The Quanitative Assay of
Antibody by Haemagglutination-inhibition: Studies
by Sedimentation-enumeration of RBC Aggregates

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The sedimentation-enumeration method described (Cameron & Bradish, 1972) for quantitative studies of haemagglutination by Semliki Forest virus (SFV) has been used for the quantitative assay of antibody through its activity for combination with virus haemagglutinin.

SFV haemagglutinin was prepared by inoculating the brains of suckling mice with the v13 strain of SFV (Bradish, Allner & Maber, 1971) and treating the harvested brains with fluorocarbon. Studies by gel filtration and equilibrium density gradient centrifugation showed that the haemagglutinin was homogeneous and similar to the infective particle in size and density (Cameron, 1969). Dilutions of a rabbit hyperimmune serum prepared against the specified strain (v13) of SFV (R. B. Fitzgeorge & C. J. Bradish, to be published) were reacted with different dilutions of SFV haemagglutinin in borate buffer (pH 9.0), containing 0.2% (w/v) bovine serum albumin, at room temperature (23 °C) for 30 min. An equal volume of a suspension of goose red blood cells (RBC) in phosphate buffered saline (PBS) was then added to each reaction mixture to give a final optimum pH 6.3 and an overall RBC concentration of 10^7/ml. Each mixture was then sampled and observed microscopically by the sedimentation-enumeration method (Cameron & Bradish, 1972) for the formation under standard conditions of RBC aggregates of various sizes. From the observed concentration and distribution of size-specified aggregates, the total numbers of RBC–RBC bonds (B) and of red blood cells (R) were determined and from these the extent of agglutination was calculated (Cameron & Bradish, 1972) as the average number of haemagglutinin-specific RBC–RBC bonds/red blood cell. This is indicated by \( (B/R)_{ab} \), where the second term expresses the correction for spontaneous agglutination in the absence of haemagglutinin. Residual haemagglutinating activities calculated in this way are presented in Fig. 1 and appear as a series of parallel lines displaced (δ) from the haemagglutinin-only control according to the antiserum dilution or antibody concentration used. Clearly, and as interpreted through Fig. 2 below, the greater the initial concentration of antibody, the lesser the quantity of haemagglutinin remaining in suspension to be detected by the agglutination of the RBC finally added.

We have shown (Cameron & Bradish, 1972) that the linear region (Fig. 1) of the haemagglutination characteristic represents by its position the virus-specific haemagglutinating activity (H) or concentration of available haemagglutinin. Thus the horizontal or vertical displacement of the reaction lines (δ in Fig. 1) due to the increasing concentration of antibody indicates the reduction of haemagglutinating activity or concentration of available haemagglutinin. In quantitative terms, the reduction of the logarithm of the haemagglutinating activity is proportional to the depression of the extent of agglutination,

\[
\log \left( \frac{H_0}{H_{ab}} \right) = \alpha \left( B/R \right)_H - \left( B/R \right)_{ab} = \alpha \delta.
\]

Here \( \alpha \) is a reaction constant, and \( (B/R)_{ab} \) or \( (B/R)_H \) the extents of agglutination in reactions with or without antibody, respectively. The dependence of this reduction of agglutination upon the concentration of antibody is shown in Fig. 2 for a number of experiments under
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Fig. 1. The residual haemagglutinating activity expressed by the extent of agglutination as bonds/RBC due to SFV haemagglutinin previously reacted for 30 min at 23 °C with various dilutions of a rabbit anti-SFV serum: [D], 0.1; ■, 0.5; △, 1; ▲, 3; ○, 5; ▲, 10. First reaction: haemagglutinin-antibody reaction in borate buffer, pH 9.0, with 0.2% (w/v) bovine serum albumin. Second reaction: agglutination of goose RBC (10^7/ml) by residual haemagglutinating activity in medium adjusted to optimum pH 6.3 by PBS.

The reduction of haemagglutinating activity due to the first reaction with antibody is shown by the displacement

$$\delta = (B/R)_H - (B/R)_{Ab}$$

Fig. 2. Inhibition by antibody of haemagglutination by SFV presented in terms of the percentage law. Reaction conditions as for Fig. 1. $D_{Ab}$, reciprocal of overall dilution of antiserum in primary reaction with haemagglutinin: ○, □, 32; ●, 128; △, 512; ▲, 1024. $(B/R)_H$ or $(B/R)_{Ab}$: extent of agglutination as bonds/RBC due to haemagglutinin only (suffix $H$) or haemagglutinin previously reacted with antibody (suffix $Ab$). The serum ‘haemagglutination-inhibition’ index is shown by the intersection on the ordinate of the straight line fitted by the method of least squares.

Different conditions with dilutions of a single rabbit anti-SFV serum. The relationship indicated by the line in Fig. 2 is characteristic (Bradish, Farley & Ferrier, 1962) of the percentage law proposed by Andrews & Elford (1933) for the neutralization of virus infectivity by reaction with antibody. This implies that the reduction of haemagglutinating activity by antibody in excess is determined by the concentration of antibody but is independent of the initial concentration of haemagglutinin.

Thus, by analogy with the mechanism and analysis of virus neutralization by antibody in excess (Bradish et al. 1962) we may write,

$$\text{Serum 'haemagglutination-inhibition' index} = \beta \delta + \log D_{Ab}$$

Here $D_{Ab}$ is the overall dilution (denominator) of antiserum in the reaction system. The serum ‘haemagglutination-inhibition’ index, like the serum neutralization index (SNI), is the logarithm of a constant times the concentration of antibody and is indicated for these experiments as $3.6 \pm 0.3$ by the intersection of the ‘best-fit’ line with the ordinate. The slope, $\beta$, of the relationship in Fig. 2 characterizes the mechanisms of agglutination and of the prior combination of antibody and haemagglutinin according to the percentage law.
apart from the merits of a quantitative analysis and a potentially absolute interpretation, information of the present type is not available through current pattern or photometric tests which depend upon unspecified distributions of aggregates and quantal observations of convenient but arbitrary reaction mixtures and end-points.

It is of interest to note that the typical rabbit anti-SFV serum (Bradish et al. 1971) quoted in this study showed a serum neutralization index (SNI) of about 4 log units in tests based on plaque reduction in agar suspensions of primary chick-embryo cells (Bradish et al. 1971). This near-identity of the neutralization and haemagglutination-inhibition indices suggests that the early mechanism through which antibody blocks virus infection of the chick cell is similar to that by which antibody blocks virus agglutination of the goose red blood cell. Since picorna- and arbo-virus particles generally combine rapidly with antibody molecules (Bradish & Crawford, 1960; Bradish et al. 1962) to form stable complexes showing both antigen- and antibody-sites (amphoteric), it is probable that the complexes of antibody with virus haemagglutinin are formed equally rapidly. The subsequent agglutination of RBC is then by stable, amphoteric complexes which, as anticipated by the percentage law, are not inhibited further by the excess of unadsorbed antibody.

Although the method of sedimentation-enumeration may be applied to the quantitation of haemagglutination or haemagglutination-inhibition in myxo- or other virus systems (Cameron, 1969), the mechanisms of inhibition by antibody in these systems may not follow the percentage law as represented by Fig. 2 and the equations above. In such cases the serum ‘haemagglutination-inhibition’ index would require to be replaced by an alternative constant appropriate to the reaction-kinetics of the system.

Microbiological Research Establishment
Porton Down
Salisbury, Wiltshire, England

REFERENCES


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* This work formed part of a thesis submitted for the Ph.D. degree of the University of Edinburgh (1969). Present address: Department of Virology, The Medical School, Birmingham B15 2TJ.