THE USE OF THE HAEMAGGLUTINATION-INHIBITION TEST IN EPIDEMIOLOGICAL INFLUENZA-VIRUS STUDIES

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The importance of the haemagglutination-inhibition test does not need to be stressed. It is a fact that the inhibition titre of a single serum has no constant value, but may vary in repeated estimations. This may be due to the use of different virus or erythrocyte suspensions or to differences in environmental circumstances, such as temperature. Duplicate tests carried out at the same time and with the same materials yield identical results. This makes it necessary to compare a serum from the acute phase of the illness with a serum obtained during convalescence from the same case. The ratio between the two titres shows whether an influenza infection has taken place or not. Although it is commonly accepted that a titre rising by two twofold dilutions (a fourfold rise) is significant, some workers will accept even a twofold rise (Sartwill & Long, 1948; Rasmussen, Stokes & Smadel, 1948). Such observations have been made on pairs of sera taken from the same individual with not more than a 5-week interval between them.

The purpose of the present investigation was to decide whether it is possible to use agglutination-inhibition as a test of infection if one compares sera obtained at the beginning and at the end of the influenza season.

This question can be divided into two questions: Does the titre of antibodies after an infection diminish so rapidly as to have disappeared altogether after an interval of 5 months? What is the range of fluctuations in titre of pairs of sera from normal individuals taken with intervals of 5–6 months?

Although some authors answer the first of these questions in the negative, no definite data are available. A statistical study is in progress and will be published.

The second question is answered by the present investigation, which was made possible by the co-operation of more than 200 medical students. Each student was bled twice, once in November 1947 and again in March 1948. Only sporadic cases of influenza occurred during that winter. Serum from each sample of blood was ampouled. After inactivation at 56° C. (133° F.) for half an hour, all ampoules were stored at 4° C. (40° F.).

The haemagglutination-inhibition test was carried out by the pattern-reading method (Dinger & Wolff, 1948). Both sera from each subject were tested simultaneously on the same virus and erythrocyte suspensions.

The results were arranged for statistical analysis.

Three subjects were known to have suffered from influenza on clinical and on immunological grounds. Their serum pairs were eliminated. Those serum pairs were also eliminated in which one or both samples showed a titre of less than 30. No sera in the rejected pairs had titres exceeding 45. After this elimination of more than 250 serum pairs examined, 177 remained. The ratios between the titres of the first and the second samples of these 177 pairs are shown in Table 1.

Table 1. *Ratios of agglutination-inhibition titres for influenza of pairs of sera from* 177 *apparently normal individuals taken with an interval of* 5 *months*

Ratio between titres of first and second sera	No.	Ratio between titres of second and first sera	No.
1	90	1·2	10
1·2	22	1·4	7
1·4	14	1·6	7
1·6	7	1·8	1
1·8	4	2·0	1
2·0	6	2·5	2
2·7	1	3·3	2
3·0	1	5·0	1
		6·0	1

As we are dealing with ratios it is necessary to present the curve logarithmically. The standard deviation (S.D.) of the logarithms from their mean was calculated. This is permitted as the curve shows a symmetrical shape, the geometrical mean (M) lying quite close to one (1·0243).

The S.D. of the logarithms is 0·16087. A ratio of 4 equals $M + 3{\cdot}8$ S.D. ($3{\cdot}8$ S.D. $= 0{\cdot}61146$; $M + 3{\cdot}8$ S.D. $= 0{\cdot}60100$). The probability of a ratio equal to or greater than this number being due to chance is

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therefore very small (1 : 13830). We may therefore assume that a rise in titre of a factor 4 or larger is significant, and the logical deduction is that an infection with influenza virus may have been the cause of it. On the contrary, a twofold rise in titre is not significant. This value only differs by 1·9 times S.D. from the calculated mean, which may be due to mere chance, the odds against it being only 33:1.

We may add that this calculation is valid only for sera obtained with an interval of 5 months. Should the interval between the first and second serum be shorter, a twofold rise in titre might be significant.

The result described above does not mean that no cases of influenza could be found without a significant rise in titre. The figures only show that it is necessary to keep the factor 4 as a minimum for conclusions of epidemiological value.

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