[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

A Theory of Antibody–Antigen Reactions. II. Theory for Reactions of Multivalent Antigen with Multivalent Antibody[1]

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By means of the methods described in the first paper of this series, TA-ARI, equations are developed for a theory involving reactions of g-valent antibody with f-valent antigen. An increase in the antibody valence produces an increase in the weight-average molecular weight of the system, broadens the region of precipitation and decreases the antibody–antigen ratio of large aggregates at the critical point except for the region over which an "inversion effect" occurs. This change in breadth of the region of precipitation is not sufficient to account for the observed differences in the behavior of equine and rabbit antibody systems with respect to antibody-excess inhibition.

Part A. Introduction

In the first paper of this series TA-ARI, a theory describing the reactions of f-valent antigen molecules with univalent and bivalent antibody molecules was developed.[2] It rests upon the calculation of the most probable distribution for a model system in which all valence sites are equally reactive and any cyclical or ring structure is forbidden in the formation of aggregates. The distribution is a function of the composition of the system, the fraction of the antigen sites which have reacted, and the antigen valence.

It is the purpose here to describe the reactions of f-valent antigen molecules with g-valent antibody molecules using the previous approach. It might be well to note that a valence g for an antibody molecule does not imply that all g sites are used up in antibody–antigen bond formation. As long as the possibility exists for any of the g sites to be used up in reactions, the antibody molecule must be considered g-valent.

Part B. Theory

With the use of the concepts presented in TA-ARI, the equations describing the system of G molecules of f-valent antigen and A molecules of g-valent antibody can be derived. After a given fraction of antigen sites p has reacted with a fraction of the antibody sites rp, the distribution of species in the system is

$$m_{ik} = fG \frac{(gi - i)!(fk - k)!r^{k-1}p^{k+i-1}}{(gi - i - k + 1)!(fk - k - i + 1)!i!k!} \times (1 - p)^{fk-k-i+1}(1 - rp)^{gi-i-k+1},\ r = fG/gA \quad (1)$$

where m_{ik} is the number of aggregates in the system each containing i antibody molecules combined with k antigen molecules. The allowed values of i and k may be determined from the antibody and antigen sites in the aggregate available for combination. This information is provided in Appendix A. The allowed values are given by

$$i = (k - 1)/(g - 1) + q$$
$$0 \leq q \leq fk - k + 1 - (k - 1)/(g - 1) \quad (2)$$

In case $(k - 1)/(g - 1)$ is not a whole number, the whole number just above it is the appropriate one. The derivation of equation 1 involves, as in TA-ARI, the evaluation of certain sums and the combinatorial factor W_{ik}, the latter being the number of ways to construct a simple i,k-aggregate from i, g-valent antibody molecules and k f-valent antigen molecules. These are described in the Appendix.

Equation 1 may be derived more easily by the direct method orginally used by Flory in obtaining the distribution of linear polymers.[3] Flory's recent direct treatment of branched polymers[4] was unknown to this author at the time the latter developed the following argument. The probability of finding in the system an antigen or antigen site connected to an i,k-aggregate is km_{ik}/G. The quantity $(1 - p)^{fk-i-k+1}(1 - rp)^{gi-i-k+1}$ is the probability of finding $fk - (i + k - 1)$ and $gi - (i + k - 1)$ free antigen and antibody sites, respectively, the number required on an i,k-aggregate. Furthermore, $p^i(rp)^{k-1}$ is the probability of finding i reacted antigen sites, and $k - 1$ reacted antibody sites not bonded directly to the former i antigen sites. The product of these probabilities with fk gives the probability of finding an antigen site on an i,k-aggregate in a specified configuration. Since i,k-aggregates can arise in many ways, depending on the arrangement of the molecules in the aggregate, a weighting factor corresponding to the different configurations available must be included for determining the probability of finding an antigen site on any i,k-aggregate. This factor is merely $R_{ik}/i!k!$ (see equation A2 in Appendix A). Therefore, $km_{ik}/G = fk(1 - p)^{fk-i-k+1}(1 - rp)^{gi-k-i+1}p^i(rp)^{k-1}R_{ik}/i!k!$, which reduces to equation 1.

If neither f nor g is unity the system will exhibit a critical point at the critical extent of reaction p_c.[2] This is the extent of reaction at which the system changes rapidly from one composed chiefly of small aggregates into one composed of relatively few enormous aggregates. The system can be characterized by a tremendous increase in its weight-average molecular weight, $(M)_w$, at this point. The weight-average molecular weight may be evaluated from the definition, equation 42 of TA-ARI. The required sums may be computed like the example in Appendix B. They are found to be

$$\sum_{i,k} i^2 m_{ik} = A + gArp^2(f - 1)\frac{1 - [rp^2(f - 1)(g - 1]^N}{1 - rp^2(g - 1)(f - 1)}$$
$$\sum_{i,k} k^2 m_{ik} = G + fGrp^2(g - 1)\frac{1 - [rp^2(g - 1)(f - 1)]^N}{1 - rp^2(g - 1)(f - 1)}$$
$$\sum_{i,k} ikm_{ik} = fGp\frac{1 - [rp^2(g - 1)(f - 1)]^N}{1 - rp^2(g - 1)(f - 1)} \quad (3)$$
$$N = (g - 1)(f - 1)k_{max}$$

Therefore

$$[(M)_w - (M)_{wo}]W = pfG[M_A^2 p(f - 1) + M_G^2 rp(g - 1) + 2M_A M_G]\frac{1 - [rp^2(f - 1)(g - 1)]^N}{1 - rp^2(f - 1)(g - 1)} \quad (4)$$

(1) This investigation was supported by the National Science Foundation, Contract NSF-G22.

(2) R. J. Goldberg, THIS JOURNAL, **74**, 5715 (1952).

(3) P. J. Flory, *ibid.*, **58**, 1877 (1936).

(4) P. J. Flory, *ibid.*, **74**, 2718 (1952).

where $(M)_{wo}$ is the initial weight-average molecular weight of the system and W is its mass. The critical extent of reaction is seen from equation 4 to be

$$p_c^2 = 1/[r(f-1)(g-1)] = \frac{A}{G}\frac{g}{f(f-1)(g-1)} \quad (5)$$

Equations 1, 4 and 5 correspond to a special case of the expressions written down by Stockmayer in his extension of the theory of three-dimensional condensation polymers.[5] Figure 1 shows the dependence of $[(M)_w - (M)_{wo}]W$ on the valence of the antibody. The values used for M_A, M_G, G, A and f are given in the legend of Fig. 1.

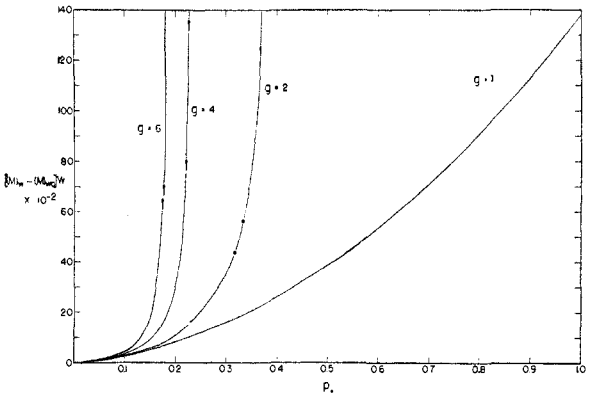

Fig. 1.—The effect of the extent of the reaction p, on a function of the weight-average molecular weight, $[(M)_w - (M)_{wo}]W$, for systems containing g-valent antibody and 7-valent antigen. $(M)_{wo}$ is the weight-average molecular weight for p zero. W is the mass of the system. $M_A = 1.7 \times 10^5$, $M_G = 0.7 \times 10^5$, $G = 10^{-8}$ mole, $r = 1$.

The attainment of the critical point is made impossible if either p_c or rp_c exceeds unity as calculated from equation 5. Consequently, the composition of the system can be so adjusted that the critical point is not attainable. Under these conditions the system exhibits complete inhibition of the critical point. The limiting values, beyond which this inhibition occurs, are defined by

$$f/[g(g-1)(f-1)] \leq A/G \leq f(f-1)(g-1)/g \quad (6)$$

If the system is prepared in such a manner that the antibody–antigen ratio lies outside the limits given by equation 6, then p_c is not attainable. Figures 2 and 3 illustrate how the lower and upper limits, respectively, are affected by the valence of the antibody as well as the valence of the antigen. The difference between the ordinate values of Figs. 2 and 3 (for the same values of f and g) gives the range of ratios for which the critical point can be attained. This range increases as the valence of the antigen increases and also as the valence of the antibody increases.

The next step is the evaluation of the average number of antibody molecules in all aggregates containing k antigen molecules. As before we denote this average as $\bar{\imath}_k$.

$$\bar{\imath}_k = \frac{\sum_i i m_{ik}}{\sum_i m_{ik}} = (fk - k + 1)\left\{1 - \left[\frac{\left(\frac{\partial}{\partial Z}\right)^{k-1}(1 + Lz^{g-1})^{fk-k}}{\left(\frac{\partial}{\partial Z}\right)^{k-1}(1 + Lz^{g-1})^{fk-k+1}}\right]_{z=1}\right\} \quad (7)$$

$$L = p(1 - rp)^{g-1}/(1 - p)$$

In the case of bivalent antibody molecules, equation 7 reduces to

$$\bar{\imath}_k = k - 1 + (fk - 2k + 2)\frac{p - rp^2}{1 - rp^2} \quad (8)$$

For the general case, equation 7 may be evaluated in straightforward fashion for small values of k.

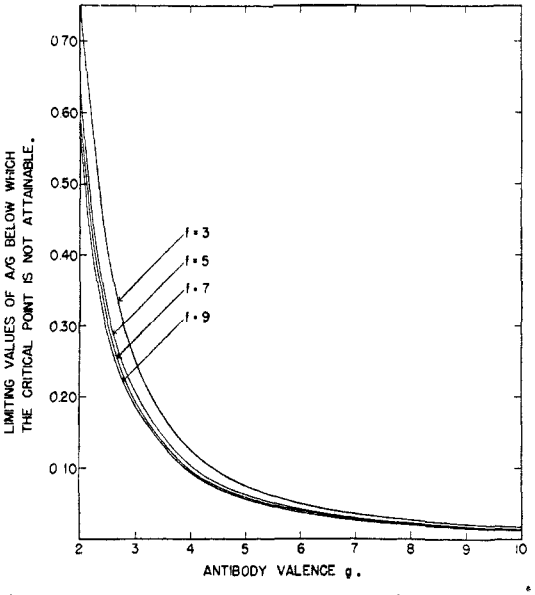

Fig. 2.—The effect of antibody and antigen valences on the critical point lower limit. This limit is defined by $A/G \geq f/[g(g-1)(f-1)]$.

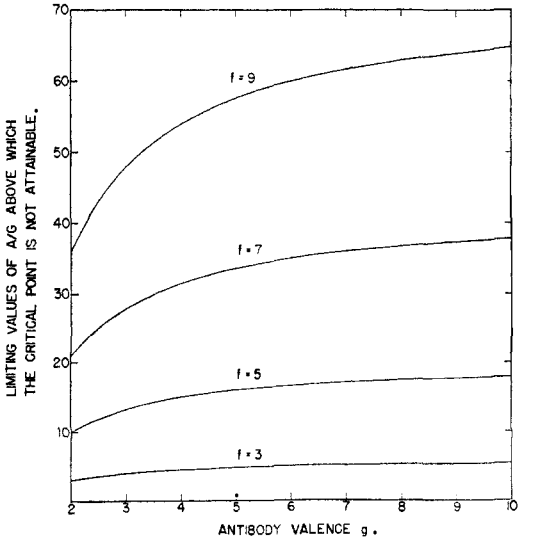

Fig. 3.—The effect of antibody and antigen valences on the critical point upper limit. This limit is defined by $A/G \leq f(f-1)(g-1)/g$.

For large values of k an approximate solution can be found with the help of the expression

(5) W. H. Stockmayer, *J. Polymer Sci.*, **9**, 69 (1952).

$$\left[\left(\frac{\partial}{\partial Z}\right)^{k-1}(1+Lz^{g-1})^{fk-k}\right]_{z=1} = (1+L)^{fk-k}(fk-k)!\sum_{j=\frac{k-1+g-2}{g-1}}^{k-1}\left(\frac{L}{1+L}\right)^{j}\frac{g_j}{(fk-k-j)!} \quad (9)$$

Equation 9 in equation 7 with the subsequent use of the Stirling formula yields approximately

$$\bar{\imath}_k = k - 1 + (fk - 2k + 2)\frac{p(1-rp)^{g-1}}{1-p+p(1-rp)^{g-1}} \quad (10)$$

for

$$\frac{L}{1+L}(fk-k+1)\left(1-\frac{j}{fk-k+1}\right) > 1$$

$$\left(1-\frac{1}{fk-k+1-j}\right)^{fk-k+1-j} \approx 1/e$$

Equation 10 can be rewritten in ratio form for very large aggregates

$$(\bar{\imath}/k) = 1 + (f-2)\frac{p(1-rp)^{g-1}}{1-p+p(1-rp)^{g-1}} \quad k \gg 1 \quad (11)$$

These ratios have been evaluated at the critical point of reaction for valences of the antibody 2, 3, 5 and 7, and a valence of 7 for the antigen. They are shown in Fig. 4. As the extent of reaction increases beyond the critical point the antibody–antigen ratio $\bar{\imath}/k$ increases in some cases and decreases in others. The increase or decrease depends on the relative numbers of antibody and antigen sites available for reaction. The range of values of A/G for which $\bar{\imath}/k$ decreases beyond the critical point decreases as the antibody valence increases.

We may define maximum and equivalent antibody–antigen ratios $(i/k)_{max}$ and $(i/k)_e$, with the use of equation 2 which describes the values of i permitted for a given k value.

$$(i/k)_{max} = (k-1)/(g-1)k + q_{max}/k = f - 1 + 1/k$$

$$q_{max} = fk - k + 1 - (k-1)/(g-1) \quad (12)$$

which is independent of g as is to be expected.

$$(i/k)_e = (k-1)/(g-1)k + q_e/k = \frac{(f-1)(g-1)}{2} + \frac{k-1}{k}\frac{1}{g-1} - \frac{1}{2}\left(1-\frac{g}{k}\right)$$

$$q_e = q_{max}\frac{(g-1)}{2} \quad (13)$$

Equations 12 and 13 yield

$$(i/k)_{max} = \frac{2}{g-1}(i/k)_e + \frac{k-1}{k}\frac{g-3}{(g-1)^2} \quad (14)$$

Part C. Discussion

The most probable distribution for a given fraction of reacted antigen sites has been calculated for a system containing g-valent antibody and f-valent antigen. The assumptions of equal reactivities and no intra-aggregate reactions have been invoked with the same justification as before.[2]

Some of the effects obtained by varying the valence of the antibody molecule are seen in the figures presented here. An increase in the valence of the antibody produces an increase in the state of aggregation as manifested by the weight-average molecular weight of the system. The distinction between univalent and higher than univalent antibody on this basis rests with the determination of the specificity of the reactions involving combination of aggregates. Figures 2 and 3 indicate that an increase in the valence of the antibody increases the range of composition of the system over which precipitation can occur. Nevertheless, this increase does not adequately account for the apparent lack of antibody-excess inhibition in systems containing rabbit antibody. *Such inhibition limits do not prevail in systems containing antibody molecules which are entirely univalent.* Consequently, the existence of inhibition is important conceptually. The only classical precipitin test data which indicate the existence of higher than univalent antibody molecules are those revealing inhibition in the antibody-excess region. Antibody–antigen systems containing equine antibody portray this inhibition.[2] Unfortunately, systems containing rabbit antibody have not demonstrated this inhibition in a clear-cut manner. This writer believes that antibody-excess inhibition exists with rabbit antibody, but that it is masked by non-specific characteristics. The superposition of solubility conditions on the theory described here leads to interesting results which can include very large displacements of inhibition regions. This problem will be discussed in a later paper. The precipitin test data which do reveal the presence of this inhibition with rabbit antibody have been described by Pauling, *et al.*,[6] and, in a more obscure manner, by the time of flocculation data of Boyd.[7] Figure 4 indicates that an increase in antibody valence decreases the antibody–antigen ratio of the large aggregates at the critical point over most of the precipitating region. When the antibody–antigen ratio of the system is in the neighborhood of unity, the slope of the $(\bar{\imath}/k)_c$ curve increases with decreasing antibody valence. This situation causes the curves to cross,

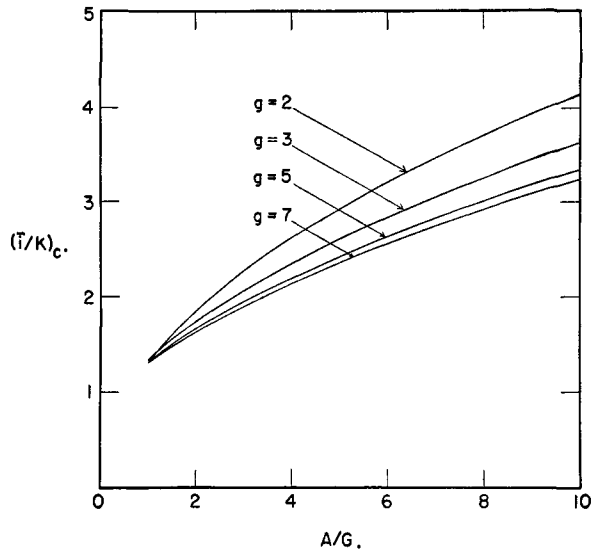

Fig. 4.—The relation between $\bar{\imath}/k$ and A/G is shown for the critical extent of reaction p_c, for various antibody valences. $\bar{\imath}/k$ is the average antibody–antigen ratio of all aggregates containing k antigen molecules. A/G is the antibody–antigen ratio of the entire system. The antigen valence has been taken as seven.

(6) L. Pauling, D. Pressman and D. Campbell, THIS JOURNAL, **66**, 330 (1944).

(7) W. C. Boyd, *J. Exper. Med.*, **74**, 369 (1941).

resulting in an "inversion effect." This effect depends on the relative excess of antigen sites over antibody sites for systems with antibody molecules of different valences.

Equation 2 permits us to make a few positive statements with regard to the antibody and antigen valences. The existence of specific aggregates each containing a number of molecules large compared to unity requires the responsible antibody and antigen molecules to be at least bivalent. If the antibody–antigen molecular ratio of these aggregates can take on values less than or greater than unity, the responsible antibody and antigen molecules are required to have valences greater than two. If this ratio cannot be less than unity but is variable, the responsible antigen molecules are required to be trivalent or more, all antibody molecules are required to be bivalent or less. If this ratio cannot exceed unity but is variable, the responsible antibody molecules are required to be trivalent or more, all antigen molecules are required to be bivalent or less. A system containing antigen molecules of any valence and univalent antibody molecules demands the use of non-specific forces for the formation of large aggregates. If non-specific combination is limited to aggregates, then the antibody–antigen ratio of any aggregate is required to be no less than unity.

The most probable distribution of species for g-valent antibody and f-valent antigen described by equation 1 can be generalized as Stockmayer has done[5] to give

$$m_{i_1 \cdots i_t k_1 \cdots k_s} = (\Sigma f_n G_n) \frac{(\Sigma f_n k_n - \Sigma k_n)!(\Sigma g_j i_j - \Sigma i_j)!}{(\Sigma f_n k_n - \Sigma k_n - \Sigma i, + 1)!(\Sigma g_j i_j - \Sigma k_n - \Sigma i_j + 1)!} \frac{(1-p)(1-rp)}{rp} \times \prod_n \left[\frac{f_n G_n}{\Sigma f_n G_n} \frac{rp(1-p)^{f_n-1}}{(1-rp)}\right]^{k_n} \frac{1}{k_n!} \prod_j \left[\frac{g_j A_j}{\Sigma g_j A_j} \frac{p(1-rp)^{g_j-1}}{1-p}\right]^{i_j} \frac{1}{i_j!} \quad (15)$$

$$r = \Sigma f_n G_n / \Sigma g_j A_j$$

where $m_{i_1 \cdots i_t k_1 \cdots k_s}$ is the number of aggregates in the system each of which consists of $i_1,\ldots,i_t$ $g_1,\ldots,g_t$-valent antibody molecules, respectively, and $k_1,\ldots,k_s$ $f_1,\ldots,f_s$-valent antigen molecules, respectively. The number of g_j-valent antibody molecules in the system is A_j, and the number of f_n-valent antigen molecules in the system is G_n. The critical point is defined by

$$p_c^2 = 1/[r(\bar{f} - 1)(\bar{g} - 1)]$$
$$\bar{f} = \Sigma f_n^2 G_n / \Sigma f_n G_n \quad (16)$$
$$\bar{g} = \Sigma g_j^2 A_j / \Sigma g_j A_j$$

where the average valences, $\bar{f}$ and $\bar{g}$, are valence-average valences. If the valence is proportional to the molecular weight, these averages are then weight averages.

Acknowledgment.—I wish to thank Professor J. W. Williams for his kind coöperation throughout this work. Personal communication with Professor W. H. Stockmayer regarding the use of appropriate generating functions and other problems connected with the treatment described in this paper is gratefully acknowledged.

Appendix

A. Derivation of the Combinatorial Factor W_{ik}

We wish to evaluate W_{ik}, defined as the number of ways in which i g-functional units (called S_g-units) and k f-functional units (called S_f-units) can be formed into a single i, k-aggregate containing no cyclic structures. All units and all functional sites thereon are distinguishable. All sites on the S_f-units are equivalent. All sites on the S_g-units are equivalent. Furthermore, sites on S_g-units are permitted to react only with sites on S_f units and *vice versa*.

Let us first picture the i S_g-units and k S_f-units laid out before us, each with a check mark on any one of its sites. The number of ways to accomplish this is $g^i f^k$. We pick up any unit first. We pick up a second unit and attach it to the first by connecting the marked sites on both. We pick up a third unit and attach it to the dimer by connecting the marked site on the third unit to any *available* site on the dimer. We continue this procedure until all the units are attached, always remembering that the marked site is the one used for attachment, and also that sites on S_g-units can be attached only to sites on S_f-units. The number of ways to put the aggregate together in this manner is R_{ik}. Therefore

$$W_{ik} = R_{ik} f^k g^i \quad (A1)$$

The use of i and k units in the process requires $k + i - 1$ bonds or $2k + 2i - 2$ sites. Since one site on each unit has already been picked for use, $k + i - 2$ sites remain to be selected. That is to say, $k - 1$ sites must be selected from $gi - i$ sites on the S_g-units, and $i - 1$ sites must be selected from $fk - k$ sites on the S_f-units. The number of ways to accomplish this task is R_{ik}. Consequently,

$$R_{ik} = \frac{(fk-k)!}{(fk-i-k+1)!} \frac{(gi-i)!}{(gi-i-k+1)!} \quad (A2)$$

The substitution of equation A2 in equation A1 yields the desired results.[8] It should be clear that the minimum number of S_g-units required for this job is that number which does not permit g sites or more on S_g-units to be left over on the finished aggregate. It can be expressed by

$$(g-1)i_{min} = k - 1 \quad (A3)$$

In case $(k-1)/(g-1)$ is not a whole number, the whole number just above it is taken. A symmetrical relation exists for k_{min}, which actually represents i_{max} for a given k value.

B. Summations

In this section we shall evaluate $\bar{\imath}_k$ and a typical sum required for the expression of the weight-average molecular weight of the system. The evaluation of $\sum_{i,k} k^2 m_{ik}$ is required for the weight-average molecular weight. It is convenient to evaluate $\sum_{i,k} k(k-1)m_{ik}$. With the following substitutions in equation 1

$$k = m + 2$$
$$(1-p)^{(f-1)(m+2)-i+1} = \sum_n (-p)^n \binom{(f-1)(m+2)-i+1}{n} \quad (B1)$$
$$(1-rp)^{(g-1)i-m-1} = \sum_e (-rp)^e \binom{(g-1)i-m-1}{e}$$
$$a = m + e$$
$$b = i + n$$

we obtain

$$\sum_{i,k} k(k-1)m_{ik} = \frac{fG}{rp} \sum_{a,b} \frac{(-rp)^{a+2}(-p)^b}{a!b!} \sum_{i,m} (-1)^{i+m} \frac{a!b!(gi-i)![(f-1)(m+2)]!}{[(f-1)(m+2)+1-b]!(gi-i-1-a)!i!(b-i)!(a-m)!} \quad (B2)$$

Now consider the function

$$H(X,Y) = X^{2f-2}(1 - X^{f-1})^a(1 - Y^{g-1})^b =$$

(8) Personal communication with Professor P. J. Flory has indicated that our methods of handling this combinatorial problem, although independent, are very much alike (see reference 9). This writer used the above method for a proposition submitted with his Ph.D. dissertation to the California Institute of Technology, June, 1951.

$$\sum_{i,m}(-1)^{i+m}X^{(f-1)(m+2)}Y^{gi-i}\frac{a!b!}{(a-m)!(b-i)!i!m!} \quad \text{(B3)}$$

Repeated differentiation of the double sum in equation B3 in the manner

$$\left[\left(\frac{\partial}{\partial X}\right)^{b-1}\left(\frac{\partial}{\partial Y}\right)^{a+1}H\right]_{X=Y=1}$$

gives just the i,m-sum in equation B2. If $b > a + 1$ or $a > b - 1$ the factor $(1 - Y^{g-1})$ or $(1 - X^{f-1})$ is present in every term after differentiation. Hence the sum in equation B2 vanishes except for the terms in which $b = a + 1$. Consequently, equation B2 becomes

$$\sum_{i,k}k(k-1)m_{ik} = -fGrp^2\sum_a\frac{(rp^2)^a}{(a+1)!a!}\times \sum_{i=1}^{a+1}(-1)^i\frac{(gi-i)!(a+1)!}{(gi-i-1-a)!i!(a+1-i)!}\times \sum_{m=0}^{a}(-1)^m\frac{[(f-1)(m+2)]!a!}{[(f-1)(m+2)-a]!m!(a-m)!} \quad \text{(B4)}$$

The i-sum in equation B4 can be evaluated with the use of

$$H_1(X) = (1 - X^{g-1})^{a+1} = \sum_{i=0}^{a+1}(-1)^iX^{gi-i}\binom{a+1}{i} \quad \text{(B5)}$$

Repeated differentiation of the sum in equation B5 in the manner

$$\left[\left(\frac{\partial}{\partial X}\right)^{a+1}H_1\right]_{X=1}$$

yields just the i-sum in equation B4. Furthermore, the only term which survives this operation is that which reduces the exponent $a + 1$ to zero. Therefore, the i-sum has the value $(a + 1)![-(g - 1)]^{a+1}$. A similar treatment with the m-sum in equation B4 yields $a![-(f - 1)]^a$. Consequently, equation B4 becomes

$$\sum_{i,k}k(k-1)m_{ik} = fGrp^2(g-1)\sum_{a=0}^{N-1}[rp^2(g-1)(f-1)]^a \quad \text{(B6)}$$

where we have picked the maximum value of a to be N, a large number approaching G, the number of antigen molecules in the system. The a-sum in equation B6 is a standard form. It gives

$$\sum_{i,k}k(k-1)m_{ik} = fGrp^2(g-1)\frac{[rp^2(g-1)(f-1)]^N - 1}{rp^2(g-1)(f-1) - 1} \quad \text{(B7)}$$

$$N = (g-1)(f-1)k_{max}$$

where k_{max} is the total number of antigen molecules in the system, if $1/(g-1) \leq A/G \leqslant f - 1$. If $rp^2(g-1)(f-1)$ has a value less than unity, it vanishes essentially when it is raised to the power N. When it approaches unity, the sum in equation B7 approaches the value $(fG/(f-1))N$. The value of p for which $rp^2(g-1)(f-1)$ is unity is known as the critical value. Below the critical point, equation B7 becomes

$$\sum_{i,k}k(k-1)m_{ik} = \frac{fGrp^2(g-1)}{1-rp^2(g-1)(f-1)} \quad \text{(B8)}$$

The evaluation of $\bar{i}_k$ is accomplished in the following manner. From equation 1

$$m_{ik} = C_kL^i\frac{(gi-i)!}{(gi-i-k+1)!(fk-i-k+1)!i!}$$

$$i = i_{min} + q$$

$$0 \leq q \leq i_{max} - i_{min} = fk - k + 1 - i_{min} \quad \text{(B9)}$$

$$C_k = fG\left(\frac{rp}{1-rp}\right)^{k-1}\frac{(1-p)^{fk-k+1}}{k!}$$

$$L = \frac{p}{1-p}(1-rp)^{g-1}$$

Equation B9 yields

$$\bar{i}_k = \frac{\sum_i im_{ik}}{\sum_i m_{ik}} = L\frac{\partial}{\partial L}\log\sum_i m_{ik}/C_k \quad \text{(B10)}$$

$$= L\frac{\partial}{\partial L}\log\sum_{q=0}^{fk-k+1-i_{min}}L^{(i_{min}+q)}\frac{[(g-1)(i_{min}+q)]!(fk-k+1-i_{min})!}{[(g-1)q]!(fk-k+1-i_{min}-q)!(i_{min}+q)!}$$

Now consider the function

$$J = Z^{k-1}(1 + LZ^{g-1})^{fk-k+1-i_{min}} = \sum_{q=0}^{fk-k+1-i_{min}}\left[L^qZ^{(g-1)q+k-1}\binom{fk-k+1-i_{min}}{q}\right] \quad \text{(B11)}$$

The following operations on the sum in equation B11

$$\left\{\left(\frac{\partial}{\partial Z}\right)^{k-1}[\int\cdots\int J(dL)^{i_{min}}]\right\}_{Z=1}$$

give just the q-sum in equation B10. Consequently, equations B10 and B11 yield

$$\bar{i}_k = L\frac{\partial}{\partial L}\left[\log\left(\frac{\partial}{\partial Z}\right)^{k-1}(1+LZ^{g-1})^{fk-k+1}\right]_{Z=1} = (fk-k+1)\left\{1 - \left[\frac{\left(\frac{\partial}{\partial Z}\right)^{k-1}(1+LZ^{g-1})^{fk-k}}{\left(\frac{\partial}{\partial Z}\right)^{k-1}(1+LZ^{g-1})^{fk-k+1}}\right]_{Z=1}\right\} \quad \text{(B12)}$$

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