ters are dark-yellow or brown viscous oils. The methallyl and allyl esters display little tendency to polymerize.

The vinyl esters have been copolymerized with vinyl acetate and also with styrene, the 2-chloroallyl esters with vinyl acetate only and the allyl esters with diallyl phthalate. The wide range of properties attainable in the copolymers suggests numerous potential uses for the products.

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Equilibrium Equations for a Model of Antibody–Antigen Combination

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Application of statistical procedures to models of antibody–antigen combination yields expressions involving certain thermodynamic constants of the equilibrium; such expressions enable the calculation of these constants from conventional measurements, and may therefore be of interest. We wish to present here certain relations of this sort based on a useful model proposed recently by Teorell.[1,2,3]

In Teorell's model, antibody (A) is assumed univalent,[4] and antigen (G) is assumed n-valent; the aggregate compounds have therefore the formulas, A_iG, and the equilibrium is formally similar to that of ampholyte dissociation[5]

$$\left.\begin{aligned} &A + A_{i-1}G \rightleftarrows A_iG \\ &K_i = [A_iG]/[A][A_{i-1}G] \end{aligned}\right\} \quad i = 1, 2, \ldots, n \qquad (1)$$

We shall assume in what follows that the total concentrations of antibody and antigen, A_0 and G_0, respectively, are known experimentally. Deferring until later a discussion of the matter, we shall also suppose that the concentration ratio of total bound antibody to total bound antigen in the initial solution

$$R = \sum_{i=1}^{i=n} i[A_iG] / \sum_{i=1}^{i=n} [A_iG]$$

is measurable (actually this ratio is measurable only in the precipitate which subsequently forms). Clearly, $\lim_{A_0 \to \infty} R = n$, the antigen valence. In certain cases it will be further required to know the amounts of bound A and G. Various assumptions regarding the aggregation will now be considered separately.

I. The reactivity of a vacant reactive site on the surface of an A–G aggregate is assumed to be completely independent of the remainder of the structure on which it exists. In this case it may be shown that

$$R = \frac{K_1[A](1 + K_1[A]/n)^{n-1}}{(1 + K_1[A]/n)^n - 1} \qquad (2)$$

If A_0 and G_0 be given special values, A_0' and G_0' such that [A] becomes equal to n/K_1, then R takes on a special value, $R' = (n/2)/(1 - 1/2^n)$, which is very nearly $n/2$ for $n \geqq 4$. Conversely, one may vary A_0 and G_0 experimentally until R becomes, say, $n/2$; at that point [A] equals n/K_1, and one may also show that on this account

$$K_1 = n/(A_0' - R'G_0') \qquad (3)$$

K_1 is thus obtainable from the usual concentration measurements. All other equilibrium constants are derivable from K_1 by means of the formula

$$K_i = (K_1/n)(n - i + 1)/i \qquad (4)$$

II. The antibody molecules on the surface of the same antigen are assumed to attract or repel one another. It is assumed (rather reasonably) that these interactions can be represented as an A–A bond energy, E_{AA}, and that they operate only between nearest neighbor molecules. To treat this case one must make specific assumptions about the surface lattice formed by the reactive sites on the antigen. We shall here consider three such lattices, corresponding to the contact points on any sphere[6] in the (a) hexagonal closest packing of spheres, (b) cubic closest packing of spheres, and (c) simple cubic packing of spheres. In this case we have

$$R = \frac{\sum_{i=1}^{i=n} \sum_{p} i(K_1[A]/n)^i W_i^{(p)} \exp(-pE_{AA}/kT)}{\sum_{i=1}^{i=n} \sum_{p} (K_1[A]/n)^i W_i^{(p)} \exp(-pE_{AA}/kT)} \qquad (5)$$

where, for a given lattice, $W_i^{(p)}$ is the number of microscopically different ways in which i antibody molecules may be placed on an antigen molecule in such a manner that among the antibody molecules there will be p nearest neighbor pairs. The calculation of the $W_i^{(p)}$ is considered elsewhere.[7]

(1) Teorell, *Nature*, **151**, 696 (1943).

(2) Teorell, *J. Hyg.*, **44**, 227 (1946).

(3) Teorell, *ibid.*, **44**, 237 (1946).

(4) The unsettled rivalry between this model, which goes back to the concepts of Bordet, and the framework model proposed independently by Marrack and Heidelberger and later greatly elaborated by Pauling (THIS JOURNAL, **62**, 2643 (1940)) is acknowledged. The same methods here used, however, appear applicable to the latter case, although with more difficulty.

(5) Analogs to our cases I and II below have been given for ampholyte dissociation by Kirkwood in Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, pp. 290–294. The specific model treated, however, is quite different.

(6) Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1940.

(7) Morales and Botts, *J. Chem. Phys.*, **16**, 587 (1948).

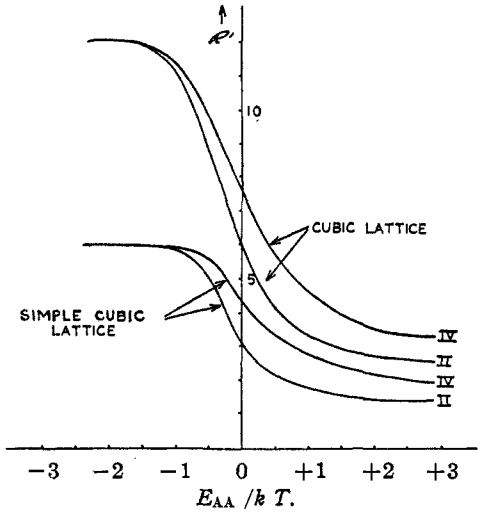

Fig. 1.—Critical value of R as a function of E_{AA}/kT, for two different lattices and for two degrees of approximation (cases II and IV of the text).

Suffice to say here that the value of R, *viz.*, R', at which $K_1 = n/(A_0' - R'G_0')$ is now no longer $n/2$, but depends also upon E_{AA}. Once E_{AA} is known, as from colligative property measurements on concentrated solutions of pure antibody, then R' is calculable (see, for example, Fig. 1, curves II). The relation between the K_i and K_1 is given by

$$K_i = \frac{K_1 \sum_p W_i^{(p)} \exp(-pE_{AA}/kT)}{n \sum_p W_{i-1}^{(p)} \exp(-pE_{AA}/kT)} \quad (6)$$

III. A and G are assumed to be spherical molecules of approximately the same mass and radius, and the moment of inertia of A_iG is assumed to be the same as that of an equivalent sphere having the aggregate mass and volume of $i + 1$ molecules. The inclusion now demanded of the translational and rotational effects upon the equilibrium can only be done very approximately, employing gas-type partition functions, but the results have some comparative value over those derived in section I. One finds that

$$R = \frac{Q(Q+1)^4(1 + K_1[A]/16n)^n}{(Q+1)^4(1 + K_1[A]/16n)^n - 1} \quad (7)$$

where Q^k stands for the operation, $\partial/\partial \log[A]$. In this case $K_1 = 16n/[A]$ when R takes on a definite value $R'(n)$ readily calculable from (7). However, it will in general be required to measure [A] at this point (as distinct from simply knowing A_0). The formula for K_i is

$$K_i = (K_1/16n)[(i+1)/i]^4(n - i + 1)/i \quad (8)$$

IV. The conditions in II and III above are to be combined. For this case

$$R' = \frac{\sum_{i=1}^{i=n} i(i+1)^4 \sum_p W_i^{(p)} \exp(-pE_{AA}/kT)}{\sum_{i=1}^{i=n} (i+1)^4 \sum_p W_i^{(p)} \exp(-pE_{AA}/kT)} \quad (9)$$

the value of R when $K_1 = 16n/[A]$, can be plotted for a given lattice (Fig. 1, curves IV) as a function of E_{AA}. It is, as in Case III, required to know [A] experimentally. For K_i we have

$$K_i = (K_1/16n)[(i+1)/i]^4 \frac{\sum_p W_i^{(p)} \exp(-pE_{AA}/kT)}{\sum_p W_{i-1}^{(p)} \exp(-pE_{AA}/kT)} \quad (10)$$

In the foregoing cases, I–IV, we have considered varying degrees of approximation to the description of the equilibrium (1) presumably in the *solution* phase; however, we have already indicated that total amounts, ratios, etc., are actually measured in the precipitate which appears upon completion of the "secondary reaction" or flocculation, this secondary reaction customarily being aided by the application of a centrifugal field. It is now necessary to discuss the relation between the solution composition (as described by the equations above) and the precipitate composition. The simplest hypothesis of all, and the one with which we will content ourselves for the present, is to assume (as have Teorell and others) that $[A_iG]_{sol'n.} = [A_iG]_{ppt.}$ Rash as this may sound, it is nevertheless possible to imagine situations in which it may be approximately true. On the basis of a monovalent antibody the qualitative reason for the insolubility of the A-G aggregates (compared to A and G) is ascribed to the occlusion[8] by A-G bond formation of sites which ordinarily would interact strongly with the solvent, and confer an appreciable solubility on the molecule. If we suppose that the "primary" reaction (1) reaches equilibrium and then a precipitating condition (*e. g.*, centrifugation or flocculation by quite a different mechanism) is *suddenly* applied, one would obtain

$$R_{ppt} = \frac{\sum_i i\{[A_iG]_{sol'n.} - [A_iG]_{sat.}\}}{\sum_i \{[A_iG]_{sol'n.} - [A_iG]_{sat.}\}} \quad (11)$$

where $[A_iG]_{sat.}$ is the molar solubility of the A_iG aggregate in the presence of the suddenly applied condition. If, in particular, all $[A_iG]_{sat.}$ are near zero,[9] then we are left with the R dealt with in cases I–IV. Quite a different justification for the assumption $[A_iG]_{sol'n.} = [A_iG]_{ppt.}$ has been suggested by Teorell,[2] namely, that molecules of the size and nature (number of reactive sites) involved here may, as it were, be "precipitated in part" or, more specifically, that sites on the structure which have not reacted may participate in the solution equilibrium; the fact that certain enzymes (*e. g.*, beef catalase) when acting as antigens can catalyze their specific reaction even after having been pre-

(8) Boyd, "Fundamentals of Immunology," Interscience Publishers, New York, N. Y., 1947.

(9) This restriction may be lightened by developing approximate expressions for $[A_iG]_{sat.}$ based on Meyer's solubility equation (see Mark, "The Physical Chemistry of High Polymeric Systems," Interscience Publishers, New York, N. Y., 1940, p. 249).

cipitated in A-G form is cited by him as indicating the plausibility of the phenomenon. It is certainly true that neither of the two foregoing arguments is thoroughly convincing, and the assumption must be regarded as provisional, even though the experimental comparison of equations based upon it is quite encouraging.[9] Probably the most satisfactory treatment would be to introduce definite solubilities for the aggregate molecules and treat the solution–precipitate equilibrium in the standard way. This has been done for the restricted case of bivalent antibody and bivalent antigen (corresponding, so far as mechanical considerations are concerned, to our case I) by Pauling, *et al.*[10] It can also be done, although with considerable awkwardness, for the present cases.

So long as the numerical values are not taken too literally but viewed, rather, in a comparative sense, it may be of some interest in closing to examine numerically the effect on the equilibrium constants of including the perturbations of cases II, III, and IV. Previous treatments (corresponding to case I) do not appear to have included them, or, when allowing for perturbations of any sort, have not specified how they can be calculated. Let us consider, for example, a system where A and G are spherical molecules of molecular weight, 100,000 g., and radius, 75 Å., the temperature is 37°, and the surface lattice of reactive sites on G is the hexavalent lattice, (c). To estimate the effect of translation and rotation we may compare the results of case I with those of case III. By the method of case I the K_1 are determined only up to a multiplicative constant (involving the translational and rotational factors) which is supposed to be independent of i. This is also clearly the case in ordinary thermodynamic formulations and in so-called "kinetic" derivations, wherein no method is provided for calculating the proportionality constant on the basis of mechanical information about the reactant molecules. Actually, as a result of translation and rotation and A-A interactions (cases II–IV), the "constant" does depend on i. Since we cannot compute the absolute value of $K_i^{(I)}$, we cannot examine the effect (say, translation and rotation) of this dependence upon the ratio, $K_i^{(I)}/K_i^{(III)}$; however, we may consider instead the function $(K_1^{(III)}/K_6^{(III)})/(K_1^{(I)}/K_6^{(I)})$, in which the proportionality "constant" does not appear, and which, if translational–rotational effects are negligible, should be unity. Actually, it turns out to be about 8.7 in this example. The effect of even very slight A-A interactions can be estimated by a comparison of case I and case II.[7] Assuming a repulsion, $E_{AA} = +kT$, one finds $K_1^{(I)}/K_1^{(II)} = 1$, $K_3^{(I)}/K_3^{(II)} = 4.9$, and $K_6^{(I)}/K_6^{(II)} = 328$, again emphasizing that such interactions are not to be disregarded. Still a third instructive computation is an estimate of the absolute value of the equilibrium constants and therefore of the standard free energy change per reaction site. (This is admittedly quite rough, because the system for which a good value of ΔH has been measured does not conform too well with our particular numerical example.) Using a hemocyanin as an antigen and horse antibody, Boyd, *et al.*,[11] found the ΔH (or what we shall here assume as equivalent, ΔE) per site to be about $-40{,}000$ cal. This leads[12] to a $K_1^{(III)}$ value of 1.18×10^8 li. mole^{-1}, and a ΔF_1° of $-11{,}400$ cal. Assuming that an A–G bond is really the composite of several "weak" bonds of bond energy *ca.* 5,000 cal.,[6] one finds for our example a free energy change per individual weak bond of about -1400 cal., which is not an unreasonable value.

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Summary

Assuming a polyvalent antigen and a monovalent antibody, there are derived by conventional statistical methods certain relations between measurable concentrations of the reactants and the dissociation constants characterizing the equilibrium. From these relations it is possible to calculate the constants, given the appropriate concentration data. This is done for various assumptions regarding the nature of the reaction. Rough estimates made on the basis of existing information suggest that certain perturbations of the equilibrium, such as the interaction between nearest neighbor antibody molecules on the same antigen molecule or the effects of translation and rotation, may not be negligible as usually has been assumed in the past.

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(10) Pauling, *et al.*, THIS JOURNAL, **64**, 3003 (1942).

(11) Boyd, *et al.*, *J. Biol. Chem.*, **139**, 787 (1941).

(12) Under the assumptions of case III, the absolute expression for K_1 is

$$K_1 = \frac{2^4 N_0 n e^{-E_{AG}/kT}}{(2\pi mkT/h^2)^{3/2}(8\pi^2 ma^2 kT/h^2)^{3/2}\sqrt{\pi}}$$

where N_0 is the Avogadro number, m the mass, k the Boltzmann constant, T the absolute temperature, h the Planck constant, and a the radius of the molecule.