown, sera became progressively less and less efficient in their abilities to neutralize the virus as they were diluted more and more. When experimental end points obtained in neutralization tests were plotted logarithmically, it became apparent that a linear relationship existed between the quantity of human serum used and the quantity of virus neutralized. This relationship seemed to be identical with that found previously (1) in experiments with ferret and rabbit antisera. Moreover, the relationship remained the same in tests with two antigenically different (8) strains of influenza A virus.

By means of this relationship it is possible to calculate the maximum quantity of virus which a particular serum can neutralize. It has been shown (1) that the whole course of the neutralization of influenza A virus by either ferret or rabbit antisera can be expressed by the equation

$$y = bx^a$$

in which y is the quantity of virus neutralized, b the intercept on the y axis, x the serum dilution end point, and a an experimental constant which has been found to be 1.44. This equation and the constant 1.44 also can be applied to human sera. It is apparent that the neutralizing capacity, b, of a serum can be estimated most accurately when a number of serum dilution end points are determined against various amounts of virus. A straight line, with the proper slope, drawn through these points intercepts the y axis at a point which indicates the greatest quantity of virus the serum can neutralize. It is obviously impractical to determine a number of dilution end points with every serum since the number of mice required would be very large. However, under the conditions of these experiments, the accuracy of individual dilution end points was found to be sufficiently high to permit the calculation of the neutralizing capacity of a serum from a single end point, providing the quantity of virus neutralized was also known. By means of the equation

$$\log b = \log y - (a \log x)$$

the neutralizing capacity of a given serum can be determined directly from the logarithm of the serum dilution end point. It is of importance to point out that x, the serum dilution end point, is a fractional quantity and, therefore, that $\log x$ is always negative.

The neutralizing capacity of a serum appears to be a fixed quantity which is independent of the amount of virus used in the neutralization test. It serves to characterize definitely the antibody level of a serum and circumvents the numerous practical and theoretical difficulties which arise from the use of neutralization titers.

SUMMARY

A linear relationship exists between the quantity of human serum used and the quantity of influenza A virus neutralized. By means of this relationship it is possible to determine the maximum quantity of virus which a given human serum can neutralize. This quantity, the neutralizing capacity, is a fixed value and, unlike the serum dilution end point, is independent of the amount of virus used in the neutralization test.

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