BIKEN JOURNAL 5, 259-296 (1962)

A New Statistical-Mechanical Theory on the Antigen-Antibody Reaction

TSUNEHISA AMANO

Department of Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka

ITIRO SYOZI AND TADAKAZU TOKUNAGA

Marine Technical College, Ashiya, Hyogo

SHOJI SATO

College of General Education, Osaka University, Toyonaka, Osaka

(Received for publication, December 31, 1962)

SUMMARY

This new theory describes the most probable distribution of antigen-antibody complexes resulting from the reactions of f-valent antigens, of which the antigen sites are distinctly different from others, with the f-kind site-specific bivalent antibodies at various concentrations. It is assumed, as in Goldberg's theory, that no intra-aggregate reactions occur which would yield cyclic structures. In a special case, in which the f-kind site-specific antibodies are at the same concentration, a range in the amount of added antigen, in which the critical condition can be attained, is obtained and the result is quite similar to that of Goldberg.

INTRODUCTION

A general theory for antigen-antibody reactions was first presented by Goldberg (1952, 1953) who applied the method of statistical mechanics, which had been successfully applied to derive the distributions of three-dimensional branched-chain polymers by Flory (1936, 1941a, b, c) and Stockmayer (1943). Goldberg made the three following assumptions to simplify the mathematical treatment: (1) Intra-aggregate reactions yielding cyclic structures do not occur, (2) all antigen sites of f-valent antigen are identical in specificity and therefore all antibody sites are also identical, regardless of the valency of the antibody molecules, and (3) any unreacting site on an antigen or antibody is as reactive as any other site, regardless of the size and phase of the aggregate to which it is attached. The second assumption, which was first proposed by Heidelberger and Kendall (1935) in deriving the theoretical relationship between the precipitating antibody and the added antigen, seems no longer tenable, because experimental evidences strongly suggest that the antigen sites of a protein molecule are not identical in specificity (Lapresle, 1955; Lapresle *et al.*, 1957a, b, 1958; Raynaud, 1958; Fujio *et al.*, 1958, 1959, 1962;

Shinka *et al.*, 1962). Therefore, Goldberg's theory must be modified in a direction to permit heterogeneity in specificity of the antigen sites.

Palmiter and Aladjem (1962) succeeded in partly modifying the second assumption. In their theory, the antigen is considered to be f-valent and antibody bivalent, and the reaction between a pair of sites (one antigen site reacting with one antibody site) is considered in terms of two rate constants which are independent of the rate constants of any other pair of sites. However, essentially their theory did not overcome the limitations of Goldberg's second assumption, because one antibody site can combine with any one of the f antigen sites giving varying rate constants.

In our present paper, Goldberg's second assumption was completely changed: each antigen site of f-valent antigen is assumed to differ in specificity from others and the antisera contain f-kind site-specific bivalent antibodies. Therefore an antigen site k can only combine with one of two combining sites on a k-type antibody molecule and one antigen molecule cannot combine with two or more antibody molecules of identical specificity. The other two assumptions made by Goldberg are still held in this theory.

A Theory for the Reactions of Multivalent Antigen Molecules with Bivalent Antibody Molecules

The theory presented here is concerned with the most probable distribution of antigen-antibody complexes, as is Goldberg's theory. In the theory the following terminology will be used.

N = number of f-valent antigen molecules in the system.

$N_1, N_2, \ldots, N_f$ (written generally as N_k) = number of distinct bivalent antibody molecules in the system.

$L_1, L_2, \ldots, L_f$ (written generally as L_k) = number of distinct antibody molecules forming bridges between antigen molecules in the system.

$I_1, I_2, \ldots, I_f$ (written generally as I_k) = number of distinct antibody molecules attached to antigen molecules (such as univalent antibodies) in the system.

M = number of aggregates in the system plus the number of free antibody and antigen molecules.

f = number of effective reactive sites on each antigen molecule (f-valent), of which no two sites are identical in specifity. These sites are located respectively at definite loci on the surface.

$m_{n,}\begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}$ (abbreviated to m) = number of aggregates each composed of n antigen molecules, which are connected by $l_1, l_2, \ldots, l_{f-1}$ and l_f antibody molecules of the respective specificities and, in addition, $i_1, i_2, \ldots$ and i_f antibody molecules are attached to the aggregate.

$m'_{0,k}$ (abbreviated to m') = number of free k-type antibodies ($k = 1, 2, \ldots, f$).

$W_{n,}\begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}$ (abbreviated to W) = number of ways of constructing a single $n, l_1, i_1, l_2, i_2, \ldots, l_f, i_f$-aggregate containing no cyclic structures from n-given antigen molecules, l_1-, l_2-, $\ldots$, l_f-given bridge making antibody molecules and i_1-, i_2-, $\ldots$, i_f-given attached antibody molecules.

$p_1, p_2, \ldots, p_f$ (written generally as p_k) = fraction of respective antigen sites in the system which have reacted; this is called the extent of the reaction of the respective antigen site.

p = fraction of total antigen sites in the system which have reacted; this is called the "overall extent of the reaction".

$r_1, r_2, \ldots, r_f$ (written generally as r_k) $= N/2N_1, N/2N_2, \ldots, N/2N_f$.

$r = fN/2 \sum_{k=1}^{f} N_k$.

When the number of the aggregates, composed of $n(n \geqq 1)$ antigen molecules and $l_k(l_k \geqq 0)$ bond-forming, $i_k(i_k \geqq 0)$ merely attached antibody molecules ($k=1, 2, \ldots, f$) is expressed as $m_{n,\left\{\begin{smallmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{smallmatrix}\right\}}$ and the number of k-type ($k=1,2,\ldots,f$) free antibody molecules as $m'_{0,k}$, the number of ways to form a given distribution of m and $m'_{0,k}$ from $N, N_1, N_2, \ldots, N_f$ molecules is

$$P = \frac{N!N_1!N_2!\ldots N_f!\, \Pi \left(Wn,\left\{\begin{smallmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{smallmatrix}\right\}\right)^m}{\Pi_n (n!)^{\underline{\Sigma m}_n}\, \Pi_{l_1} (l_1!)^{\underline{\Sigma m}_{l_1}}\, \Pi_{i_1} (i_1!)^{\underline{\Sigma m}_{i_1}} \ldots \Pi (l_f!)^{\underline{\Sigma m}_{l_f}}\, \Pi (i_f!)^{\underline{\Sigma m}_{i_f}}\, \Pi m! \prod_{k=1}^{f} (m'_{0,k}!)}, \quad (1)$$

where $\underline{\Sigma m}_n, \underline{\Sigma m}_{l_1}, \underline{\Sigma m}_{i_1} \ldots$ implies the summations over other values of suffixes of m fixing $n, l_1, i_1, \ldots$, respectively.

Here, n antigen molecules are connected with l_1 antibodies of the 1-type, l_2 antibodies of the 2-type, $\ldots$, l_f antibodies of the f-type and i_1 antibodies of the 1-type, i_2 antibodies of the 2-type, $\ldots$, i_f antibodies of the f-type are attached to antigen sites of the respective types. Then $N, N_1, N_2, \ldots, N_f$ and M values can be expressed by

$$\Sigma n\, m_{n,\left\{\begin{smallmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{smallmatrix}\right\}} = N, \quad (2)$$

$$\Sigma (l_k + i_k)\, m_{n,\left\{\begin{smallmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{smallmatrix}\right\}} + m'_{0,k} = N_k, \ (k=1, 2, \ldots, f)$$

and

$$\Sigma\, m_{n,\left\{\begin{smallmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{smallmatrix}\right\}} + \sum_{k=1}^{f} m'_{0,k} = M. \quad (3)$$

Here, all values up to n are allowed for n except zero and the values of l_k and $i_k (k=1, 2, \ldots, f)$ for a given n are restricted as follows

$$n - 1 = \sum_{k=1}^{f} l_k,$$

$$0 \leqq \sum_{k=1}^{f} (l_k + i_k) \leqq fn - n + 1, \quad (4)$$

$$0 \leqq i_k \leqq n - 2l_k.$$

Among the l_k values, the largest value of any l_k cannot exceed $(n-1)/2$ when n is an odd number or $n/2$ when n is an even number. In the sums and products which follow, these relations express the limits, unless otherwise specified.

When N antigen molecules and N_k antibody molecules $(k=1, 2, \ldots, f)$ are mixed, the number of molecules and aggregates at that time is identical to $N+\Sigma N_k$. After a certain reaction time, the number of free k-type antibody molecules is $m'_{0,k}$ and the number of aggregates, composed of n $(n\geqq 1)$ antigen molecules and n_k (of which l_k are connecting antigens and i_k are merely attached to the aggergates) antibody molecules of the k-type $(n_k\geqq 0)$, is m and the distribution of m and $m'_{0,k}$ is not necessarily the most probable one. When the reacted fraction of antigen sites (the overall extent of the reaction $=p$) is considered under such conditions, we have (cf. Eqs. 2, 3, 4)

$$p = \frac{\Sigma\{\Sigma_k(2l_k+i_k)\}m}{fN} = \frac{\Sigma\{\Sigma_k(l_k+i_k)+\Sigma_k l_k\}m}{fN}$$

$$= \frac{\Sigma_k\{\Sigma(l_k+i_k)m\}+\Sigma(n-1)m}{fN}$$

$$= \frac{\Sigma_k N_k-\Sigma_k m'_{0,k} + \Sigma\, n\, m-\Sigma m}{fN}$$

$$= \frac{\Sigma_k N_k+N-M}{fN}\,. \tag{5}$$

As f, N and N_k are constants, an increase in p corresponds to a decrease in M. Therefore, the reaction process can be studied by analyzing the decrease in M. For this analysis it is assumed in the present theory that m and $m'_{0,k}$ represent the most probable distribution in the system at any moment during the reaction. The most probable distribution means that, for a certain value of M, the elements of N and N_k are distributed into M molecules (one aggregate is also counted as one molecule) in the way which involves the largest number of ways of forming the distribution of m and $m'_{0,k}$. Hereafter, $m'_{0,k}$ and $m_{n,\left\{\begin{smallmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{smallmatrix}\right\}}$ are defined to express the most probable distribution for a given value of M.

In order to find the most probable distribution, *i.e.* the set of numbers of m and $m'_{0,k}$ giving the maximal value of P in Eq. 1, one must set the total differential $d\,(\log P)$ equal to zero for constants N, N_1, N_2, $\ldots$, N_f and M with the help of Stirling's approximation and Lagrangean undetermined multipliers as Stockmayer (1943) and Goldberg (1952) did:

$$\log P = \log (N!N_1!\ldots N_f!)+\Sigma m \log W-\Sigma_n(\Sigma_{\underline{n}} m) \log n!-\Sigma_{l_1}(\Sigma_{\underline{l_1}} m) \log l_1!$$

$$- \sum_{i_1}(\sum_{\underline{i_1}} m) \log i_1! \ldots - \Sigma \log m! - \sum_{k=1}^{f} \log m'_{0,k}!,$$

$$d(\log P) = \Sigma (\log W - \log n! - \log l_1! - \log i_1 \ldots - \log m)\, dm$$
$$- \sum_{k=1}^{f} (\log m'_{0,k})\, dm' = 0,$$

under the following conditions,

$$\begin{aligned} &\lambda_o \Sigma n\, d m = 0, \\ &\lambda_k \{\Sigma(l_k+i_k)\, dm + dm'_{0,k}\} = 0, \quad (k=1, 2 \ldots, f) \qquad (6) \\ &\mu\{\Sigma\, dm + \sum_k dm'_{0,k}\} = 0. \end{aligned}$$

Vanishing the coefficients of dm and dm', we obtain

$$\log W - \log n! - \log l_1! - \log i_1! \ldots - \log m + \lambda_o n + \sum_{k=1}^{f} \lambda_k (l_k + i_k) + \mu = 0, (n \neq 0)$$

and

$$-\log m'_{0,k} + \lambda_k + \mu = 0, \quad (n=0).$$

Therefore, the most probable distribution becomes,

$$\begin{aligned} m &= e^{\mu} \frac{W}{n!\, l_1!\, i_1! \ldots l_f!\, i_f!} (e^{\lambda_o})^n \prod_{k=1}^{f} (e^{\lambda_k})^{l_k+i_k} \\ &= e^{\mu} \frac{W}{n! \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\Sigma(l_k+i_k)}} (e^{\lambda_o})^n \prod_{k=1}^{f} (2e^{\lambda_k})^{l_k+i_k} \qquad (7) \\ &= \frac{C\, W}{n! \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\Sigma(l_k+i_k)}} y^n \prod_{k=1}^{f} (x_k)^{l_k+i_k}, \end{aligned}$$

and

$$m'_{0,k} = \frac{1}{2} e^{\mu}(2e^{\lambda_k}) = \frac{C}{2} x_k, \qquad (8)$$

where the substitutions $y = e^{\lambda_o}$, $x_k = 2e^{\lambda_k}$ and $C = e^{\mu}$ are introduced.
As shown in the Appendix (A 20), we obtain

$$W_{n, \begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}} = n!\, n^{f-2} \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\sum_{k=1}^{f} n_k} \cdot \prod_{k=1}^{f} \frac{(n-l_k-1)!}{l_k!\, i_k!\, (n-2l_k-i_k)!},$$

which yields

$$m_{n, \begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}} = C n^{f-2} \prod_{k=1}^{f} \frac{(n-l_k-1)!}{l_k!\, i_k!\, (n-2l_k-i_k)!} x_1^{n_1} x_2^{n_2} \ldots x_f^{n_f} y^n. \qquad (9)$$

The summations of Eqs. 2 and 3 are also shown in the Appendix (A 41, A 43, A 50, A 56 and A 57) and the results are

$$\begin{aligned}
N &= \Sigma\, n\, m_{n,}\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} = C A y, \\
L_k &= \Sigma\, l_k\, m_{n,}\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} = \frac{C}{2} x_k \left(\frac{yA}{A_k}\right)^2, \\
I_k &= \Sigma\, i_k\, m_{n,}\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} = C x_k \frac{yA}{A_k}, \\
N_k &= L_k + I_k + \frac{C}{2} x_k = \frac{C}{2} (A_k - 1)\left(\frac{A_k + yA}{A_k}\right), \quad (A_k \geqq 1) \\
x_k &= \frac{A_k(A_k - 1)}{A_k + yA}, \\
A &= A_1 A_2 \cdots A_f .
\end{aligned} \tag{10}$$

The extent of the reaction of the respective antigen sites (p_k) can be expressed with A_k, as

$$p_k = \frac{2\Sigma l_k m + \Sigma i_k m}{N} = \frac{2L_k + I_k}{N} = 1 - \frac{1}{A_k}. \tag{11}$$

The overall extent of the reaction can be expressed with p_k's, as

$$p = \frac{2\sum_k l_k + \sum_k I_k}{fN} = \frac{\sum_k \left(1 - \frac{1}{A_k}\right)}{f} = \frac{\Sigma p_k}{f}. \tag{12}$$

From Eqs. 5, 10 and 12, we have

$$M = C\left\{A y \{1 - \frac{1}{2} \sum_k (1 - \frac{1}{A_k})\} + \frac{1}{2} \sum_k (A_k - 1)\right\}. \tag{13}$$

When the values of N_k and M in Eqs. 10 and 13 are put into Eqs. 11 and 12, we obtain

$$\sum_{k=1}^{f} A_k = \frac{f}{C}\left(\frac{N}{r} - pN + C\right). \tag{14}$$

Eqs. 11 and 14 yield

$$C = \frac{fN\left(\frac{1}{r} - p\right)}{\sum_{k=1}^{f}\left(\frac{p_k}{1 - p_k}\right)}, \tag{15}$$

and Eqs. 10, 11, 12 and 15 yield

$$x_k = \frac{p_k}{1 - p_k} \cdot \frac{f\left(\frac{1}{r} - p\right)}{f\left(\frac{1}{r} - p\right) + (1 - p_k) \sum_{k=1}^{f}\left(\frac{p_k}{1 - p_k}\right)}, \tag{16}$$

$$y = \frac{\prod_{k=1}^{f} (1-p_k) \cdot \sum_{k=1}^{f} \left(\frac{p_k}{1-p_k}\right)}{f\left(\frac{1}{r} - p\right)} .$$

The probability, that a k-antigen site has reacted with a chain-forming k-type antibody, can be calculated in relation with Eqs. 10 and 11,

$$a_k = \frac{2\,\Sigma\, l_k\, m}{N} = p_k N \cdot \frac{1}{CA_k + N} = p_k N \cdot \frac{p_k}{2N_k} = p_k^2\, r_k. \qquad (17)$$

And the overall probability, that an antigen site has reacted with a bridge-forming antibody, can be represented as

$$a = \frac{2\Sigma L_k}{fN} = \frac{\Sigma p_k^2\, r_k}{f} = \frac{\Sigma a_k}{f} . \qquad (18)$$

The equation corresponding to that of Heidelberger-Kendall can be derived,

$$Ab \text{ pptd} = \sum_{k=1}^{f} (N_k - m'_{0,k}) = N\Sigma p_k - \frac{N^2}{4}\, \Sigma \frac{p_k^2}{N_k} . \qquad (19)$$

Only in the region of extreme antibody excess can p be unity, because the concentrations of respective antibodies may vary, and hence some p_k becomes less than unity even in the antibody excess zone.

The range, in which the critical point can be attained, cannot be obtained at varied concentrations of the respective antibodies and it can only be shown in the special antisera as described below, in which the concentrations of respective antibodies are approximately identical.

i) As M is usually larger than unity and M tends to unity at the most extreme condition of the critical point, the following relation is obtained from Eq. 5,

$$M = N + fN_k - fNp \geqq 1,$$

Therefore, we have

$$\frac{1}{f} + \frac{1}{2r} - \frac{1}{fN} \fallingdotseq \frac{1}{f} + \frac{1}{2r} \geqq p\,, \text{ (curve 1 in Fig. 1)}. \qquad (20)$$

As $0 \leqq p \leqq 1$, the smallest value allowed for r on the curve 1 is $\frac{f}{2(f-1)}$ and the point corresponds to the value of $\frac{fN_k}{N} = f-1$.

ii) The fraction of reacted antigen sites must always be smaller than the ratio of the total antibody sites to the total antigen sites:

$$p \leqq \frac{1}{r} \qquad \text{(curve 2 in Fig. 1)}. \qquad (21)$$

The smallest value allowed for r on the curve 2 is obtained when p is unity, and at the point r becomes unity and $\frac{fN_k}{N} = \frac{2}{f}$.

iii) As another criterion, the weight average molecular weight of the complexes are considered. When this increases infinitely, it means that the system has already attained a critical condition. For the purpose of the theory, the numerators of the weight average molecular weights of the antigen or antibody are considered. The numerator of the weight average molecular weight of the antigen molecule, ($<n^2>$), becomes (cf. A. 59, A. 60)

$$<n^2> = \Sigma n^2 \frac{CW}{n! \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\Sigma(l_k+i_k)}} x_1^{l_1+i_1} x_2^{l_2+i_2} \cdots x_f^{l_f+i_f} y^n$$

$$= y \frac{\partial}{\partial y} N = Cy \frac{\partial}{\partial y} (YA). \tag{22}$$

As shown in the Appendix (A 66),

$$y \frac{\partial}{\partial y} (YA) = \frac{YA}{1-\Sigma}, \quad \left(\Sigma = \sum_{k=1}^{f} \frac{(A_k-1)\,YA}{A_k^2+(2A_k-1)\,YA}\right).$$

Therefore, Eq. 22 becomes

$$<n^2> = \frac{CYA}{1-\Sigma}. \tag{23}$$

The numerator of the weight average molecular weight of the 1-type antibodies ($<n_1{}^2>$), becomes (cf. A. 59, A. 60)

$$<n_1{}^2> = \Sigma n_1{}^2 \frac{CW}{n! \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\Sigma n_k}} x_1^{n_1} x_2^{n_2} \cdots\cdots x_f^{n_f} y^n + 1^2 \cdot \frac{C}{2} x_1$$

$$= x_1 \frac{\partial}{\partial x_1} N_1 = x_1 \frac{\partial}{\partial x_1} \left\{ \frac{C}{2} (A_1-1) \frac{A_1+YA}{A_1} \right\} = \frac{Cx_1}{2} \frac{\partial}{\partial x_1} \left(A_1+YA-1-\frac{YA}{A_1}\right). \tag{24}$$

From Eq. A 65, we obtain

$$\frac{\partial}{\partial x_1} = \frac{(A_1+YA)^2}{A_1\{A_1{}^2+(2A_1-1)\,YA\}} \left\{ Y\Sigma \frac{\partial A_k}{\partial y} \frac{\partial}{\partial A_k} + A_1 \frac{\partial}{\partial A_1} \right\}, \tag{25}$$

and the following relations have already been obtained in Eqs. A 60 and A 66,

$$Y\Sigma \frac{\partial A_k}{\partial y} \frac{\partial}{\partial A_k} (YA) = y \frac{\partial}{\partial y} (YA) - YA$$

$$= \frac{YA}{1-\Sigma} - YA,$$

$$Y\Sigma \frac{\partial A_k}{\partial y} \frac{\partial}{\partial A_k}\left(\frac{YA}{A_1}\right) = \frac{YA}{1-\Sigma} \cdot \frac{A_1+YA}{A_1{}^2+(2A_1-1)YA} - \frac{YA}{A_1}, \quad (26)$$

$$Y\Sigma \frac{\partial A_k}{\partial y} \frac{\partial}{\partial A_k}(A_1) = \frac{YA}{1-\Sigma} \cdot \frac{A_1(A_1-1)}{A_1{}^2+(2A_1-1)YA}.$$

Therefore, the following relations can be obtained when the results of Eq. 26 are applied to Eq. 24,

$$\frac{\partial}{\partial x_1}\left(A_1+YA-1-\frac{YA}{A_1}\right) = \frac{(A_1+YA)^2}{A_1\{A_1{}^2+(2A_1-1)YA\}} \left\{Y\Sigma \frac{\partial A_k}{\partial y} \frac{\partial}{\partial A_k}\left(A_1 + YA-1-\frac{YA}{A_1}\right) + A_1 \frac{\partial}{\partial A_1}\left(A_1+YA-1-\frac{YA}{A_1}\right)\right\}$$

$$= \frac{(A_1+YA)^2}{A_1\{A_1{}^2+(2A_1-1)YA\}} \left\{\frac{YA}{1-\Sigma} \cdot \frac{2(A_1-1)(A_1+YA)}{A_1{}^2+(2A_1-1)YA} + \frac{YA}{A_1} + A_1\right\}. \quad (27)$$

When the value is multiplied by $\frac{C}{2}x_1 = \frac{C}{2} \cdot \frac{A_1(A_1-1)}{A_1+YA}$, we have

$$<n_1{}^2> = \frac{CYA}{1-\Sigma} \cdot \frac{(A_1-1)^2(A_1+YA)^2}{\{A_1{}^2+(2A_1-1)YA\}^2} + \frac{C(A_1-1)(A_1+YA)(A_1{}^2+YA)}{2A_1\{A_1{}^2+(2A_1-1)YA\}}.$$

Similarly, we have

$$<n_k{}^2> = \frac{CYA}{1-\Sigma} \cdot \frac{(A_k-1)^2(A_k+YA)^2}{\{A_k{}^2+(2A_k-1)YA\}^2} + \frac{C(A_k-1)(A_k+YA)(A_k{}^2+YA)}{2A_k\{A_k{}^2+(2A_k-1)YA\}}. \quad (28)$$

As can be seen in Eqs. 23 and 28, the numerators of the weight average molecular weight concerned either with antigen molecules or antibody molecules diverge when the value of $1-\Sigma$ vanishes *i.e.* Σ becomes unity.

When $N_1=N_2=\dots=N_f$ (at identical concentrations of the respective antibodies), *i.e.* $A_1=A_2=\dots=A_f$, we obtain

$$\Sigma = f\frac{(A_k-1)YA}{A_k{}^2+(2A_k-1)YA} = f\frac{p^2}{\frac{1}{r}+p^2} \leqq 1,$$

which leads to

$$p \leqq \frac{1}{\sqrt{f-1}} \cdot \frac{1}{\sqrt{r}}, \quad \text{(curve 3 in Fig. 1).} \quad (29)$$

The smallest value of r becomes $\frac{1}{f-1}$, $\left[\frac{fN_k}{N} = \frac{f(f-1)}{2}\right]$, which is given by $p=1$. This value of r is the smallest value obtainable with these three limitations and hence, when the amount of antigen added is less than this value $\left[\frac{fN_k}{N}\right.$

$\leqq \dfrac{f(f-1)}{2}\Big]$, the critical condition cannot be attained.

These three curves are shown in Fig. 1.

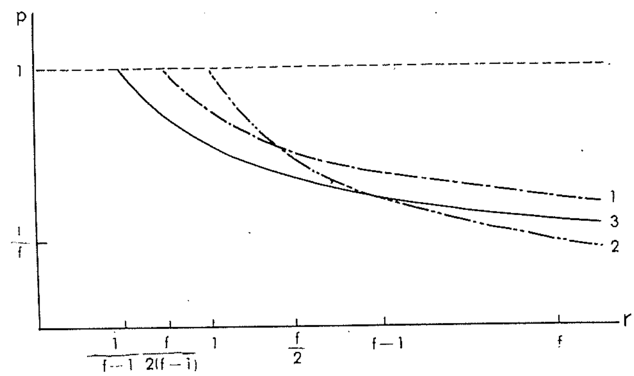

Fig. 1. Limits of p in relation to r.

The intersection of curves 2 and 3 is at $r=f-1$, $\left[\dfrac{fN_k}{N}=\dfrac{f}{2(f-1)}\right]$, and this point expresses the largest amount of antigen, which gives a precipitate with a constant amount of antibody. Therefore, antigen-antibody precipitates are given in the range of $f-1 \geqq r \geqq \dfrac{1}{f-1}$, $\left[\dfrac{f}{2(f-1)} \leqq \dfrac{fN_k}{N} \leqq \dfrac{f(f-1)}{2}\right]$. The intersection of curves 1 and 2 is $r=\dfrac{f}{2}$, $\left[\dfrac{fN_k}{N}=1\right]$. Therefore, the range, in which the critical condition can be attained, can be divided into three ranges: $\dfrac{1}{(f-1)} \leqq r \leqq \dfrac{f}{2(f-1)}$, $\left[\dfrac{f(f-1)}{2} \geqq \dfrac{fN_k}{N} \geqq f-1\right]$, $\dfrac{f}{2(f-1)} \leqq r \leqq \dfrac{f}{2}$, $\left[f-1 \geqq \dfrac{fN_k}{N} \geqq 1\right]$, and $\dfrac{f}{2} \leqq r \leqq f-1$, $\left[1 \geqq \dfrac{fN_k}{N} \geqq \dfrac{f}{2(f-1)}\right]$.

In the range of $\dfrac{1}{f-1} \leqq r \leqq \dfrac{f}{2(f-1)}$, p increases with the reaction time and attains curve 3 and still continues to increase. When p reaches curve 3, some precipitate appears, because the weight average molecular weight becomes infinite. However, the number average molecular weight of antigen ($\langle n \rangle$) does not tend to infinity,

$$\langle n \rangle = \frac{\Sigma nm}{\Sigma m} = \frac{N}{M} = \frac{N}{N+fN_k-fNp} = \frac{1}{f\left(\dfrac{1}{f}+\dfrac{1}{2r}-p\right)}. \quad (30)$$

If p could increase up to $\dfrac{1}{f}+\dfrac{1}{2r}$, $\langle n \rangle$ would tend to infinity. However, p

cannot reach $\frac{1}{f}+\frac{1}{2r}$ in this range, even when p attains the maximum value unity. Therefore, not all the antigen-antibody complexes grow to giant complexes of sufficient size to be precipitated, and only a fraction of the complexes can be precipitated as giant complexes.

In the range of $\frac{f}{2(f-1)} \leqq r \leqq \frac{f}{2}$, p cannot attain the value unity, but p can reach curve 1. When p reaches $\frac{1}{f}+\frac{1}{2r}$, M becomes unity. This means all the antigen and antibody molecules are incorporated into a single giant complex, and both $\langle n \rangle$ and $\langle n^2 \rangle$ become infinite.

In the range of $\frac{f}{2} \leqq r \leqq f-1$, p cannot reach curve 1, because it reaches curve 2 before it can reach curve 1 and it cannot exceed curve 2. Therefore, not all the antigen-antibody complexes can grow up to a single precipitable giant complex.

In the range of $f-1<r$, p cannot reach curve 3, because it reaches curve 2 and cannot exceed it. Therefore, no precipitates can be given.

In the range of $r \leqq \frac{f}{2(f-1)}$, p cannot reach any of the three curves, although it can attain the value unity. Therefore, no precipitates can be formed.

The most abundant complex in the most probable distribution is next considered. As the concentrations of the respective types of antibodies are identical, the following relations are easily found from Eq. 9 when

$$A_1 = A_2 = \ldots = A_f \text{ and } x_1 = x_2 = \ldots = x_f = x,$$

$$m_{n,\left\{\begin{matrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{matrix}\right\}} = C\frac{\Omega}{n}\prod_{k=1}^{f}\binom{n-2l_k}{i_k} x^{n_1+n_2+\ldots+n_f} y^n,$$

where $\Sigma n_k = \Sigma(l_k+i_k)$ and $\Omega = n^{f-1} \cdot \Pi \frac{(n-l_k-1)!}{l_k!(n-2l_k)!}$. (31)

When n, l_k $(k=1, 2, \ldots, f)$ are fixed,

$$\sum_i i_1\, m = \frac{C\Omega}{n} x^{\Sigma l_k} y^n \cdot \sum_{i_1=0}^{n-2l_1}\binom{n-2l_1}{i_1} i_1 x^{i_1} \cdot \sum_{i_2=0}^{n-2l_2}\binom{n-2l_2}{i_2} x^{i_2} \ldots$$

$$\times \sum_{i_f=0}^{n-2l_f}\binom{n-2l_f}{i_f} x^{i_f} = \frac{C\Omega}{n}(n-2l_1)\, x^n\, (1+x)^{nf-2n+1} y^n. \tag{32}$$

Therefore, we have

$$\sum_{k=1}^{f}\left(\sum_i i_k\, m\right) = \frac{C\Omega}{n}(fn-2n+2)\,(1+x)^{nf-2n+1}x^n y^n. \tag{33}$$

As l_k is still fixed, we obatin on the other hand,

$$\sum_{k=1}^{f}\left(\sum_{i} l_k\, m\right) = \left(\sum_{k=1}^{f} l_k\right)\left(\sum_{i} m\right) = (n-1)\frac{C\Omega}{n} x^{\Sigma l_k} y^n (1+x)^{nf-2\Sigma l_k}$$

$$= (n-1)\ \frac{C\Omega}{n}\ x^{n-1} y^n (1+x)^{nf-2n+2}\ . \tag{34}$$

The sum of Eq. 33 and Eq. 34 is the number of antibodies contained in complexes which are composed of fixed numbers of n, $l_1, l_2, \ldots, l_f$. To find the total number of antibodies in the complexes containing n antigen molecules, one must obtain the sum of the over all sets of l_k satisfying $\Sigma l_k = n - 1$ in Eqs. 33 and 34. For this purpose, $\sum_{l} \Omega$ must be obtained.

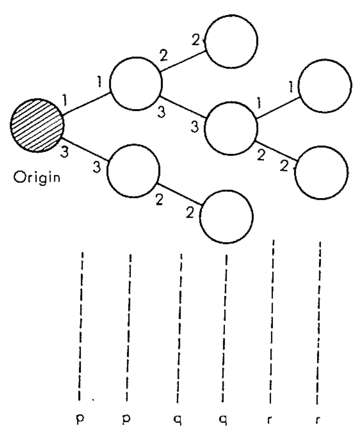

Fig. 2. Schematic presentation of the way to find ω_n

The $\sum_{l}\Omega$ is identical with ω_n which is the number of ways to construct trees with n identical balls containing f holes all of different shapes and with type-differentiated rods, of which the numbers are not indicated,

$$\omega_n = \sum_{p,q,r,\ldots} \binom{f}{p}\binom{(f-1)p}{q}\binom{(f-1)q}{r} \cdots\cdots\ . \tag{35}$$

This can be solved in a similar way as shown in other cases in Appendix 1, 2 and 3, and the result is

$$\Sigma\Omega = \frac{f(fn-n)!}{(fn-2n+2)!(n-1)!}\ . \tag{36}$$

Thus, Eqs. 33 and 34 yield

$$\sum_{l}\left\{\sum_{k=1}^{f}\left(\sum_{i} i_k\, m\right)\right\} = C\cdot\frac{f(fn-n)!}{(fn-2n+2)!n!}\ x^n(1+x)^{fn-2n+1} y^n\ , \tag{37}$$

$$\sum_l \left\{ \sum_{k=1}^{f} \left(\sum_i l_k m \right) \right\} = C \cdot \frac{f(fn-n)!}{(fn-2n+2)!n!} (n-1) x^{n-1}(1+x)^{fn-2n+2} y^n. \quad (38)$$

The sum of Eqs. 37 and 38 yields the total number of antibodies in complexes containing n antigen molecules (Σn_k),

$$\Sigma n_k = C \frac{f(fn-n)!}{(fn-2n+n)!n!} x^{n-1}(1+x)^{nf-2n+1} y^n \{(fn-n+1)x+n-1\}. \quad (39)$$

On the other hand, the total number of complexes containing n antigen molecules ,m_n, is

$$m_n = \sum_l (\sum_i m) = \sum_l C \frac{\Omega}{n} x^{n-1} y^n (1+x)^{fn-2n+2}$$

$$= C \frac{f(fn-n)!}{(fn-2n+2)!\,n!} x^{n-1} y^n (1+x)^{fn-2n+2}. \quad (40)$$

The mean antibody content in m_n is

$$<n_k> = \frac{\Sigma n_k}{m_n} = fn-n+1-\frac{fn-2n+2}{1+x}. \quad (41)$$

In this case, Eq. 16 can be written as $(x_1=x_2=\ldots\ldots=x_f=x)$

$$x = \frac{(p-p^2 r)}{1-p}, \quad (p=p_1=p_2=\ldots\ldots=p_f). \quad (42)$$

Eq. 42 is introduced into Eq. 41,

$$<n_k> = \frac{n-1+(fn-2n+2)p-(nf-n+1)p^2 r}{1-p^2 r}. \quad (43)$$

In the zone of extreme antigen excess $(f-1<r)$, the upper limit allowed for p is $\frac{1}{r}$ as shown in Fig. 1. Introducing this value into Eq. 43, we obtain

$$<n_k> = n-1. \quad (44)$$

This means that all the antibodies which reacted are playing roles in connecting antigen molecules when $f-1<r$.

In the zone of antibody excess $\left(r<\frac{1}{f-1}\right)$ where no precipitates can be expected, p can increase to unity. Putting $p=1$ into Eq. 43, we have

$$<n_k> = fn-n+1. \quad (45)$$

This means that all the antigen sites are attached to antibodies ("saturated complex"). To find the most abundant complex with regard to n, the following equations are made. Eq. 40 can be rewritten as

$$m_n = \frac{f(fn-n)!}{(fn-2n+2)!\,n!} \cdot N(p^2r)^{n-1}(1-p^2r)^{fn-2n+2}, \tag{46}$$

where $x = \frac{p-p^2r}{1-p}$, $y = \frac{(1-p)^{f-1}pr}{1-pr}$ and $C = \frac{N(1-pr)(1-p)}{pr}$.

m_n is a monotonously decreasing function with regard to n, which will be proved as follows. For this purpose, the following ratio is considered (cf. Eq. 18),

$$\begin{aligned}\frac{m_{n+1}}{m_n} &= \frac{(fn+f-n-1)!\,(fn-2n+2)!}{(fn+f-2n)!\,(fn-n)!\,(n+1)}\,p^2r(1-p^2r)^{f-2}\\ &= \frac{n(f-1)+f-1}{n(f-2)+f}\cdot\frac{n(f-1)+f-2}{n(f-2)+f-1}\cdot\dots\cdot\frac{n(f-1)+2}{n(f-2)+3}\\ &\times \frac{n(f-1)+1}{n+1}\cdot a(1-a)^{f-2},\ (a=p^2r).\end{aligned} \tag{47}$$

As $\frac{n(f-1)+k-1}{n(f-2)+k}$ $(k=3, 4, \dots, f)$ and $\frac{n(f-1)+1}{n+1}$ are all positive and monotonously increase with regard to n, their products also increase monotonously with regard to n, and, in addition, the following relation is obained,

$$\begin{aligned}&\lim_{n\to\infty}\frac{n(f-1)+f-1}{n(f-2)+f}\cdot\frac{n(f-1)+f-2}{n(f-2)+f-1}\cdot\dots\cdot\frac{n(f-1)+2}{n(f-2)+3}\cdot\frac{n(f-1)+1}{n+1}\\ &= \frac{(f-1)^{f-1}}{(f-2)^{f-2}}.\end{aligned} \tag{48}$$

The function $a(1-a)^{f-2}$ shows its maximum at $a = \frac{1}{f-1}$, $(i.e.\,p = \frac{1}{\sqrt{r(f-1)}})$, and its maximum value is $\frac{(f-2)^{f-2}}{(f-1)^{f-1}}$. From these reasons, over all the range of r,

$$\frac{m_{n+1}}{m_n} < 1. \tag{49}$$

This result is further analyzed in relation to p. When r, f and n are fixed and the fate of m_n in relation to p is considered (Eq. 46),

$$\begin{aligned}\frac{dm_n}{dp} &= \frac{(fn-n)!\,fN}{(fn-2n+2)!\,n!}\cdot r^{n-1}\cdot 2p^{2n-3}(1-rp^2)^{nf-2n+1}\\ &\times \{(n-1)-(nf-n+1)rp^2\}.\end{aligned} \tag{50}$$

The value of p giving a maximal value for m_n becomes

$$p_{\max} = \sqrt{\frac{n-1}{r(fn-n+1)}}. \tag{51}$$

When $n=1$, $p_{\max}=0$ and the maximum value of m_1 becomes N. This means m_1 decreases with increasing values of p, as shown in Fig. 3. Curve m_1 is upwardly

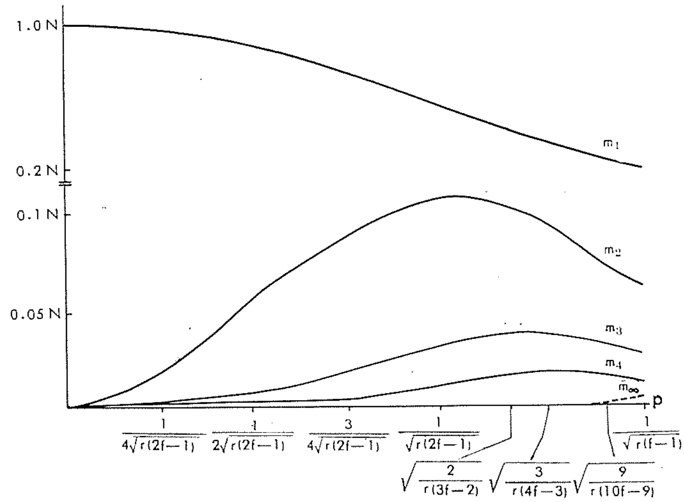

Fig. 3. Fate of M_n in relation to p

convex at the very beginning of the reaction, and then becomes upwardly concave, and the inflexion point is located at $p = \frac{1}{\sqrt{r(2f-1)}}$. When $n=2$, $p_{\max} = \frac{1}{\sqrt{r(2f-1)}}$. Curve m_2 increases at the beginning of reaction until p reaches $\frac{1}{\sqrt{r(2f-1)}}$ and then it decreases with further increase in p. Curve m_2 is always within m_1. Curve m_3 shows its maximum at $p = \sqrt{\frac{2}{r(3f-2)}}$ and at this point m_2 is in its decreasing phase. In addition, curve m_3 is always within m_2 and so on. When a large enough complex to be precipitated is formed, n can be assumed to be infinite and $p_{\max}$ becomes $\frac{1}{\sqrt{r(f-1)}}$. It was already shown that if p reaches $\frac{1}{\sqrt{r(f-1)}}$, $<n^2>$ and $<n_k{}^2>$ become infinite, *i.e.* the system attains the critical condition, and that only at this point does $\lim_{n\to\infty} \frac{m_{n+1}}{m_n}$ reach unity.

In the range of $r > f-1$, p cannot reach $\frac{1}{\sqrt{r(f-1)}}$ because the increase of p is limited by $p \leqq \frac{1}{r}$ (Eq. 21). It can be stated under such conditions that no free antibody site remains and thus the reaction cannot proceed further. This statement was already proved in Eq. 44, but it can be confirmed as follows. When the reacted fraction (p') of the total anibody sites is considered,

$$p' = \frac{\text{reacted antibody sites (r.a.s.)}}{2\,\Sigma N_k} = \frac{\text{r.a.s.}}{fN} \cdot \frac{fN}{2\,\Sigma N_k} = pr. \qquad (52)$$

As in this range p can grow to $\frac{1}{r}$, p' becomes unity. This means that no free antibody site remains and that all the antibody molecules are playing a role in connecting antigen molecules, as shown in Eq. 44. For this reason the m_1 complex in this range consists of nothing but free antigen.

In the range of $\frac{1}{f-1} \leqq r \leqq f-1$, p reaches $\frac{1}{\sqrt{r(f-1)}}$ and some precipitate appears. As described in Eq. 30, p can grow further. However it cannot be stated that the principle of the most probable distribution is still held for values of p larger than $\frac{1}{\sqrt{r(f-1)}}$. For such values of p, only Eq. 5 is still available, because Eq. 5 holds for other types of distribution besides the most probable one. Therefore it can be expected that, in the range of $\frac{f}{2(f-1)} \leqq r \leqq \frac{f}{2}$, M becomes unity (*i.e.* the number average molecular weight also becomes infinite as shown in Eq. 30). This means that no smaller complexes are present when p has increased up to $\frac{1}{f}+\frac{1}{2r}$ and no free antigen nor antibody molecules are left.

In the range of $\frac{f}{2} < r \leqq (f-1)$, p can still increase up to $\frac{1}{r}$ beyond $\frac{1}{\sqrt{r(f-1)}}$. Here, the results obtained under the concept of the most probable distribution cannot be applied any longer. However, it can be stated that p is less than unity, because even at the end of the reaction p remains at $\frac{1}{r}$, where p' (Eq. 52) becomes unity. This means that all the antibody sites are located inbetween two antigen molecules and that some antigen sites are not attached by antibodies. In addition, as described in Eq. 30, the number average molecular weight of complexes remains at a finite value in this range of r. For these reasons, it can be expected that some smaller complexes containing free antigen sites, which are not large enough to be precipitated, remain in the supernatant together with free antigen (m_1) and that these complexes and the free antigen can be precipitated by further addition of an appropriate amount of antibody.

In the range $\frac{1}{f-1} \leqq r < \frac{f}{2(f-1)}$, p can also increase up to unity beyond $\frac{1}{\sqrt{r(f-1)}}$ and, as shown in Eq. 30, the number average molecular weight of complexes remains at a finite value. For these reasons, it can be expected that some non-precipitable complexes remain in the supernatant together with free antibodies and all the antigen sites of the complexes are attached by antibodies ("saturated complex"). The saturated complexes and free antibodies can react with newly added antigen to give precipitates, when an appropriate amount of antigen is added to the supernatant for the test of excess antibodies.

In the range of $r < \frac{1}{f-1}$, it has already been shown in Eq. 45 that all the antigen sites are saturated with antibodies and no precipitates are formed.

In this range, the ratio of the amount of m_2 saturated complex in weight (W_2) to that of m_1 saturated complex (W_1), is considered,

$$\frac{W_2}{W_1} = \frac{2g+(2f-1)b}{g+fb} \cdot \frac{f}{2} r(1-r)^{f-2} = \left\{1 - \frac{1}{2\left(\frac{g}{b}+f\right)}\right\} fr(1-r)^{f-2}, \tag{53}$$

where g=molecular weight of antigen, b=molecular weight of antibody and $p=1$. In Eq. 53, we obtain

$$0 < \left\{1 - \frac{1}{2\left(\frac{g}{b}+f\right)}\right\} < 1,$$

and $fr(1-r)^{f-2}$ is evaluated at its maximal value, which is given by setting $r = \frac{1}{f-1}$,

$$\frac{f}{f-1}\left(\frac{f-2}{f-1}\right)^{f-2} = \frac{f(f-2)}{f-1} \cdot \left(\frac{f-2}{f-1}\right)^{f-3} = \frac{a^2-1}{a^2} \cdot \left(\frac{a-1}{a}\right)^{a-2}, \tag{54}$$

where $f-1 \equiv a \geqq 2$, and Eq. 54 is less than unity. Therefore, we obtain $0 < \frac{W_2}{W_1} < 1$, when $f \geqq 3$. As usual it is assumed that antigens are more than trivalent and it can be expected that by weight the most abundant saturated complex is m_1.

DISCUSSION

The experimental data in the literature on antigen-antibody systems must first be examined with regard to the theory presented here. As this theory clearly

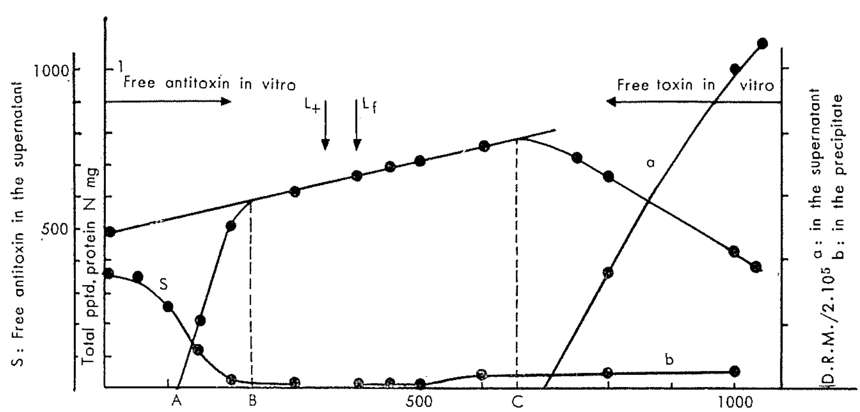

Fig. 4. Flocculation of diphtheria toxin with horse antitoxic serum No. 906. (Cited from E. H. Relyveld: Toxine et antitoxine diphtheriques p. 96, Fig. 30)

predicts the presence of a "prezone" when the respective concentrations of site-specific antibodies are approximately identical and when the valency of the antigen is not so large, such a case can be considered to correspond to the flocculation type of precipitin reaction. Among the experimental results on flocculation reactions of the diphtheria toxin-antitoxin system already reported by Relyveld (1959), a few very interesting results were included (curve 14 of Fig. 27 on page 91 and Fig. 30 on page 96). The latter example is cited in this paper (Fig. 4), in which 18.4 mμ Mol of antibody are added to tubes containing varying amounts of a purified diphtheria toxin preparation (3000 Lf per mg N). The ranges of equivalence, prezone and antibody excess giving some precipitate are calculated from this result

Table 1. Comparison of Dato in Fig. 4 with This Theory

	Values at points in Fig. 4		
	A	B	C
N/fN_k	$2/f(f-1)$	$1/(f-1)$	1
$(N/fN_k)_{f=4}$	0.17	0.33	1
Toxin added in mμ Mol (N)	3.24	6.64	18.72
N/18.4	0.17	0.36	1.02

and compared with those of the present theory, as shown in Table 1. When f is assumed to be 4, the experimental results are quite consistent with the present theory. Although a valency of 4 of diphtheria toxin is too small when compared with the value of 7 obtained by ultracentrifugal studies by Pappenheimer, Lundgren and Williams (1940), the discrepancy must be solved by further experiments.

It is well known that the Heidelberger-Kendall equation cannot directly be applied to antigen-antibody systems in the range of the prezone. In such a system the equation must be modified by subtracting the amount of antibody contained in non-precipitable saturated complexes. For this modification, Eq. 39 must be summed over n from unity to a finite value n, which means the m_n complex is soluble and the m_{n+1} complex is precipitable. However, such a summation is impossible despite the fact that Eq. 39 can be summed over n from unity to infinity as Stockmayer did.

Although the most probable distribution is obtained in the general case of varying concentrations of respective site-specific antibodies, the critical range has not yet been clarified. When this range is clarified, the difference between the precipitin reaction of usual rabbit antisera and that of usual horse antisera will be demonstrated.

ACKNOWLEDGEMENTS

The authors express their deep gratitude to Dr. Ryoyu Utiyama of the School of Science and Dr. Kenjiro Shoda of the School of Engineering Science of Osaka University for encouraging their work.

Appendix

1. Evaluation of $W_{n,\begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}}$

There are $n(n \geq 1)$ balls distinguishable from each other, which contain $f(f \geq 2)$ holes also distinguishable from each other in shape (1-, 2-, ..., f-type), and $\sum_{k=1}^{f}(l_k+i_k)$ rods, the two ends of which are differentiated into f kinds of shapes. The rods, which can be fitted into k-type holes, are named k-type rods, and both ends have the same shape, and there are l_1 rods of 1-type, l_2 of 2-type, ..., and l_f of f-type ($\sum_{k=1}^{f} l_k = n-1$) as the bridge-forming antibodies and, in addition, i_1 rods of 1-type, i_2 of 2-type, ..., and i_f of f-type as the non-bridge-forming antibodies. Each of the l_k+i_k rods ($k=1, 2, \ldots, f$) is also distinguishable from other rods of the same type.

To determine $W_{n,\begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}}$, the number of ways to form trees consisting of n balls and l_k rods ($k=1, 2, \ldots, f$) will first be determined ($Wn, l_1, l_2, \ldots, l_f$) and then the number of ways to insert i_k rods ($k=1, 2, \ldots, f$) into the empty holes in the k-type ($k=1, 2, \ldots, f$) will be considered ($Rn, i_1, i_2, \ldots, i_f$) because the latter procedure does not interfere with the former procedure. Therefore, we have

$$W_{n,\begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}} = Wn, l_1, l_2, \ldots, l_f \times Rn, i_1, i_2, \ldots, i_f. \qquad \text{(A 1)}$$

$Wn, l_1, l_2, \ldots, l_f$ can be obtained in the following way. To construct a tree with n balls and $n-1$ rods, two balls are connected with one rod and no cyclic structure is formed in any part of the tree. First pick up a ball (this ball is distinct and called the "origin") regarding other $n-1$ balls indistinguishable, and find out the number of ways ($\omega n, l_1, l_2, \ldots, l_f$) to construct trees. Then, when $n-1$ balls are distinbuishable, we obtain

$$Wn, l_1, l_2, \ldots, l_f = (n-1)!\ \omega n, l_1, \ldots, l_f. \qquad \text{(A 2)}$$

To the "origin" ball are given p_1 rods of 1-type, p_2 rods of 2-type, and p_3 rods of 3-type, ..., and these are used to connect the balls of the "first generation (F_1)." For the "second generation (F_2)," q_1 rods of 1-type, q_2 of 2-type and q_3 of 3-type, ..., are inserted into the balls of the "first genera-

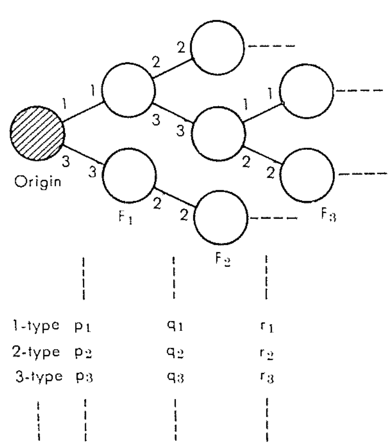

Fig. 5. Schematic presentotion of the woy to find $\omega n, l_1, l_2, \ldots, l_f$

tion." For the "third generation," r_1 rods of 1-type, r_2 of 2-type and r_3 of 3-type, ... , are inserted into the balls of the "second generation," and so on (Fig. 5). The number of trees thus formed becomes

$$\binom{1}{p_1}\binom{1}{p_2}\binom{1}{p_3}\cdots\cdots\binom{\sum p_k-p_1}{q_1}\binom{\sum p_k-p_2}{q_2}\binom{\sum p_k-p_3}{q_3}\cdots\cdots$$
$$\times\binom{\sum q_k-q_1}{r_1}\binom{\sum q_k-q_2}{r_2}\binom{\sum q_k-q_3}{r_3}\cdots\cdots. \tag{A 3}$$

The reason is as follows. As the "origin" ball contains one hole of each type, the number of ways to insert p_1 rods into one 1-type hole is $\binom{1}{p_1}$ and similar values are expected for the other types. The second generation balls ($\sum p_k$) are uniquely connected with these Σp_k rods. The number of empty holes of 1-type on the second generation balls is $\sum p_k-p_1$ and q_1 rods of 1-type can select q_1 from $\sum p_k-p_1$ holes and so on. Thus, we have

$$\omega n,\, l_1,\, l_2,\, \ldots,\, l_f=\sum\binom{1}{p_1}\binom{1}{p_2}\binom{1}{p_3}\cdots\cdots\binom{\sum p_k-p_1}{q_1}\binom{\sum p_k-p_2}{q_2}\binom{\sum p_k-p_3}{q_3}\cdots\cdots$$
$$\times\binom{\sum q_k-q_1}{r_1}\binom{\sum q_k-q_2}{r_2}\binom{\sum q_k-q_3}{r_3}\cdots\cdots, \tag{A 4}$$

where the summations of $\{p_k, q_k, r_k, \ldots (k=1, 2, \ldots, f)\}$ extend over all positive integers including zero which satisfy the following relations;

$$p_k+q_k+r_k+\ \ldots\ = l_k,\ (k=1,\ 2,\ \ldots,f)$$
$$\sum_{k=1}^{f} l_k = n-1. \tag{A 5}$$

In order to find an expression for ωn, l_1, l_2, ... , l_f, we indroduce the generating function,

$$Z=\sum\omega_n,\, l_1,\, l_2,\, \ldots\, l_f\cdot x_1^{l_1}\, x_2^{l_2}\ldots x_f^{l_f}, \tag{A 6}$$

where the summations over l_1, l_2, . . . , l_f extend from zero to infinity. Z is rewritten as,

$$Z=\sum\left\{\sum\binom{1}{p_1}\binom{1}{p_2}\cdots\cdots\binom{\sum p_k-p_1}{q_1}\binom{\Sigma p_k-p_2}{q_2}\cdots\cdots\binom{\Sigma q_k-q_1}{r_1}\binom{\Sigma q_k-q_2}{r_2}\cdots\cdots\right\}$$
$$\times x_1^{p_1+q_1+r_1+\ldots}\, x_2^{p_2+q_2+r_2+\ldots}\cdots\cdots x_f^{p_f+q_f+r_f+\ldots}. \tag{A 7}$$

The summation can be performed easily for p_1, p_2 , ... , q_1, q_2, ... , r_1, r_2, ... , separately. For example the following simplified function for $f=3$ is considered,

$$Z'=\sum\binom{1}{p_1}\binom{1}{p_2}\binom{1}{p_3}\binom{p_2+p_3}{q_1}\binom{p_1+p_3}{q_2}\binom{p_1+p_2}{q_3}\binom{q_2+q_3}{r_1}\binom{q_1+q_3}{r_2}\binom{q_1+q_2}{r_3}$$
$$\times x_1^{p_1+q_1+r_1}\, x_2^{p_2+q_2+r_2}\, x_3^{p_3+q_3+r_3}. \tag{A 8}$$

The sum over r_1 is

$$\sum_{r_1}\binom{\Sigma q-q_1}{r_1}x_1^{r_1}=(1+x_1)^{\Sigma q-q_1}=(1+x_1)^{q_2+q_3},$$

and hence

$$\sum_{r_2}=(1+x_2)^{q_1+q_3},\qquad \sum_{r_3}=(1+x_3)^{q_1+q_2}.$$

The sum over q_1 becomes

$$\sum_{q_1}\binom{\Sigma p-p_1}{q_1}x_1^{q_1}(1+x_2)^{q_1}(1+x_3)^{q_1}=\{1+x_1(1+x_2)(1+x_3)\}^{p_2+p_3},$$

and in the same way

$$\sum_{q_2} = \{1+x_2(1+x_3)(1+x_1)\}^{p_3+p_1}, \quad \sum_{q_3} = \{1+x_3(1+x_1)(1+x_2)\}^{p_1+p_2} .$$

The sum over p_1 becomes

$$\sum_{p_1} \binom{1}{p_1} x_1^{p_1} \{1+x_2(1+x_3)(1+x_1)\}^{p_1} \{1+x_3(1+x_1)(1+x_2)\}^{p_1}$$

$$= 1+x_1\{1+x_2(1+x_3)(1+x_1)\}\{1+x_3(1+x_1)(1+x_2)\} .$$

$\sum_{p_2}$, $\sum_{p_3}$ can be obtained in the same way.

Thus Z can be written as follows,

$$\begin{aligned} Z = & [1+x_1\{1+x_2(\ldots\ldots)\}\{1+x_3(\ldots\ldots)\} \ldots\ldots \{1+x_f(\ldots\ldots)\}] \\ \times & [1+x_2\{1+x_1(\ldots\ldots)\}\{1+x_3(\ldots\ldots)\} \ldots\ldots \{1+x_f(\ldots\ldots)\}] \\ \times & [1+x_3\{1+x_1(\ldots\ldots)\}\{1+x_2(\ldots\ldots)\} \ldots\ldots \{1+x_f(\ldots\ldots)\}] \\ & \ldots\ldots \\ \times & [1+x_f\{1+x_1(\ldots\ldots)\}\{1+x_2(\ldots\ldots)\} \ldots\ldots \{1+x_{f-1}(\ldots\ldots)\}] . \end{aligned} \tag{A 9}$$

Now, the following substitutions are made,

$$\begin{aligned} Z &= A'_1A'_2A'_3\ldots A'_f \equiv A' \quad \text{and} \\ A'_1 &= 1+x_1A'_2A'_3\ldots A'_f, \\ A'_2 &= 1+x_2A'_1A'_3\ldots A'_f, \qquad , (A'_k \geqq 1) \\ &\vdots \\ A'_f &= 1+x_fA'_1A'_2\ldots A'_{f-1}. \end{aligned} \tag{A10}$$

As $\omega n, l_1, l_2, \ldots, l_f$ is the coefficient of the term, $x_1^{l_1} x_2^{l_2} \ldots x_f^{l_f} y^{n-1}$, we have,

$$\omega n,\ l_1,\ l_2,\ \ldots,\ l_f = \frac{1}{(2\pi i)^f} \oint \frac{Z}{x_1^{l_1+1} x_2^{l_2+1} \ldots x_f^{l_f+1}} dx_1dx_2\ldots dx_f. \tag{A11}$$

The integral variables $x_k(k=1, 2, \ldots, f)$ are transformed to $A'_k(k=1, 2, \ldots, f)$,

$$x_k = \frac{A'_k(A'_k-1)}{A'} \quad \text{and} \quad \frac{\partial x_k}{\partial A'_j} = \begin{cases} \dfrac{A'_k}{A'} \ldots\ldots\ldots\ldots (j=k) \\ \dfrac{-A'_k(A'_k-1)}{A'A'_j} (j \neq k) , \end{cases} \tag{A12}$$

$$D \equiv dx_1dx_2\ldots dx_f = \begin{vmatrix} \frac{\partial x_1}{\partial A'_1} & \frac{\partial x_1}{\partial A'_2} & \frac{\partial x_1}{\partial A'_3} & \cdots & \frac{\partial x_1}{\partial A'_f} \\ \frac{\partial x_2}{\partial A'_1} & \frac{\partial x_2}{\partial A'_2} & \frac{\partial x_2}{\partial A'_3} & \cdots & \frac{\partial x_2}{\partial A'_f} \\ \frac{\partial x_3}{\partial A'_1} & \frac{\partial x_3}{\partial A'_2} & \frac{\partial x_3}{\partial A'_3} & \cdots & \frac{\partial x_2}{\partial A'_f} \\ \vdots & \vdots & \vdots & & \vdots \\ \frac{\partial x_f}{\partial A'_1} & \frac{\partial x_f}{\partial A'_2} & \frac{\partial x_f}{\partial A'_3} & \cdots & \frac{\partial x_f}{\partial A'_f} \end{vmatrix} dA'_1dA'_2\ldots dA'_f$$

$$= \begin{vmatrix} \frac{A'_1}{A'} & \frac{-A'_1(A'_1-1)}{A'A'_2} & \frac{-A'_1(A'_1-1)}{A'A'_3} & \cdots\cdots \\ \frac{-A'_2(A'_2-1)}{A'A'_1} & \frac{A'_2}{A'} & \frac{-A'_2(A'_2-1)}{A'A'_3} & \cdots\cdots \\ \frac{-A'_3(A'_3-1)}{A'A'_1} & \frac{-A'_3(A'_3-1)}{A'A'_2} & \frac{A'_3}{A'} & \cdots\cdots \\ \vdots & \vdots & \vdots & \end{vmatrix} dA'_1 dA'_2 \cdots dA'_f$$

$$= \prod_{k=1}^{f} \frac{-(A'_k-1)}{A'} \begin{vmatrix} a_1 & 1 & 1 & \cdots & 1 \\ 1 & a_2 & 1 & \cdots & 1 \\ 1 & 1 & a_3 & \cdots & 1 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 1 & 1 & 1 & \cdots & 1 \end{vmatrix} dA'_1 dA'_2 \cdots dA'_f, \left\{a_k = \frac{-A'_k}{(A'_k-1)} = 1 + \left(\frac{A'_k-1}{1-A'_k}\right)\right\}. \tag{A13}$$

With the useful identity:

$$\begin{vmatrix} 1+b_1 & 1 & \cdots & 1 \\ 1 & 1+b_2 & \cdots & 1 \\ \vdots & \vdots & \ddots & \vdots \\ 1 & 1 & \cdots & 1+b_f \end{vmatrix} = b_1 b_2 \cdots b_f \left(1 + \frac{1}{b_1} + \frac{1}{b_2} + \cdots + \frac{1}{b_f}\right), \tag{A14}$$

we have from Eq. A 13,

$$D = (A')^{-f} \prod_{k=1}^{f} (1-A'_k) \cdot \prod_{k=1}^{f} \left(\frac{2A'_k-1}{1-A'_k}\right) \cdot \left(1 + \sum_{k=1}^{f} \frac{1-A'_k}{2A'_k-1}\right) dA'_1\, dA'_2 \cdots dA'_f$$

$$= \prod_{k=1}^{f} \left(\frac{2A'_k-1}{A'}\right) \cdot \left(1 - \sum_{k=1}^{f} \frac{A'_k-1}{2A'_k-1}\right) dA'_1\, dA'_2 \cdots dA'_f. \tag{A15}$$

From Eq. A 15 and A 11 we can obtain,

$$\omega = \frac{1}{(2\pi i)^f} \oint \frac{A'}{\prod_{k=1}^{f} \left\{\frac{A'_k(A'_k-1)}{A'}\right\}^{l_k+1}} \cdot \prod_{k=1}^{f} \left(\frac{2A'_k-1}{A'_k}\right) \cdot \left(1 - \sum \frac{A'_k-1}{2A'_k-1}\right) \cdot$$

$$dA'_1 dA'_2 \cdots dA'_f. \tag{A16}$$

Further, by the substitution, $B_k = A'_k - 1 (k=1, 2, \ldots, f)$, Eq. A 16 becomes

$$\omega = \frac{1}{(2\pi i)^f} \oint \prod_{k=1}^{f} \frac{(B_k+1)^{n-l_k-1}(2B_k+1)}{B_k^{l_k+1}} \cdot \left(1 - \sum \frac{B_k}{2B_k+1}\right) dB_1 dB_2 \cdots dB_f$$

$$= \frac{1}{(2\pi i)^f} \oint \left(\prod_{k=1}^{f} \frac{(B_k+1)^{n-l_k-1}(2B_k+1)}{B_k^{l_k+1}} - \sum_{j=1}^{f} \left\{ \prod \frac{(B_k+1)^{n-l_k-1}(2B_k+1)}{B_k^{l_k+1}} \cdot \frac{B_j}{2B_j+1} \right\} \right) \cdot$$

$$dB_1 dB_2 \cdots dB_f$$

$$= \prod_{k=1}^{f} \left(\frac{1}{2\pi i} \oint \frac{(B_k+1)^{n-l_k-1}(2B_k+1)}{B_k^{l_k+1}} dB_k \right) - \sum_{j=1}^{f} \left\{ \left(\frac{1}{2\pi i} \oint \frac{(B_j+1)^{n-l_j-1}}{B_j^{l_j}} dB_j \right) \right.$$

$$\left. \times \prod_{\substack{k=1 \\ k \neq j}}^{f} \left(\frac{1}{2\pi i} \oint \frac{(B_k+1)^{n-l_k-1}(2B_k+1)}{B_k^{l_k+1}} dB_k \right) \right\}$$

$$= \prod_{k=1}^{f} \left\{ 2\binom{n-l_k-1}{l_k-1} + \binom{n-l_k-1}{l_k} \right\} - \sum_{j=1}^{f} \left\{ \binom{n-l_j-1}{l_j-1} \cdot \prod_{\substack{k=1 \\ k \neq j}}^{f} \left(2\binom{n-l_k-1}{l_k-1} + \binom{n-l_k-1}{l_k} \right) \right\}$$

$$= n^f \prod_{k=1}^{f} \frac{(n-l_k-1)!}{l_k!(n-2l_k)!} - n^{f-1} \sum_{j=1}^{f} \left(l_j \cdot \prod_{\substack{k=1 \\ k \neq j}}^{f} \frac{(n-l_k-1)!}{l_k!(n-2l_k)!} \right)$$

$$= n^{f-1} \prod_{k=1}^{f} \frac{(n-l_k-1)!}{l_k!(n-2l_k)!} \,. \tag{A17}$$

As each of the l_k rods, each end of a rod, and each of $n-1$ balls are respectively distinguishable from others,

$$W_{n,l_1,l_2,\ldots,l_f} = (n-1)!\omega \cdot \Pi l_k! \cdot 2^{\Sigma l_k} = n! n^{f-2} \cdot \prod_{k=1}^{f} \frac{(n-l_k-1)! l_k!}{(n-2l_k)!\, l_k!} \cdot 2^{\Sigma l_k} . \tag{A18}$$

$R_{n,\, i_1,\, i_2,\ldots,\, i_f}$ is obtained in the following way. The remaing i_k rods must be inserted into k-type holes without forming new bridges between any pair of balls. The number of empty k-type holes is $n-2l_k$. As each of the i_k rods and each end of a rod is distinguishable, the number of ways to distribute i_k rods in $(n-2l_k)$ holes is

$$R_{n,\, i_1,\, i_2,\ldots,\, i_f} = \prod_{k=1}^{f} \binom{n-2l_k}{i_k} i_k! \cdot 2^{\Sigma i_k} . \tag{A19}$$

Therefore, we obtain by Eqs. A 18 and A 19.

$$W_{n,\left\{\begin{smallmatrix} l_1,\, l_2,\, \ldots,\, l_f \\ i_1,\, i_2,\, \ldots,\, i_f \end{smallmatrix}\right\}} = n!\, n^{f-2} \cdot \prod_{k=1}^{f} \frac{(n-l_k-1)!}{(n-2l_k-i_k)!} \cdot 2^{\Sigma n_k} , \quad (n_k = l_k + i_k)$$

$$= n! n^{f-2} \cdot \prod_{k=1}^{f} (l_k!\, i_k!) \cdot \prod_{k=1}^{f} \frac{(n-l_k-1)!}{l_k! i_k!\, (n-2l_k-i_k)!} \cdot 2^{\Sigma n_k} . \tag{A20}$$

When f is 3, $W_{n,\left\{\begin{smallmatrix} l_1,\, l_2,\, l_3 \\ i_1,\, i_2,\, i_3 \end{smallmatrix}\right\}}$ can be evaluated in a similar manner as Stockmayer (1943) and Goldberg (1952). $W_{n,\left\{\begin{smallmatrix} l_1,\, l_2,\, l_3 \\ i_1,\, i_2,\, i_3 \end{smallmatrix}\right\}}$ is defined as the number of ways in which n 3-functional units (called S_n-units), l_1+i_1, l_2+i_2, and l_3+i_3 bifunctional units (S_{n1}-,S_{n2}-and S_{n3}-units) can be formed into a single n, l_1, i_1, l_2, i_2, l_3, i_3-aggregate containing no cyclic structures. All the units and all the functional sites thereon are distinguishable. The three sites on S_n-units have different shapes (1-, 2- 3-types).Both sites on S_{n1} can be fitted only into 1-type holes and those of S_{n2} into 2-type holes and those of S_{n3} into 3-type holes of S_n-units. S_n-units are represented by mechanical frames containing 3 holes of different shapes. A pair of holes of the same type beloging to different frames are connected together by a bolt of the same type. Bolts are also required to fill all the other holes. However they do not connect different frames. The n frames are connected together by l_1,l_2 and l_3 bolts and the remaining empty holes of the k-type are fitted with i'_k bolts (k=1,2,3). Here, the following relations are given

$$\begin{aligned} &l_1+l_2+l_3 = n-1, \\ &n-2l_k = i'_k, \\ &\frac{n}{2} \geqq l_k \ (k = 1, 2, 3). \end{aligned} \tag{A21}$$

The number of ways to bolt all the frames together into an n-aggregate, containing no cyclic structures, is W_n. To form an n-aggregate $n-1$ $(=l_1+l_2+l_3)$ bolts are used. The empty holes of the resulting structure are filled with i'_1,i'_2 and i'_3 bolts and this procedure does not change the number of ways of forming the n-aggregate. After the structure has been made, l_k bolts are replaced by l_k S_{nk}-units and i'_k bolts are also replaced by i_k S_{nk}-units (k=1,2,3). The number of ways to carry out the latter procedure is defind as R_n. Therefore

$$W_{n,\left\{\begin{smallmatrix} l_1,\, l_2,\, l_3 \\ i_1,\, i_2,\, i_3 \end{smallmatrix}\right\}} = W_n \times R_n . \tag{A22}$$

Wn is determined as follows. Any one of the bolted arrangements can be dissociated into each n separate frames in such a way that each bolt removed is left in one of a pair of frames and each frame contains two holes occupied by bolts and one empty hole. There will be one free bolt left over. Now the type of the most abundant bolts used to connect frames is provided as 3-type ($l_3 \geqq l_1, l_2$) and one of the 3-type bolts is chosen as the free bolt and is removed first from the structure. As the number of 3-type bolts on the structure is $n-l_3$, the number of ways of choosing the free bolt from the 3-type bolts is also $n-l_3$. If the free bolt is chosen, it uniquely determines the empty holes in each of the n frames. When P is the number of possible dissociated arrangements and Q is the number of ways of bolting each dissociated arrangement together, the number of different bolted arrangements is

$$Wn = \frac{P \cdot Q}{n-l_3} . \tag{A23}$$

P is the number of ways of leaving one empty hole on each plate. The number of newly made empty 1-type holes is l_1, that of 2-type holes is l_2 and that of 3-type holes is l_3. The total number of empty 3-type holes is l_3+1, because one empty hole of the 3-type has already been made by removing the free bolt of the 3-type. Therefore, we obtain

$$P = \binom{n}{l_1} \binom{n-l_1}{l_2} = \frac{n!}{l_1!\, l_2!\, (l_3+1)!} . \tag{A24}$$

Q is determined as follows. As i'_k bolts are also bifunctional units as l_k and can be inserted into the k-type holes, all k-type bolts ($k=1, 2, 3$) can serve to form the n-aggregate. The l_1 frames, each containing one empty 1-type hole, are named group 1 and l_2 frames, having empty 2-type holes, group 2 and l_3+1 frames, having empty 3-type holes, group 3. At first, l_1 empty 1-type holes are filled by inserting 1-type bolts contained in the frames of groups 2 and 3. From group 2 r bolts of 1-type and $l_1 - r$ bolts from group 3 are used to connect the frames. Thus the number of ways to take r from l_2 and l_1-r from l_3+1 and to insert these 1-type bolts into empty holes is,

$$Q_1 = \binom{l_2}{r} \binom{l_3+1}{l_1-r} \cdot l_1! , \quad (0 \leqq r \leqq l_2,\ 0 \leqq l_1-r \leqq l_3+1). \tag{A25}$$

Now, there are r given structures, each of which is composed of two frames connected by 1-type bolt and containing one empty 2-type hole (named the 2-1 group), and l_1-r structures, each of which is composed of two frames connected by 1-type bolt and containing one empty 3-type hole (named the 3-1 group). In addition to these, there remain l_2-r unconnected frames containing empty 2-type holes (named the 2-2 group) and l_3+1-l_1+r unconnected frames with empty 3-type holes (named the 3-2 group). First, ways to fill r empty 2-type holes of the 2-1 group are considered. Each structure contains one empty hole. To the first selected 2-type hole of a structure of the 2-1 group one 2-type bolt must be inserted. Such a 2-type bolt can be selected from $r-1$ bolts of the 2-1 group, $2(l_1-r)$ bolts of the 3-1 group, and (l_3+1-l_1+r) bolts of the 3-2 group. Therefore, the number of ways to select such a bolt is l_1+l_3. This procedure is repeated r times. Thus, the number of ways to fill the 2-type holes of the 2-1 group is ${}_{l_1+l_3}P_r$ (when r is zero, there is still only one way, in which there is nothing to do). Next, the ways to fill $l_2 - r$ empty 2-type holes are considered. At present, $l_1+l_3+1- r$ bolts of the 2-type are available. The number of ways can be expressed as ${}_{l_1+l_3+1-r}P_{l_2-r}$. Therefore, the total number of ways to fill l_2 empty holes, Q_2, can be calculated,

$$Q_2 = {}_{l_1+l_3}P_r \times {}_{l_1+l_3+1-r}P_{l_2-r} = \frac{(l_1-l_3)!}{(l_1+l_3+1-l_2)!} (l_1+l_3+1-r). \tag{A26}$$

Then, the empty 3-type holes are filled. Though the number of 3-type bolts in each of the structures formed cannot be determined, it is obvious that the number of structures formed is l_3+1 and that each structure contains one empty 3-type hole and that there remains one free bolt of the 3-type. As the number of 3-type bolts in the system (including the free bolt) is l_1+l_2+1, l_3 washers are distributed to l_1+l_2+1 of the 3-type bolts. In this process, a washer may or may not be given to the free bolt. The number of ways to distribute the washers and to fit another structure through the empty 3-type hole is,

$$Q_3 = {}_{l_1+l_2+1}P_{l_3} = \frac{(l_1+l_2+1)!}{(l_1+l_2+1-l_3)!}. \tag{A27}$$

Therefore, Q can be obtained as follows,

$$Q = Q_3 \sum_r Q_1 Q_2 = \frac{l_1!\, l_2!\, (l_3+1)!\, (l_1+l_3)!\, (l_1+l_2+1)!}{(l_1+l_3+1-l_2)!\, (l_1+l_2+1-l_3)!}$$

$$\times \sum_r \frac{(l_1+l_3+1-r)}{r!(l_1-r)!(l_2-r)!(l_3+1-l_1+r)!}. \tag{A28}$$

From Eqs. A 23, A 24 and A 28 we obtain,

$$Wn = \frac{P\cdot Q}{n-l_3} = \frac{n!(n-l_2-1)!(n-l_3-1)!}{(n-2l_2)!(n-2l_3)!}$$

$$\times \sum_r \frac{(l_1+l_3+1-r)}{r!(l_1-r)!(l_2-r)!(l_3+1-l_1+r)!}. \tag{A29}$$

R_n is the product of the number of ways to replace the l_k bolts by l_k S_{nk}-units with the number of ways to replace the remaining $n-2l_k$ bolts by i_k of S_{nk}-units and with $2^{\sum_{k=1}^{3}(l_k+i_k)}$,

$$R_n = 2^{\sum_{k=1}^{3}(l_k+i_k)} \cdot \prod_{k=1}^{3} l_k! \left({}_{n-2l_k}P_{i_k} \right)$$

$$= 2^{\sum_{k=1}^{3}(l_k+i_k)} \cdot \prod_{k=1}^{3} \frac{(n-2l_k)!\, l_k!}{(n-2l_k-i_k)!}. \tag{A30}$$

From Eqs. A 29 and A 30, we have,

$$W_{n,\left\{\begin{smallmatrix} l_1,l_2,l_3 \\ i_1,i_2,i_3 \end{smallmatrix}\right\}} = 2^{\sum_{k=1}^{3}(l_k+i_k)} \cdot \frac{n!l_1!l_2!l_3!(n-l_2-1)!(n-l_3-1)!}{(n-2l_1-i_1)(n-2l_2-i_2)!(n-2l_3-i_3)!}$$

$$\times \left\{(n-2l_1)! \sum_r \frac{(l_1+l_3+1-r)}{r!(l_1-r)!(l_2-r)!(l_3+1-l_1+r)!}\right\}. \tag{A31}$$

Here, the following summation must be performed,

$$S = (n-2l_1)! \sum_r \frac{(l_1+l_3+1-r)}{r!(l_1-r)!(l_2-r)!(l_3+1-l_1+r)!}$$

$$= \frac{(n-2l_1)!}{l_2!(l_3+1)!} \sum_r \frac{l_2!}{r!(l_2-r)!} \cdot \frac{(l_3+1)!}{(l_1-r)!\{l_3+1-(l_1-r)\}!} \cdot (l_1+l_3+1-r)$$

$$= \frac{(n-2l_1)!}{l_2!(l_3+1)!} \sum_r \left\{ \binom{l_2}{r}\binom{l_3+1}{l_1-r} \cdot (l_1+l_3+1-r) \right\} \tag{A32}$$

$$= \frac{(n-2l_1)!}{l_2!(l_3+1)!} \cdot U, \left(U \equiv \sum_r \binom{l_2}{r}\binom{l_3+1}{l_1-r} \cdot (l_1+l_3+1-r) \right).$$

In oder to find U, the following functions are considered,

$$A = (1+x)^{l_2} = \sum \binom{l_2}{r} x^r,$$

$$B = x^{l_3}(1+x)^{l_3+1} = \sum \binom{l_3+1}{s} x^{s+l_3+1},$$

$$B'=\frac{dB}{dx}=(l_3+1)\,x^{l_3}(1+x)^{l_3}(1+2x)=\sum\binom{l_3+1}{s}x^{s+l_3}(s+l_3+1)$$

$$=x^{l_3}\left\{\sum\binom{l_3+1}{s}x^{s}\,(s+l_3+1)\right\}.$$

When $s=l_1-r$, B' becomes

$$(B')_{s=l_1-r}=x^{l_3}\left\{\sum\binom{l_3+1}{l_1-r}x^{l_1-r}\cdot(l_1+l_3+1-r)\right\}$$

We obtain from A and B',

$$A\times B'=(l_3+1)x^{l_3}(1+x)^{l_2+l_3}(1+2x)$$

$$=x^{l_3}\left\{\sum_r\sum_s\binom{l_2}{r}\binom{l_3+1}{s}x^{s+r}(s+l_3+1)\right\}.$$

Now we consider the term $x^{l_1+l_3}$ of $A\times B'$ setting $s=l_1-r$, which can be expressed as

$$(A\times B')_{s=l_1-r}=\left\{\sum_r\binom{l_2}{r}\binom{l_3+1}{l_1-r}x^{l_1-r+r}\cdot(l_1+l_3+1-r)\right\}x^{l_3}$$

$$=\left\{\sum_r\binom{l_2}{r}\binom{l_3+1}{l_1-r}(l_1+l_3+1-r)\right\}x^{l_1+l_3} \tag{A33}$$

$$=U\cdot x^{l_1+l_3}.$$

$A\times B'$ can be rewritten as,

$$A\times B'=x^{l_3}\left\{(l_3+1)\ (1+2x)\ (1+x)^{l_2+l_3}\right\}$$

$$=x^{l_3}\left\{(l_3+1)\ (1+2x)\ \sum\binom{l_2+l_3}{t}x^{t}\right\}. \tag{A34}$$

U of Eq. A 33 corresponds to the coefficient of the term $x^{l_1+l_3}$ of Eq. A 34,

$$U=(l_3+1)\left\{\binom{l_2+l_3}{l_1}+2\binom{l_2+l_3}{l_1-1}\right\}=\frac{(l_2+l_3)!n\cdot(l_3+1)}{l_1!(n-2l_1)!}. \tag{A35}$$

S of Eq. A 32 can be calculated from this result,

$$S=\frac{n(n-l_1-1)!}{l_1!\,l_2!\,l_3!}.$$

Therefore, we obtain,

$$W_{n,}\left\{\begin{matrix}l_1,\ l_2,\ l_3\\ i_1,\ i_2,\ l_3\end{matrix}\right\}=n!\,n\cdot\prod_{k=1}^{3}l_k!\,i_k!\cdot\prod_{k=1}^{3}\frac{(n-l_k-1)!}{l_k!i_k!(n-2l_k-i_k)!}\cdot 2^{\Sigma i_k}, \tag{A36}$$

and the validity of the solution in the general case of $W_{n,}\left\{\begin{matrix}l_1,\ l_2,\ \dots,\ l_f\\ i_1,\ i_2,\ \dots,\ i_f\end{matrix}\right\}$ is proved in the case of $f=3$.

2. Summations

(1) $\sum n\,m_{n,}\left\{\begin{matrix}l_1,\ l_2,\ \dots,\ l_f\\ i_1,\ i_2,\ \dots,\ i_f\end{matrix}\right\}=N$

In this summation, the following figure (Fig. 6) is considered, where l_k and i_k rods are included to consider the number of ways to form trees, and the ways to connect the first(F_1) and second generation (F_2) balls are not uniquely determined. Therefore, we obtain

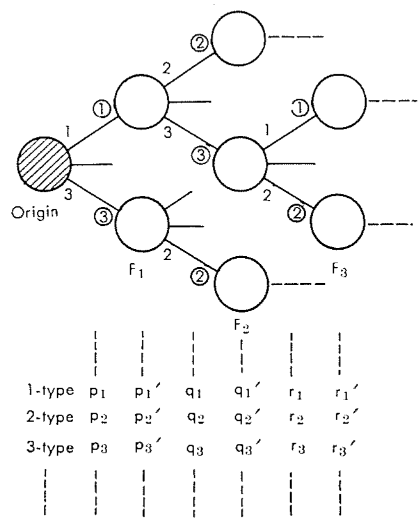

Fig. 6. Schematic presentation of the way to find $\omega_{n,}\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, l_f \end{Bmatrix}$

$$\omega_{n,}\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} = \sum \binom{1}{p_1}\binom{1}{p_2}\cdots\binom{p_1}{p'_1}\binom{p_2}{p'_2}\cdots\cdots\binom{\sum p'_k - p'_1}{q_1}\binom{\Sigma p'_k - p_2}{q_2}\cdots\cdots \binom{q_1}{q'_1}\binom{q_2}{q'_2}\cdots, \tag{A37}$$

and the following relations hold in the summation,

$$\begin{aligned} p_k' + q_k' + \cdots &= l_k, \\ (p_k + q_k + \cdots) - (p'_k + q'_k + \cdots) &= i_k, \\ \sum_{k=1}^{f} (p'_k + q'_k + \cdots\cdots\cdots) &= n-1. \end{aligned} \tag{A38}$$

Here, the following function is considered,

$$F = \sum \omega_{n,}\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} x_1^{l_1+i_1} x_2^{l_2+i_2} \cdots x_f^{l_f+i_f} y^{n-1}, \tag{A39}$$

where the summations over $n, l_1, l_2, \dots, l_f, i_1, i_2, \dots, i_f$ extend from zero to infinity with the relation, $\sum_{k=1}^{f} l_k = n-1$. F is rewritten as,

$$F = \sum \left\{ \sum \binom{1}{p_1}\binom{1}{p_2}\cdots\binom{p_1}{p'_1}\binom{p_2}{p'_2}\cdots\binom{\Sigma p'_k - p'_1}{q_1}\binom{\Sigma p'_k - p'_2}{q_2}\cdots\binom{q_1}{q'_1}\binom{q_2}{q'_2}\cdots \right\} \times x_1^{p_1+q_1+\cdots} x_2^{p_2+q_2+\cdots} x_3^{p_3+q_3+\cdots} \cdots y^{p'_1+p'_2+\cdots+q'_1+q'_2+\cdots}.$$

We can perform the summations easily. As an example, we have for $f = 3$,

$$F' = \sum \binom{1}{p_1}\binom{1}{p_2}\binom{1}{p_3}\binom{p_1}{p'_1}\binom{p_2}{p'_2}\binom{p_3}{p'_3}\binom{p'_2+p'_3}{q_1}\binom{p'_1+p'_3}{q_2}\binom{p'_1+p'_2}{q_3} x_1^{p_1+q_1} x_2^{p_2+q_2}$$
$$\times\, x_3^{p_3+q_3}\, y^{p'_1+p'_2+p'_3} .$$

The sum over q_1 is,

$$\sum_{q_1} \binom{p'_2+p'_3}{q_1} x_1^{q_1} = (1+x_1)^{p_2'+p_3'} , \text{ and hence } \sum_{q_2} = (1+x_2)^{p_1'+p_3'} , \sum_{q_3} = (1+x_3)^{p_1'+p_2'} .$$

The sum over p_1' is

$$\sum_{p_1'} \binom{p_1}{p_1'} \left\{ y(1+x_2)(1+x_3) \right\}^{p_1'} = \left\{ 1+y(1+x_2)(1+x_3) \right\}^{p_1},$$

and hence

$$\sum_{p_2'} = \left\{ 1+y(1+x_1)(1+x_3) \right\}^{p_2}, \quad \sum_{p_3'} = \left\{ 1+y(1+x_1)(1+x_2) \right\}^{p_3}.$$

The sum over p_1 is

$$\sum_{p_1} \binom{1}{p_1} \left(x_1 \left\{ 1+y(1+x_2)(1+x_3) \right\} \right)^{p_1} = 1+x_1 \left\{ 1+y(1+x_2)(1+x_3) \right\}$$

and hence

$$\sum_{p_2} = 1+x_2 \left\{ 1+y(1+x_1)(1+x_3) \right\}, \quad \sum_{p_3} = 1+x_3 \left\{ 1+y(1+x_1)(1+x_2) \right\}.$$

Thus, F can be written as follows,

$$\begin{aligned} F = & \left[1+x_1 \left\{ 1+y(1+x_2(1+y\ldots)) \right\} \left\{ 1+x_3(1+y\ldots) \right\} \ldots \right] \\ & \times \left[1+x_2 \left\{ 1+y(1+x_1(1+y\ldots)) \right\} \left\{ 1+x_3(1+y\ldots) \right\} \ldots \right] \\ & \times \left[1+x_3 \left\{ 1+y(1+x_1(1+y\ldots)) \right\} \left\{ 1+x_2(1+y\ldots) \right\} \ldots \right] \\ & \cdots\cdots\cdots\cdots \\ & \times \left[1+x_f \left\{ 1+y(1+x_1(1+y\ldots)) \right\} \left\{ 1+x_2(1+y\ldots) \right\} \ldots \right] . \end{aligned} \tag{A40}$$

Now, the following substitutions are made,

$$\begin{aligned} F &= A_1 A_2 \cdots A_f \equiv A, \\ A_1 &= 1+x_1 \ (1+y\, A_2\, A_3 \cdots A_f), \\ A_2 &= 1+x_2 \ (1+y\, A_1\, A_3 \cdots A_f), \\ &\cdots\cdots\cdots \\ A_f &= 1+x_f \ (1+y\, A_1\, A_2 \cdots A_{f-1}). \end{aligned} \quad , \ (A_k \geqq 1) \tag{A41}$$

The permutation of $n-1$ balls, except for the origin, and l_k and i_k rods being considered, we have,

$$W_{n,} {\scriptstyle \begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}} = \omega_{n,} {\scriptstyle \begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}} \cdot (n-1)! \cdot 2^{\sum_{k=1}^{f} (l_k+i_k)} \cdot \prod_{k=1}^{f} l_k! i_k! ,$$

and hence,

$$\omega_{n,} {\scriptstyle \begin{Bmatrix} l_1, l_2, \ldots, l_f \\ i_1, i_2, \ldots, i_f \end{Bmatrix}} = n \times \frac{W_{n,} {\scriptstyle \begin{Bmatrix} l_1, l_2, l_3, \ldots, l_f \\ i_1, i_2, i_3, \ldots, i_f \end{Bmatrix}}}{n! \prod_{k=1}^{f} (l_k!\, i_k!) \cdot 2^{\Sigma(l_k+i_k)}} . \tag{A42}$$

From Eqs. 2, 7, A 39, A 42, the following relation is obtained,

$$\sum n \frac{CW_{n,\left\{ l_1, l_2, \dots, l_f \atop i_1, i_2, \dots, i_f \right\}}}{n!\prod_{k=1}^{f}(l_k!\, i_k!)\cdot 2^{\Sigma(l_k+i_k)}}\, x_1^{l_1+i_1} x_2^{l_2+i_2} \cdots x_f^{l_f+i_f}\, y^n = \sum n\, m_{n,\left\{ l_1, l_2, \dots, l_f \atop i_1, i_2, \dots, i_f \right\}}$$

$$= N = C y A_1 A_2 \cdots A_f = C y A. \qquad \text{(A43)}$$

(2) $\sum l_1\, m_{n,\left\{ l_1, l_2, \dots, l_f \atop i_1, i_2, \dots, i_f \right\}} = L_1$

In this case, one of the l_1 rods is picked up as the origin and the directions of the two ends of the origin are fixed, for instance upper and lower. Two balls (first generation (F_1)) are connected to it by each 1-type hole. The rods inserted into the first generation balls are not of the 1-type. Therefore, the number of ways to form trees is,

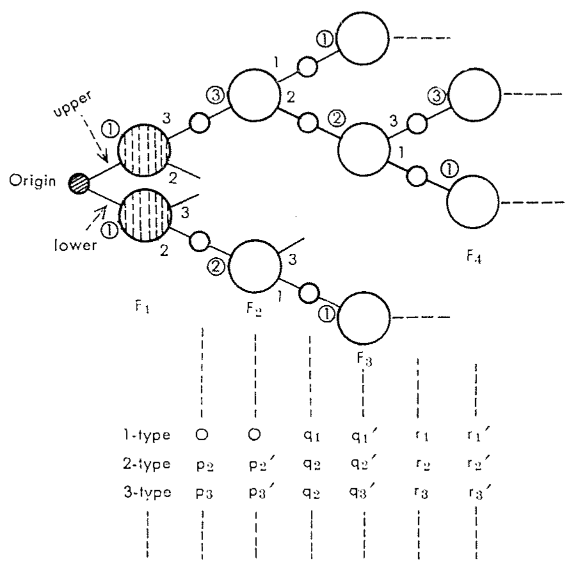

Fig. 7. Schematic presentation of the way to find $\omega'_{n,\left\{ l_1, l_2, \dots, l_f \atop i_1, i_2, \dots, i_f \right\}}$

$$\omega'_{n,\left\{ l_1, l_2, \dots, l_f \atop i_1, i_2, \dots, i_f \right\}} = \sum \binom{2}{p_2}\binom{2}{p_3}\cdots\binom{p_2}{p'_2}\binom{p_3}{p'_3}\cdots\cdots\binom{\sum p_k' - p_1'}{q_1}\binom{\Sigma p_k' - p_2'}{q_2}\cdots\cdot, \qquad \text{(A44)}$$

subject to the relations,

$$\begin{aligned}
&p_1' + q_1' + \dots = l_1 - 1 \quad , (p_1' = 0) \\
&(p_1 + q_1 + \dots) - (p_1' + q_1' + \dots) = i_1 \quad , (p_1 = 0) \\
&p_k' + q_k' + \dots = l_k \qquad , (k = 2, 3, \dots, f) \\
&(p_k + q_k + \dots) - (p_k' + q_k' + \dots) = i_k, \\
&\sum_{k=1}^{f} (p_k' + q_k' + \dots) = n - 2.
\end{aligned} \qquad \text{(A45)}$$

The following function is considered, which is easily summed,

$$\sum \omega_{n}',\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} \cdot x_1^{l_1+i_1-1} x_2^{l_2+i_2} \dots x_f^{l_f+i_f} y^{n-2} = (A_2 A_3 \dots A_f)^2 . \tag{A46}$$

And the following relation is easily seen,

$$\omega_{n}',\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} \cdot n! \cdot (l_1-1)! \cdot i_1! \cdot \prod_{k=2}^{f} l_k! i_k! \cdot 2^{\{\sum_{k=1}^{f} (l_k+i_k)\}-1} = W_{n},\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} ,$$

$$\omega_{n}',\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} = 2l_1 \cdot \frac{W_{n},\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix}}{n! \cdot \prod_{k=1}^{f} l_k! i_k! \cdot 2^{\Sigma(l_k+i_k)}} . \tag{A47}$$

Eq. A 46 becomes

$$\sum 2l_1 \cdot \frac{C \cdot W_{n},\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix}}{n! \cdot \prod_{k=1}^{f} l_k! i_k! \cdot 2^{\sum(l_k+i_k)}} x_1^{l_1+i_1} x_2^{l_2+i_2} \dots x_f^{l_f+i_f} y^{n} = C x_1 y^2 (A_2 A_3 \dots A_f)^2 . \tag{A48}$$

Therefore, we obtain

$$\sum l_1 m = L_1 = \frac{C}{2} x_1 y^2 \left(\frac{A}{A_1}\right)^2 . \tag{A49}$$

Thus, analogously we obtain

$$\sum l_k m = L_k = \frac{C}{2} x_k y^2 \left(\frac{A}{A_k}\right)^2 . \tag{A50}$$

(3) $\sum i_1 m_{n},\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} = I_1$

In this case, one of the i_1 rods hanging from an n-aggregate is picked up as the origin. As the first generation ball, only one ball is connected to one of the two ends of the origin through the 1-type hole in the ball. The rods, which can be inserted into any of the empty holes in the first generation balls (F_1), are not of the 1-type. The number of ways to form trees is

$$\omega_{n}'',\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} = \sum \binom{1}{p_2}\binom{1}{p_3} \dots \binom{p_2}{p_2'}\binom{p_3}{p_3'} \dots \binom{\sum_{k=1}^{f} p_k'}{q_1}\binom{\sum_{k=2}^{f} p_k' - p_2'}{q_2} \dots$$
$$\times \binom{q_1}{q_1'}\binom{q_2}{q_2'} \dots \binom{\Sigma q_k' - q_1'}{r_1}\binom{\Sigma q_k' - q_2'}{r_2} \dots , \tag{A51}$$

subject to the relations,

$$\begin{aligned} & q_1'+r_1'+ \dots = l_1 , \quad (p_1'=0) \\ & (q_1+r_1+ \dots) - (q_1'+r_1'+ \dots) = i_1 - 1, \\ & \left.\begin{aligned} & p_k'+q_k'+r_k' + \dots = l_k \\ & (p_k+q_k+r_k+ \dots) - (p_k'+q_k'+r_k'+ \dots) = i_k \end{aligned}\right\} , \quad (k = 2, 3, \dots, f) \\ & p_2'+p_3' + \dots + (q_k'+r_k'+ \dots) = n-1. \end{aligned} \tag{A52}$$

The following function is easily summed,

$$\sum \omega_{n}'',\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} \cdot x_1^{l_1+i_1-1} x_2^{l_2+i_2} \dots x_f^{l_f+i_f} y^{n-1} = A_2 A_3 \dots A_f . \tag{A53}$$

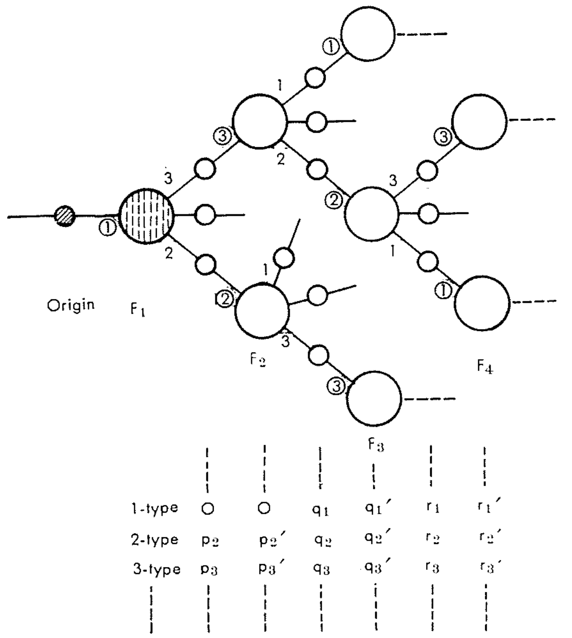

Fig. 8. Schematic presentation of the way to find $\omega_n'',\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix}$

As before, we have,

$$\omega_n'',\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} \cdot n! l_1 (!i_1 - 1)! \prod_{k=2}^{f} (l_k!\, i_k!) \cdot 2^{\{\sum_{k=1}^{f} (l_k + i_k)\} - 1} \times 2 = W_n,\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix}$$

$$\omega_n'',\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix} = i_1 \frac{W_n,\begin{Bmatrix} l_1, l_2, \dots, l_f \\ i_1, i_2, \dots, i_f \end{Bmatrix}}{n! \cdot \prod_{k=1}^{f} (l_k!\, i_k!) \cdot 2^{\Sigma(l_k + i_k)}} . \tag{A54}$$

From Eqs. A 53 and A 54, we get,

$$C \cdot \sum \omega'' \cdot x_1^{l_1+i_1} x_2^{l_2+i_2} \dots x_f^{l_f+i_f} y^n = C x_1 y A_2 A_3 \dots A_f$$

$$= \sum i_1 m = I_1 = C x_1 y \frac{A}{A_1} . \tag{A55}$$

Hence similarly the following solution is given for I_k ($k = 1, 2, \dots, f$),

$$I_k = C x_k y \frac{A}{A_k} . \tag{A56}$$

(4) The value of N_k.

From Eq. 2, N_k is given as,

$$N_k = L_k + I_k + m'_{0,k} = \frac{C}{2} x_k \left\{ \left(\frac{yA}{A_k} \right)^2 + 2 \frac{yA}{A_k} + 1 \right\} = \frac{C}{2} \left(A_k - 1 \right) \left(\frac{A_k + yA}{A_k} \right) . \tag{A57}$$

(5) The summation of $\Sigma m_{n,\{l_1, l_2, \ldots, l_f\} \atop \{i_1, i_2, \ldots, i_f\}} + \Sigma m'_{0,k} = M$

Although M has already been obtained in Eq. 13, we can find an expression for M in a different fashion. Eqs. 2 and 3 are written here, where $n_k = l_k + i_k$ $(k=1,2,\ldots,f)$,

$$N = \sum n \frac{CW}{n! \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\Sigma n_k}} x_1^{n_1} x_2^{n_2} \ldots x_f^{n_f} y^n ,$$

$$N_k = \sum n_k \frac{CW}{n! \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\Sigma n_k}} x_1^{n_1} x_2^{n_2} \ldots x_f^{n_f} y^n + \frac{Cx_k}{2} ,$$

$$M = \sum \frac{CW}{n! \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\Sigma n_k}} x_1^{n_1} x_2^{n_2} \ldots x_f^{n_f} y^n + \sum_{k=1}^{f} \frac{Cx_k}{2} .$$

From these relations, it is obvious that M satisfies the following $f+1$ partial differential equations,

$$y \frac{\partial}{\partial y} M = N,$$

$$x_k \frac{\partial}{\partial x_k} M = N_k, \quad (k=1, 2, \ldots, f). \tag{A58}$$

As the values of N and N_k have already been obtained in Eqs. A 43 and A 57 as functions of $C, y, A_1, A_2, \ldots, A_f$, the differential operators, $y\frac{\partial}{\partial y}$ and $x_k \frac{\partial}{\partial x_k}$, can be transformed into those on $(A_1, A_2, \ldots, A_f, Y)$-space (cf. Eq. A 41).

$$x_k = \frac{A_k - 1}{1 + YA_1A_2 \ldots \underline{\boldsymbol{k}} \ldots A_f} , \; y = Y, \; (\underline{\boldsymbol{k}} \text{ means the lack of } A_k), \tag{A59}$$

$$y \frac{\partial}{\partial y} = Y \frac{\partial}{\partial Y} + Y \sum_{k=1}^{f} \frac{\partial A_k}{\partial y} \frac{\partial}{\partial A_k} ,$$

$$x_i \frac{\partial}{\partial x_i} = x_i \left(\frac{\partial Y}{\partial x_i} \frac{\partial}{\partial Y} + \sum_{k=1}^{f} \frac{\partial A_k}{\partial x_i} \frac{\partial}{\partial A_k} \right) . \tag{A60}$$

The differential operator, $\frac{\partial}{\partial y}$, which is the partial differentiation by y when $x_1, x_2, \ldots, x_f$ are fixed, must be distinguished from $\frac{\partial}{\partial Y}$, which means the partial differentiation by y when $A_1, A_2, \ldots, A_f$ are fixed. From this reason y is expressed by Y after transformations of $x_k \to A_k$ $(k=1, 2, \ldots, f)$ are performed. The change of $y \to Y$ does not mean changes in the values of A_k $(k=1, 2, \ldots, f)$ from those in Eq. A 41. The differential coefficients, $\frac{\partial A_k}{\partial y}$ and $\frac{\partial A_k}{\partial x_i}$, must be obtained from Eq. A 60. As $\frac{\partial x_i}{\partial A_k}$, $\frac{\partial x_i}{\partial Y}$ and others can easily be obtained from Eq. A 59, $\frac{\partial A_k}{\partial y}$ and $\frac{\partial A_k}{\partial x_i}$ are obtained by evaluating the inverse matrix of the matrix, $(J) = \frac{\partial(x_1, x_2, \ldots, x_f, y)}{\partial(A_1, A_2, \ldots, A_f, Y)}$. From Eq. A 59, the following relations are obtained,

$$\frac{\partial x_i}{\partial A_i} = \frac{1}{1 + YA_1 \ldots \underline{\boldsymbol{i}} \ldots A_f} = \frac{A_i}{A_i + YA} ,$$

$$\frac{\partial x_i}{\partial A_k} = -\frac{(A_i - 1)YA_1 \ldots \underline{\boldsymbol{i}} \ldots \underline{\boldsymbol{k}} \ldots A_f}{(1 + YA_1 \ldots \underline{\boldsymbol{i}} \ldots A_f)^2} = \frac{-A_i\,(A_i - 1)\,YA}{A_k(A_i + YA)^2} , \quad (i \neq k)$$

$$\frac{\partial x_i}{\partial Y} = -\frac{(A_i-1)A_1 \ldots \underline{i} \ldots A_f}{(1+YA_1 \ldots \underline{i} \ldots A_f)^2} = \frac{-A_i(A_i-1)YA}{Y(A_i+YA)^2}\,. \tag{A61}$$

Eq. A 61 yields

$$(J) = \begin{pmatrix} \frac{\partial x_1}{\partial A_1} & \frac{\partial x_1}{\partial A_2} & \frac{\partial x_1}{\partial A_3} & \cdots & \frac{\partial x_1}{\partial A_f} \\ \frac{\partial x_2}{\partial A_1} & \frac{\partial x_2}{\partial A_2} & \frac{\partial x_2}{\partial A_3} & \cdots & \frac{\partial x_2}{\partial A_f} \\ \frac{\partial x_3}{\partial A_1} & \frac{\partial x_3}{\partial A_2} & \frac{\partial x_3}{\partial A_3} & \cdots & \frac{\partial x_3}{\partial A_f} \\ \vdots & \vdots & \vdots & & \vdots \\ \frac{\partial x_f}{\partial A_1} & \frac{\partial x_f}{\partial A_2} & \frac{\partial x_f}{\partial A_3} & \cdots & \frac{\partial x_f}{\partial A_f} \end{pmatrix}$$

$$= \begin{pmatrix} \frac{A_1}{A_1+YA} & -\frac{A_1(A_1-1)YA}{A_2(A_1+YA)^2} & -\frac{A_1(A_1-1)YA}{A_3(A_1+YA)^2} & \cdots & -\frac{A_1(A_1-1)YA}{A_f(A_1+YA)^2} \\ -\frac{A_2(A_2-1)YA}{A_1(A_2+YA)^2} & \frac{A_2}{A_2+YA} & -\frac{A_2(A_2-1)YA}{A_3(A_2+YA)^2} & \cdots & -\frac{A_2(A_2-1)YA}{A_f(A_2+YA)^2} \\ -\frac{A_3(A_3-1)YA}{A_1(A_3+YA)^2} & -\frac{A_3(A_3-1)YA}{A_2(A_3+YA)^2} & \frac{A_3}{A_3+YA} & \cdots & -\frac{A_3(A_3-1)YA}{A_f(A_3+YA)^2} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \cdots & 1 \end{pmatrix}$$

$$= (-1) \times \begin{pmatrix} \frac{A_1(A_1-1)YA}{(A_1+YA)^2} & & \mathbf{0} \\ & \frac{A_2(A_2-1)YA}{(A_2+YA)^2} & \\ \mathbf{0} & & \ddots \; Y \end{pmatrix} \begin{pmatrix} \frac{-A_1(A_1+YA)}{(A_1-1)YA} & 1 & \cdots & 1 \\ 1 & \frac{-A_2(A_2+YA)}{(A_2-1)YA} & \cdots & 1 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & -1 \end{pmatrix}$$

$$\times \begin{pmatrix} \frac{1}{A_1} & & \mathbf{0} \\ & \frac{1}{A_2} & \\ \mathbf{0} & & \ddots \; \frac{1}{Y} \end{pmatrix} = (-1) \times \begin{pmatrix} \frac{A_1(A_1-1)YA}{(A_1+YA)^2} & & \mathbf{0} \\ & \frac{A_2(A_2-1)YA}{(A_2+YA)^2} & \\ \mathbf{0} & & \ddots \; Y \end{pmatrix} \begin{pmatrix} 1+a_1 & 1 & \cdots & 1 \\ 1 & 1+a_2 & \cdots & 1 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & -1 \end{pmatrix}$$

$$\times \begin{pmatrix} \frac{1}{A_1} & & \mathbf{0} \\ & \frac{1}{A_2} & \\ \mathbf{0} & & \ddots \; \frac{1}{Y} \end{pmatrix}, \quad \left(a_k = \frac{A_k^2+(2A_k-1)YA}{(A_k-1)YA} \right). \tag{A62}$$

From Eq. A 62, we obtain,

$$
(J)^{-1}=(-1)\times\begin{pmatrix} A_1 & & & 0 \\ & A_2 & & \\ & & \ddots & \\ 0 & & & Y \end{pmatrix}
\begin{pmatrix}
\dfrac{1}{a_1}\cdot\dfrac{1+\sum\limits_{k\neq 1}\dfrac{1}{a_k}}{1+\sum\dfrac{1}{a_k}}, & -\dfrac{1}{a_1}\cdot\dfrac{\dfrac{1}{a_2}}{1+\sum\dfrac{1}{a_k}}, & -\dfrac{1}{a_1}\cdot\dfrac{\dfrac{1}{a_3}}{1+\sum\dfrac{1}{a_k}}, & \dots, & \dfrac{1}{a_1}\cdot\dfrac{1}{1+\sum\dfrac{1}{a_k}} \\
\dfrac{-1}{a_1}\cdot\dfrac{\dfrac{1}{a_1}}{1+\sum\dfrac{1}{a_k}}, & -\dfrac{1}{a_2}\cdot\dfrac{1+\sum\limits_{k\neq 2}\dfrac{1}{a_k}}{1+\sum\dfrac{1}{a_k}}, & -\dfrac{1}{a_2}\cdot\dfrac{\dfrac{1}{a_3}}{1+\sum\dfrac{1}{a_k}}, & \dots, & \dfrac{1}{a_2}\cdot\dfrac{1}{1+\sum\dfrac{1}{a_k}} \\
\vdots & \vdots & \vdots & \ddots & \vdots \\
0 & 0 & 0 & \cdots & -1
\end{pmatrix}
$$

$$
\times\begin{pmatrix} \dfrac{(A_1+YA)^2}{A_1(A_1-1)YA} & & & 0 \\ & \dfrac{(A_2+YA)^2}{A_2(A_2-1)YA} & & \\ & & \ddots & \\ 0 & & & \dfrac{1}{Y} \end{pmatrix} \equiv (b_{ij}),\ (i,j=1,2,\dots,f+1). \tag{A63}
$$

Since Eq. A 63 can be rewritten as

$$
(J)^{-1}=\frac{\partial(A_1, A_2, \dots, A_f, Y)}{\partial(x_1, x_2, \dots, x_f, y)}=(b_{ij}),
$$

we have

$$
\begin{aligned}
\frac{\partial A_i}{\partial x_i} &= b_{ii}=(-1)A_i\cdot\frac{1}{a_i}\cdot\frac{1+\sum\limits_{k\neq i}\dfrac{1}{a_k}}{1+\sum\dfrac{1}{a_k}}\cdot\frac{(A_i+YA)^2}{A_i(A_i-1)YA} \\
&= \frac{(A_i+YA)^2}{A_i^2+(2A_i-1)YA}\cdot\frac{1-\Sigma'}{1-\Sigma}, \\
\frac{\partial A_i}{\partial x_j} &= b_{ij}=(-1)A_i\cdot\left(-\frac{1}{a_i}\cdot\frac{\dfrac{1}{a_j}}{1+\sum\dfrac{1}{a_k}}\right)\cdot\frac{(A_j+YA)^2}{A_j(A_j-1)YA} \\
&= \frac{A_i(A_i-1)(A_j+YA)^2YA}{A_j\{A_i^2+(2A_i-1)YA\}\{A_j^2+(2A_j-1)YA\}}\cdot\frac{1}{1-\Sigma}, \qquad \text{(A64)} \\
\frac{\partial A_i}{\partial y} &= b_{i,f+1}=(-1)A_i\cdot\frac{1}{a_i}\cdot\frac{1}{1+\sum\dfrac{1}{a_k}}\cdot\frac{1}{Y} \\
&= \frac{A_i(A_i-1)A}{A_i^2+(2A_i-1)YA}\cdot\frac{1}{1-\Sigma},
\end{aligned}
$$

where $1-\Sigma=1-\sum\limits_{k=1}^{f}\dfrac{(A_k-1)YA}{A_k^2+(2A_k-1)YA}$ and $1-\Sigma'=1-\sum\limits_{\substack{k=1\\k\neq i}}^{f}.$

Applying the results of Eq. A 64 to Eq. A 60, we obtain

$$y\frac{\partial}{\partial y}=Y\frac{\partial}{\partial Y}+Y\cdot\frac{A}{1-\Sigma}\sum\frac{A_k(A_k-1)}{A_k^2+(2A_k-1)YA}\cdot\frac{\partial}{\partial A_k},$$

$$x_i\frac{\partial}{\partial x_i}=x_i\cdot\frac{(A_i+YA)^2}{A_i^2+(2A_i-1)YA}\frac{\partial}{\partial A_i}+x_i\sum_{k=1}^{f}\frac{A_k(A_k-1)\ (A_i+YA)^2YA}{A_i\{A_k^2+(2A_k-1)YA\}\{A_i^2+(2A_i-1)YA\}}\cdot\frac{1}{1-\Sigma}$$

$$\times\frac{\partial}{\partial A_k}=x_i\cdot\frac{(A_i+YA)^2}{A_i^2+(2A_i-1)YA}\cdot\frac{\partial}{\partial A_i}+x_i\cdot\frac{(A_i+YA)^2Y}{A_i\{A_i^2+(2A_i-1)YA\}}\sum_{k=1}^{f}\cdot\frac{\partial A_k}{\partial y}\cdot\frac{\partial}{\partial A_k}. \qquad \text{(A65)}$$

From Eqs. A 58 and A 59, the particular solution, $G(A_1, A_2, \ldots, A_f, Y)$, of M satisfying $y\frac{\partial M}{\partial y}=N$ is sought. For this purpose, the following differentiations are obtained by applying Eqs. A 60 and A 65,

$$y\frac{\partial}{\partial y}(YA)=Y\frac{\partial}{\partial Y}(AY)+Y\cdot\frac{A}{1-\Sigma}\sum_{k=1}^{f}\frac{A_k(A_k-1)}{A_k^2+(2A_k-1)YA}\cdot\frac{\partial}{\partial A_k}(YA)$$

$$=YA+\frac{YA}{1-\Sigma}\sum_{k=1}^{f}\frac{(A_k-1)}{A_k^2+(2A_k-1)YA}\cdot YA=YA+\frac{YA}{1-\Sigma}\Sigma$$

$$=\frac{YA}{1-\Sigma},$$

$$y\frac{\partial}{\partial y}\left(\frac{YA}{A_i}\right)=\frac{YA}{A_i}+\frac{YA}{1-\Sigma}\sum_{k\neq i}^{f}\frac{A_k-1}{A_k^2+(2A_k-1)YA}\cdot\frac{YA}{A_i}$$

$$=\frac{YA}{1-\Sigma}\cdot\frac{1}{A_i}\left\{1-\Sigma+\Sigma-\frac{(A_i-1)YA}{A_i^2+(2A_i-1)YA}\right\}$$

$$=\frac{YA}{1-\Sigma}\cdot\frac{A_i+YA}{A_i^2+(2A_i-1)YA}, \qquad \text{(A66)}$$

$$y\frac{\partial}{\partial y}\left(\sum_{i=1}^{f}\frac{YA}{A_i}\right)=\sum_{i=1}^{f}y\frac{\partial}{\partial y}\left(\frac{YA}{A_i}\right)=\frac{YA}{1-\Sigma}\sum_{i=1}^{f}\frac{Ai+YA}{A_i^2+(2A_i-1)YA},$$

$$y\frac{\partial}{\partial y}(A_i)=\frac{YA}{1-\Sigma}\cdot\frac{A_i(A_i-1)}{A_i^2+(2A_i-1)YA},$$

$$y\frac{\partial}{\partial y}\left(\sum_{i=1}^{f}A_i\right)=\frac{YA}{1-\Sigma}\cdot\sum_{i=1}^{f}\frac{A_i(A_i-1)}{A_i^2+(2A_i-1)YA}.$$

From these we have the particular solution;

$$G=C\left\{AY\left(1-\frac{f}{2}\right)+\frac{1}{2}\sum_{i=1}^{f}\frac{YA}{A_i}+\frac{1}{2}\sum_{i=1}^{f}A_i\right\}. \qquad \text{(A67)}$$

The results of Eq. A 66 being applied to the equation;

$$y\frac{\partial}{\partial y}G=C\left\{\left(1-\frac{f}{2}\right)y\frac{\partial}{\partial y}(YA)+\frac{1}{2}y\frac{\partial}{\partial y}\left(\sum_{i=1}^{f}\frac{YA}{Ai}\right)+\frac{1}{2}y\frac{\partial}{\partial y}\left(\sum_{i=1}^{f}A_i\right)\right\}, \qquad \text{(A68)}$$

following result is derived,

$$y\frac{\partial}{\partial y}G=CYA=N. \qquad \text{(A69)}$$

Therefore, we obtain

$$M=G+H, \qquad \text{(A70)}$$

where H is an arbitrary function of $x_1, x_2, \ldots, x_f$.
The second equation of Eq. A 58 is now considered in relation to Eq. A 70,

$$x_i \frac{\partial}{\partial x_i} M = x_i \frac{\partial}{\partial x_i} G + x_i \frac{\partial}{\partial x_i} H. \tag{A71}$$

The first term of Eq. A 71 is calculated in applying Eq. A 65,

$$x_i \frac{\partial}{\partial x_i} G = x_i \cdot \frac{(A_i + YA)^2}{A_i^2 + (2A_i - 1) YA} \frac{\partial}{\partial A_i} G + x_i \cdot \frac{(A_i + YA)^2 Y}{A_i\{A_i^2 + (2A_i - 1) YA\}} \times \sum_{k=1}^{f} \frac{\partial A_k}{\partial y} \cdot \frac{\partial}{\partial A_k} G. \tag{A72}$$

From Eq. A 60, we have

$$\sum_{k=1}^{f} \frac{\partial A_k}{\partial y} \cdot \frac{\partial}{\partial A_k} = \frac{y \frac{\partial}{\partial y} - Y \frac{\partial}{\partial Y}}{Y}. \tag{A73}$$

Eq. A 73 is applied to Eq. A 72,

$$x_i \frac{\partial}{\partial x_i} G = x_i \cdot \frac{(A_i + YA)^2}{A_i^2 + (2A_i - 1) YA} \cdot \frac{\partial}{\partial A_i} G + x_i \frac{(A_i + YA)^2}{A_i\{A_i^2 + (2A_i - 1) YA\}} \times \left\{ y \frac{\partial}{\partial y} G - Y \frac{\partial}{\partial Y} G \right\}. \tag{A74}$$

When the result of Eq. A 69 is applied to Eq. A 74, we obtain (cf. Eqs. 10 and A 57)

$$\begin{aligned} x_i \frac{\partial}{\partial x_i} G &= x_i \cdot \frac{(A_i + YA)^2}{A_i\{A_i^2 + (2A_i - 1) YA\}} \left\{ A_i \frac{\partial}{\partial A_i} G + CYA - Y \frac{\partial}{\partial Y} G \right\} \\ &= x_i \cdot \frac{(A_i + YA)^2 C}{A_i\{A_i^2 + (2A_i - 1) YA\}} \left\{ YA\left(1 - \frac{f}{2}\right) + \frac{1}{2} \sum_{i=1}^{f} \frac{YA}{A_i} - \frac{1}{2} \frac{YA}{A_i} + \frac{1}{2} A_i + YA \right. \\ &\qquad \left. - YA\left(1 - \frac{f}{2}\right) - \frac{1}{2} \sum_{i=1}^{f} \frac{YA}{A_i} \right\} \\ &= \frac{C}{2} x_i \cdot \left(\frac{A_i + YA}{A_i} \right)^2 = N_i. \end{aligned} \tag{A75}$$

However, $x_i \frac{\partial}{\partial x_i} M = N_i$ has already been obtained. Thus, $x_i \frac{\partial}{\partial x_i} H$ must be zero. This means H is not a function of $x_1, x_2, \ldots, x_f$ but a constant. Eq. A 70 can be rewritten as

$$\begin{aligned} M &= G + H \\ &= C \left\{ YA\left(1 - \frac{f}{2}\right) + \frac{1}{2} \sum_{i=1}^{f} \frac{YA}{A_i} + \frac{1}{2} \sum_{i=1}^{f} A_i \right\} + H. \end{aligned} \tag{A76}$$

On the other hand, M is expressed by Eqs. 3, 7 and 8 as

$$M = \sum \frac{CW}{n! \cdot \prod_{k=1}^{f} l_k!\, i_k! \cdot 2^{\sum n_k}} x_1^{n_1} x_2^{n_2} \ldots x_f^{n_f} y^n + \sum_{k=1}^{f} \frac{C x_k}{2}.$$

In this equation, when $x_1 = x_2 = \ldots = x_f = y = 0$ (*i. e.* $A_1 = A_2 = \ldots = A_f = 1$), M must be zero. When these value are put into Eq. A 76, we obtain

$$H = -\frac{Cf}{2}.$$

Therefore, Eq. A 77 becomes identical with Eq. 13,

$$M=C\left\{YA\left(1-\frac{1}{2}\sum_{k=1}^{f}\frac{A_k-1}{A_k}\right)+\frac{1}{2}\sum_{k=1}^{f}(A_k-1)\right\}. \tag{A77}$$

REFERENCES

Flory, P.J. (1936). Molecular size distribution in linear condensation polymers. *J. Am. Chem. Soc.* **58,** 1877-1885.

Flory, P.J. (1941a). Molecular size distribution in three dimensional polymers. I. Gelation. *J. Am. Chem. Soc.* **63,** 3083-3090.

Flory, P.J. (1941b). Molecular size distribution in three dimensional polymers. II. Trifunctional branching units. *J. Am. Chem. Soc.* **63,** 3091-3096.

Flory, P.J. (1941c). Molecular size distribution in three dimensional polymers. III. Tetrafunctional branching units. *J. Am. Chem. Soc.* **63,** 3096-3100.

Fujio, H., Kishiguchi, S., Noma, Y., Shinka, S., Saiki, Y. and Amano T. (1958). The antigenic relationship between the T-component of diphtheria antitoxic antiserum and antitoxic globulin modified with protease. *Biken's J.* **1,** 138-156.

Fujio, H., Kishiguchi, S., Shinka, S., Saiki, Y. and Amano, T. (1959). Immunochemical studies on lysozyme. I. Comparative studies of lysozyme and lysozyme methyl ester. *Biken's J.* **2,** 55-76.

Fujio, H., Saiki, Y., Imanishi, M., Shinka, S. and Amano, T. (1962). Immunochemical studies on lysozyme. III. Cross reactions of hen and duck lysozymes and their methyl esters. *Biken J.* **5,** 201-226.

Goldberg, R.J. (1952). A theory of antibody-antigen reactions. I. Theory for reactions of multivalent antigen with bivalent and univalent antibody. *J. Am. Chem. Soc.* **74,** 5715-5725.

Goldberg, R.J. (1953). A theory of antibody-antigen reactions. II. Theory for reactions of multivalent antigen with multivalent antibody. *J. Am. Chem. Soc.* **75,** 3127-3131.

Heidelberger, M. and Kendall, F.E. (1935). The precipitin reaction between type III penumococcus polysaccharide and homologous antibody. III. A quantitative study and a theory of the reaction mechanism. *J. Exptl. Med.* **61,** 563-591.

Lapresle, C. (1955). Étude de la dégradation de la sérum albumine humaine par éxtrait de rate de lapin. II. Mise en évidence de trois groupements spécifiques differents dans le motif antigénique de l'albumine humaine et de trois anticorps correspondants dens le sérum de lapin antialbumine humaine. *Ann. Inst. Pasteur* **89,** 654-655.

Lapresle, C. and Durieux, J. (1957a). Étude de la dégradation de la sérum albumine humaine par un éxtrait de rate de lapin. III. Modifications immunologiques de l'albumine en fonction du stade dégradation. *Ann. Inst. Pasteur* **92,** 62-73.

Lapresle, C. and Durieux, J. (1957b). Étude de la dégradation de la sérum albumine humaine par un éxtrait de rate de lapin. IV. Variations individuelles de la reaction de sérum de lapin antialbumine avec l'albumine dégradée. *Bull. Soc. Chim. Biol.* **39,** 833-841.

Lapresle, C. and Durieux, J. (1958). Étude de la dégradation de la sérum albumine humaine par un éxtrait de rate de lapin. V. Antigenicité de l'albumine dégradée. *Ann. Inst. Pasteur* **94,** 38-48.

Palmiter, M. T. and Aladjem, F. (1962). The antigen-antibody reaction. V. A quantitative theory of antigen-antibody reactions. in press.

Pappenheimer, A. M. Jr., Lundgren, H.P. and Williams, J.W. (1940). Studies on the molecular weight of diphtheria toxin, antitoxin, and their reaction products. *J. Exptl. Med.* **71,** 247-262.

Raynaud, M. (1958). Heterogeneity of diphtheria antitoxin. In Mechanisms of hypersensitivity, p.19. Little Brown & Co.

Relyveld, E.H. (1959). Toxine et antitoxine diphtheriques. Hermann, Paris.

Shinka, S., Imanishi, M., Kuwahara, O., Fujio, H. and Amano, T. (1962). Immunochemical studies on lysozyme. II. On the non-neutralizing antibodies. *Biken J.* **5,** 181-200.
Stockmayer, W.H. (1943). Theory of molecular size distribution and gel formation in branched-chain polymers. *J. Chem. Phys.* **11,** 45-55.