

Biochemical Aspects of Immune Complex Formation and Immune Complex Diseases

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Formation of immune complexes is a normal part of the immune defence against soluble antigens. Immune complexes may nevertheless play a pathogenic role of their own. The present review discusses antigen antibody interactions with special regard to immune complex formation. A new classification of antigen antibody interactions is proposed, based on the antigenic valence. Oligovalent antigens and bivalent antibodies form genuine immune complexes. Experimental observations and theoretical considerations indicate that although a vast variety of complexes is possible genuine immune complex formation is a self-limiting process, so it is typical that the smallest possible complexes are formed in the largest amounts. Formation of immune complexes differs in mechanism from most other biological ligand binding reactions, and the extent to which immune complex formation follows its own laws is discussed. To explain the mechanism of precipitin reactions, a two-stage model is proposed. The outcome of antigen antibody interactions is further complicated because antibody preparations are typically heterogeneous, and the impact of antibody heterogeneity is also discussed.

Soluble antigens are normally processed and rendered harmless by a sequence of processes that starts with direct interactions between the antigen and its corresponding antibodies. The processes which eventually lead to inactivation or neutralization of the antigen comprise two stages of different nature. In the first stage the antigen combines with appropriate antibodies forming immune complexes. In the second stage the immune complexes are phagocytosed either directly or upon reaction with the complement system and thus removed from circulation.

Formation of soluble circulating immune complexes is therefore a normal part of the humoral immune defence against soluble antigens, irrespective of their nature. Nevertheless, circulating immune complexes seem to be involved themselves in the pathogenesis of some diseases. The classical examples, i.e. those grouped as type III in the classification of Coombs & Gell (6), are the

serum sickness situation and the Arthus situation. More recently, evidence has accumulated which shows that immune complexes are heavily involved in the development of rheumatoid arthritis and systemic lupus erythematosus (for review see Lambert & Casali (18)). Still other conditions are regarded as diseases associated with immune complexes in the terminology of WHO (29). Great efforts are presently being undertaken to develop and refine analytical means for identification and quantification of circulating immune complexes (18).

The formation of immune complexes is a unique biochemical process. Antibodies as exemplified by IgG are bivalent whereas antigens may have several antigenic determinants, which means that the antigen and the antibody can combine in many different ways. Accordingly, the outcome of antigen-antibody interactions is a mixture of compositionally different complexes, and not a

single reactant as is otherwise typical of macromolecular reactions. Moreover, the different kinds of complexes are likely to have different properties. The understanding of immune complex formation requires a theoretical treatment that is different from the usual theory of macromolecular binding processes.

The purpose of the present review is mainly to discuss the special problems of immune complex formation under different circumstances. A graphic illustration of different distributions of immune complexes will be presented with examples of immune complexes formed at different antigen-antibody ratios. The problems of precipitating antibodies and of antibody heterogeneity will be discussed. Special emphasis will be placed on the natural laws of immune complex formation.

ANTIGENS IN IMMUNE COMPLEXES

Several different substances have been identified as the antigen components of circulating immune complexes. One might distinguish between antigens of endogenous origin and antigens of exogenous origin. Typical of the first group is immunoglobulin (IgG) as seen in rheumatoid arthritis (17), nuclear material (DNA) as in systemic lupus erythematosus (1, 3) and specific cellular antigens in some tumours and auto-immune diseases (12). Occasionally some very specialized compounds like alkaline phosphatase or milk proteins may be observed as the antigen (4). In the second group iatrogenic antigens play a role of their own because the antigen is well known and administered intentionally, as for instance in serum sickness or drug allergy. Antigens from infectious organisms also belong to the group of exogenous antigens (21). Thus in hepatitis B the coat protein of the virus is a quantitatively dominating antigen, but other parts of the virion also elicit production of specific antibodies. In post-streptococcal glomerulonephritis bacterial debris is the likely antigen. Other antigens from other infectious

organisms are known or under suspicion (29).

The antigens involved in circulating immune complexes thus constitute a very broad spectrum of substances. Chemically, these antigens may be proteins, glycoproteins, lipoproteins, polysaccharides, nucleic acids and aggregates of these compounds. Their only conspicuous common feature is that they are soluble, and that they are macromolecules or macromolecular aggregates.

CLASSIFICATION OF ANTIGEN-ANTIBODY INTERACTIONS

Antigens constitute a far more variable group than do antibodies. The outcome of antigen-antibody interactions depends very much on the nature of the antigen, and to discuss antigen-antibody interactions fully it is important to differentiate between different types of interactions. The present proposal for classification of antigen-antibody interactions is based on the nature of the antigen, and to further simplify the problem of classification of antigen-antibody interactions only antigen-antibody interactions of bivalent antibodies (mainly IgG) will be discussed here. The classification can, however, be extended to cover IgA and IgM interactions also by use of the same basic rules.

The basic criterion of the present classification of antigen-antibody interactions is the antigenic valence, i.e. the number of determinants on each antigen molecule. Within each type, distinction will be made between antigens with repeating identical determinants and antigens with different determinants. The most important types of antigen-antibody interactions will be discussed shortly in the following, and a schematic illustration of the classification is given in Fig. 1.

Type I (monovalent antigens, free haptens)

Interactions between strictly monovalent antigens (free haptens) and antibodies that are produced against the hapten when coupled to

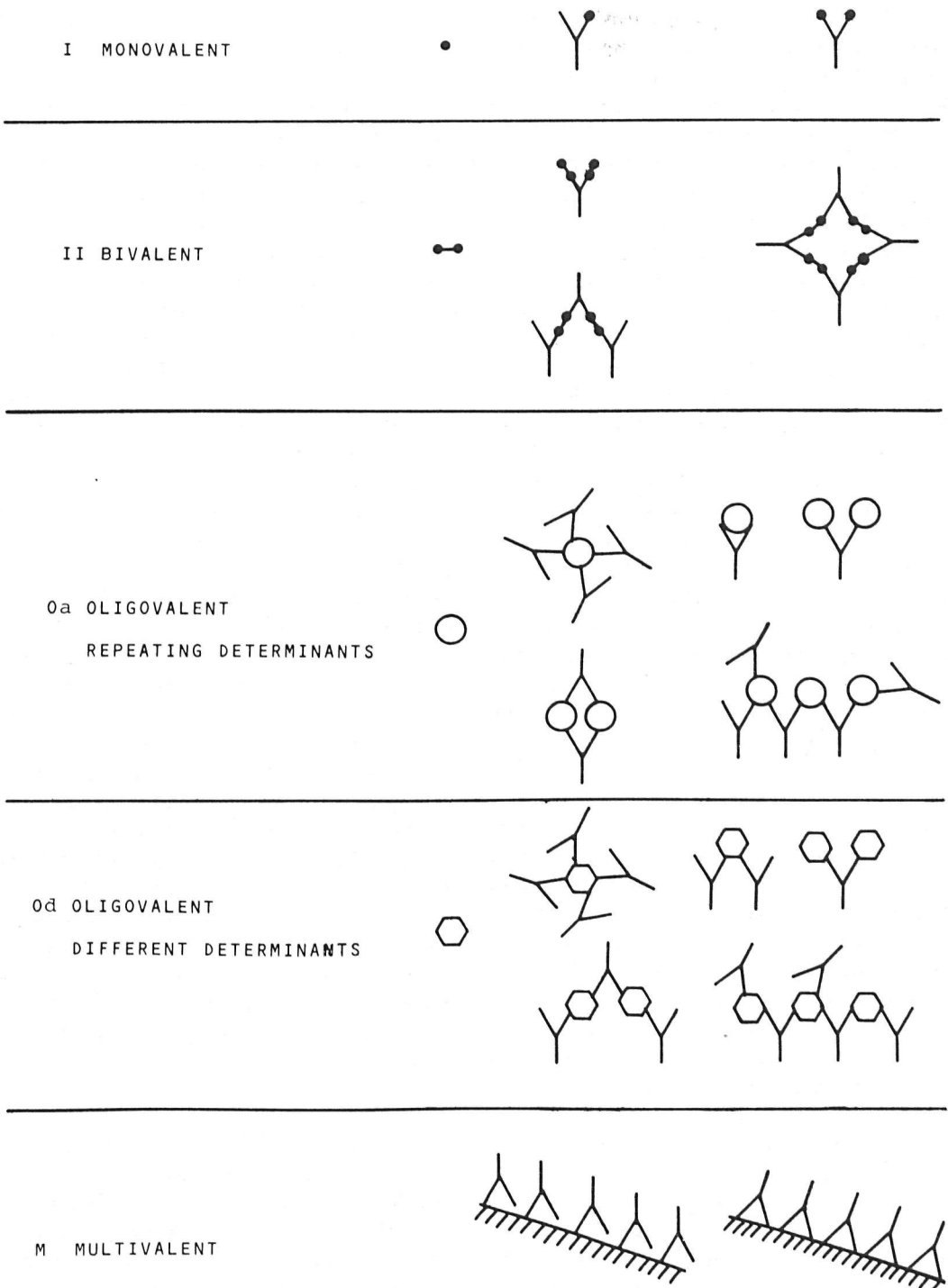


Fig 1. *Classification of antigen-antibody interactions.* Antigen-antibody interactions are classified primarily after antigenic valence. Thus monovalent antigens appear in type I of antigen-antibody interactions. Inside each group, distinction can be made between antigens with repeating identical determinants (subtype a) and antigens with different antigenic determinants (subtype d).

a carrier protein are frequently used as model systems for the study of kinetics and thermodynamics of antigen-antibody interactions. Because of the antigenic monovalency this type is rather easy to deal with, and the important feature is that only two different complexes, namely AgAb and Ag₂Ab can be formed in this system.

Type I interactions resemble in mechanism the binding of ligands to transporting plasma-proteins, binding of hormones to their receptors and binding of substrates to their enzymes. Type I interactions are the only antigen-antibody interactions for which the classical enzyme-antibody analogy is valid. The theory of type I interactions has been discussed in several studies (e.g. 7, 8, 37, 46, 47). An important feature of the theory of type I interactions is that graphical methods are developed for estimation of association constants, antibody concentrations and other parameters (cf. Fig. 7). Most radioimmuno-logical assays are based on the theory of type I interactions.

Type II interactions (bivalent antigens, bivalent haptens)

If antibodies raised against a monovalent hapten coupled to an indifferent carrier protein are allowed to react with a bivalent version of the hapten, several different complexes may appear. As illustrated in Fig. 1, some of these may be cyclical in structure. Complexes formed in this system are especially interesting because they form complexes that can be seen very clearly by electron microscopy (48). The theory of type II interactions is complicated because cyclic structures are involved (31).

Type O interactions (oligovalent antigens).

Oligovalent is here used to denote a limited number of antigenic determinants, say from 4 to 50. Thus, included in this group are interactions of natural protein antigens (including serum proteins), nucleic acid

antigens, polysaccharide antigens and artificial antigens produced by coupling a number of haptenic groups to a carrier protein. Within this group, distinction is made between antigens where all antigenic determinants are alike (type O/a) and antigens with different non-repeating determinants (type O/d).

Type O/a interactions are frequently used as model systems for the study of immune complex formation and to study the fate of immune complexes *in vivo* (16, 19, 20). Though type O/a interactions have an apparent look of simplicity it is a very difficult system to treat theoretically. A complicating aspect is that cyclical complexes are a highly likely outcome of type O/a interactions (cf. Fig. 1). This system also involves the possible binding of antibodies to the antigen by use of both antibody sites (monogamous bivalent binding (7)) or incestuous binding (8). So far no completely dependable theory of type O/a interactions has been given.

Type O/d interactions are here regarded as the type O interactions that are typical of immune complex formation *in vivo*, and this type of antigen-antibody interaction will be discussed in detail in the following sections. The hallmark of type O/d interactions is that the interactions lead to formation of many different kinds of complexes with different contents of antigen and antibody. Type O/d interactions should not be treated theoretically in the same way as Type I interactions. It is characteristic that the outcome of type O/d interactions is dependent on the absolute concentration of antibody, in contrast to type I interactions (42).

Type M interactions (multivalent antigens)

Multivalent antigens are here used to denote antigens with a very large number of antigenic determinants as virus particles or micro-organisms. Type M interactions differ from type O interactions in that the antigen is very much larger than the antibody molecules, and the antibody molecules will tend to cover the surface of the antigen whenever possible.

IMMUNE COMPLEX FORMATION (INTERACTION TYPE O/d)

To discuss the problems of immune complex formation further we shall examine the formation of complexes between a bivalent antibody and an oligovalent antigen, as for example a serum protein. The typical antigen is supposed to possess a few (5 to 15) antigenic determinants, all of which are different. Cyclical complexes are *a priori* excluded because it is assumed that the antigen, like most proteins, is too asymmetric to be able to enter cyclical complexes.

A reaction scheme for interactions of type O/d is shown in Fig. 2 (39, 40). The reaction scheme shows the most important path of reactions in immune complex formation, and it also provides a method for systematizing the many possible complexes according to composition and size. As the first step, antigen and antibody together form the simplest possible immune complex, AgAb. This complex can react further in several different ways. However, three ways are most typical. First the complex AgAb may accept another antigen molecule yielding the complex Ag₂Ab and this process is shown with vertical arrows in the reaction scheme. Second, the complex AgAb may interact with another antibody molecule giving the complex AgAb₂, as denoted by a diagonal arrow. Third, any complex may react with another complex giving a new and larger complex. Complex growth by interactions between complexes are here exemplified by horizontal arrows specifically denoting complex growth by addition of the complex AgAb. Although many other mutual interactions are possible, these three are sufficient to account for the formation of all possible complexes (42).

Apart from showing the most important ways of forming immune complexes, the reaction scheme of type O/d interactions gives a review of the composition of all theoretical immune complexes. No complexes can be formed beyond the top row, which contains the most antigen-rich complexes, because all

available antibody sites are saturated here. Similarly, no complexes are possible below the lowest diagonal row of complexes. Complexes can only grow to the right of the reaction scheme. However, as shall be shown later the growth of complexes seems to be self-limiting in this direction.

The reaction scheme is also arranged to show the complexes systematically. The complexes in the first column comprise all the complexes that are structurally possible with one antibody molecule. The second column contains the complexes that can be made from two antibody molecules, and so forth. Inside each column, the complex with the highest content of antigen molecules is given at the top, whereas the complex with the minimum content of antigen is given at the bottom. The top row therefore comprises complexes with the maximum possible antigen-antibody ratio, whereas the lower diagonal comprises the complexes with the minimum antigen-antibody ratio.

The reaction scheme as shown in Fig. 2 also has the advantage of systematizing all possible complexes in a way that makes it possible to calculate the amounts of each individual complex when concentrations of antigen and antibody are known, combined with information on the antigenic valence and knowledge of the affinity of interactions between individual antigenic determinants and their corresponding antibody sites. Several ways of performing such calculations have been described (10, 27, 41, 42, 49). To illustrate the calculations we shall make the assumption that the antigen is a molecule with six different antigenic determinants, and that the antibody preparation is homogeneous with respect to affinity towards the antigenic determinants. It is further assumed that all interactions can be described by one association constant (K), being the average equilibrium constant as measurable by standard techniques. The problems that arise when antibody heterogeneity is to be included shall be shortly treated later. For this system the concentration of a complex of the

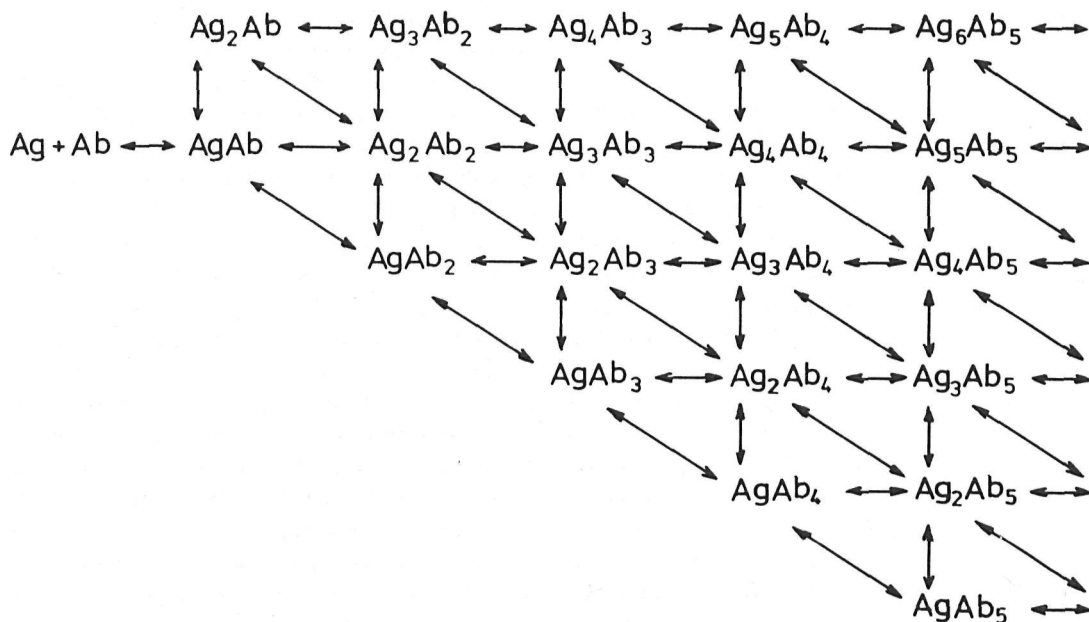


Fig. 2. Reaction scheme of type O/d antigen-antibody interactions. The simplest possible complex (AgAb) as most other complexes can grow in different ways: (1) by addition of one antigen molecule (vertical arrows), (2) by addition of one antibody molecule (diagonal arrows) and (3) by addition of the simplest possible complex (horizontal arrows).

composition Ag_iAb_j at equilibrium is given by

$$[\text{Ag}_i\text{Ab}_j] = K^{i+j-1} [\text{Ag}]^i [\text{Ab}]^j$$

where $[\]$ denote equilibrium concentrations (31, 42). This formula allows calculation of the concentration of all theoretically possible complexes under defined conditions, and it also gives the possibility of calculating the sum of all antigens and antibodies that exist in form of complexes for known equilibrium concentration of free antigen and antibody. It is further possible to calculate concentrations of individual complexes from total concentrations of antigen and antibody. One way of doing this is to use a computer simulation technique as described previously (41, 42, 43).

In order to find an uncomplicated method for describing the distribution of different complexes formed by defined concentrations of antigens and antibodies, the complex patterns in Fig. 3 have been devised. In this figure each box represents one complex, and the com-

position of that complex is given by its position in the complete pattern so that each box corresponds to the complex in the reaction scheme with the same position. Thus the top boxes are the antigen-rich complexes Ag_2Ab , Ag_3Ab_2 and so forth. The relative concentration of each complex shown is given by the number of skew lines in the box, so that a high concentration is shown as a very dark box and a low concentration as a light box. The actual concentrations are directly proportional to the number of shading lines in each box.

A series of different calculated complex distribution patterns are shown in Fig. 3 computed for total antigen-antibody ratios from 1.7 (antigen excess) to 0.2 (i.e. 1 antigen to 5 antibody molecules). In the heaviest antigen excess example it appears that the most likely complexes are those included in the top row (also cf. Fig. 2). In the antibody excess examples the most likely complexes are those corresponding to the lowest diagonal of the reaction scheme. In between these two

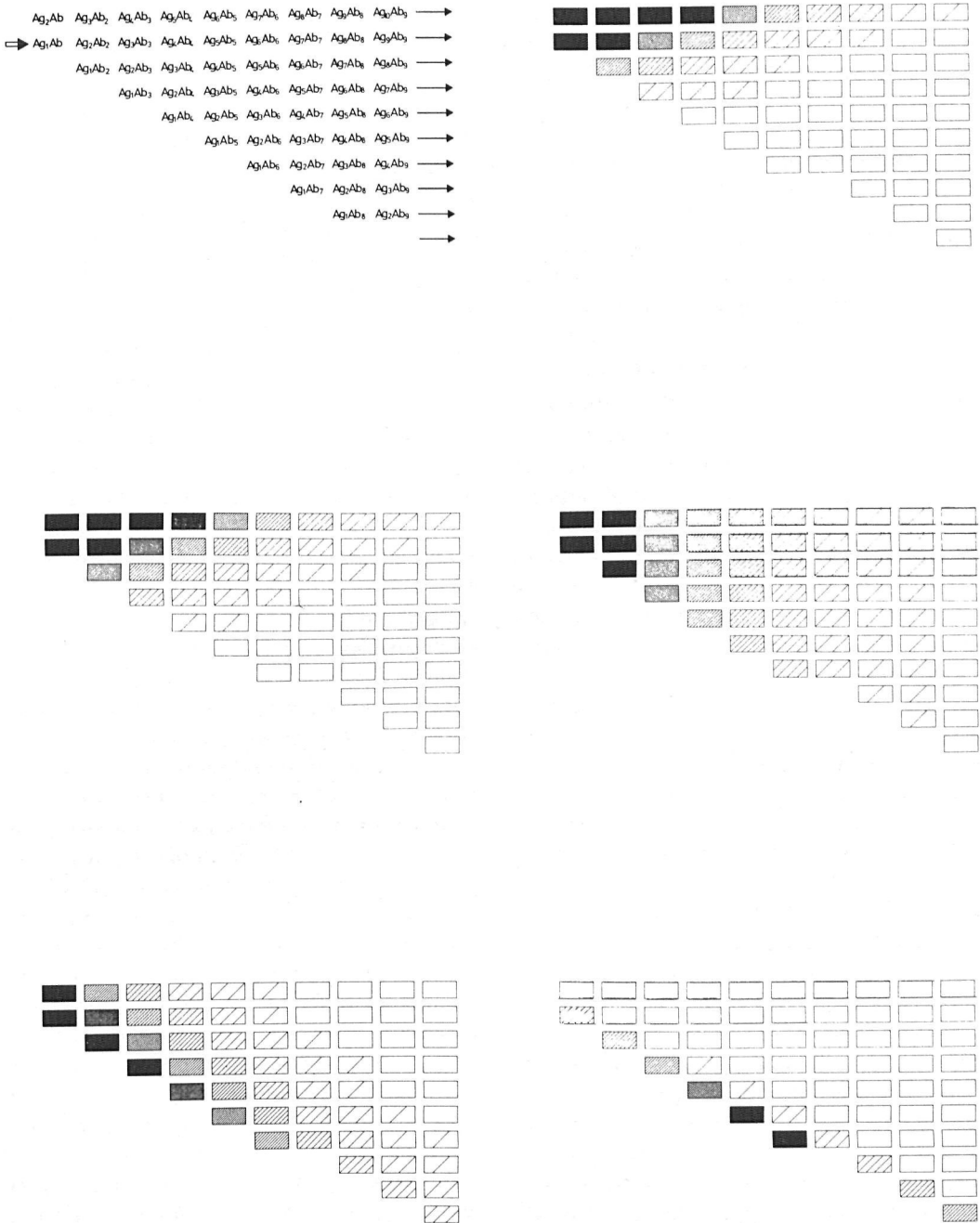


Fig. 3. Calculated immune complex distribution patterns for different antigen/antibody ratios. In each of the five schematic patterns each small box represents one specific immune complex. The composition of each complex is given by its position corresponding to the reaction scheme in the upper left corner. For example, the top row of complexes in each figure section contains the most antigen-rich complexes. The concentration of each complex is calculated according to the number of shading lines in each box, i.e. the darkness of the box. The total antigen/antibody ratios in the five patterns are (1) 1.7 (upper right figure), (2) 1.5 (middle left figure), (3) 0.9 (middle right figure), (4) 0.6 (lower left figure) and (5) 0.2 (lower right figure).

extremes a richer variety of different complexes is formed, and in the present example the maximum number of different kinds of complexes are seen at an antigen-antibody ratio near 0.6.

An important feature of these complex distribution patterns is that they demonstrate that complex formation is self-limiting. It has been a consistent feature in all the calculated patterns that the concentration of complexes decreases with increasing complex size. Experimental findings to be discussed in a later section support the concept that immune complex formation as such is self-limiting with respect to the maximal size of complexes.

The distribution patterns of immune complexes formed at different total antigen-antibody ratios in Fig. 3 are believed also to reflect the progression of an immune response against a soluble antigen *in vivo*. At the beginning a situation where the ratio of total antigen to total antibody is very high is likely. The complexes which will be formed as antibody is synthesized and liberated in circulation are the antigen excess type. As more and more antibody is synthesized the antigen-antibody reaction ratio will decrease, and the distribution of complexes will change. More different kinds of complexes will be formed, and the average complex size will increase. Some of the larger and more antibody-rich complexes may directly or indirectly trigger phagocytosing cells, and these complexes will be removed from circulation. The antigen-antibody ratio (as the total ratio) will decrease further because antigen (in the form of complexes) is being steadily removed and also because the antibody synthesis is still active. Thus, finally, the complex distribution will change to a genuine antibody excess pattern, where the complexes are those in the lowest diagonal of the reaction scheme. These complexes are probably the complexes which basically are the best triggers of phagocytotic cells, and they should be destroyed automatically. Eventually, all antigen is cleared from the circulation.

EXPERIMENTAL ANALYSES OF IMMUNE COMPLEX DISTRIBUTIONS

Sucrose density gradient centrifugation can be used to study the composition of immune complex containing materials. Such separations can be done with zonal rotors of Anderson's design (2) or with conventional swing-out rotors. Both techniques can be used to calculate sedimentation coefficients for identified complexes, thus giving a good estimate of the molecular weight of immune complexes (44). An example is given in Fig. 4. Immune complexes in slight antigen excess were made by mixing human IgG (here as the antigen) and rabbit anti-human IgG against the Fc-portion of human IgG. The mixture was incubated at 37°C for 1 h and left overnight at 5°C before centrifugation, to ensure that equilibrium was reached. The antigen was labelled radioactively. The sample was centrifuged for 3 h at 48,000 rev/min in a shallow 3–23% (w/w) sucrose gradient. The results were subjected to a computer based analysis leading to the plot shown where the concentration of complexes is put in relation to their size, as given by their calculated sedimentation coefficients. Moreover, an estimate of the composition of the complexes is given. This has been achieved by calculating the corresponding location of complexes containing 2, 3, 4 and more IgG molecules. It appears from this Figure that a rather large number of different complexes can be seen. However, it is also clear that the smallest complexes are present in the largest amounts, and that the amounts of complexes decrease with increasing complex size, thus supporting the theoretical prediction that immune complex formation is self-limiting with respect to the size of complexes. Similar results for other antigen-antibody interactions have been found also (25, 38) by the same method, by analytical ultracentrifugation (32) and by gel chromatography (19, 20).

Serum samples from patients with rheumatoid arthritis and other pathological conditions supposed to involve immune

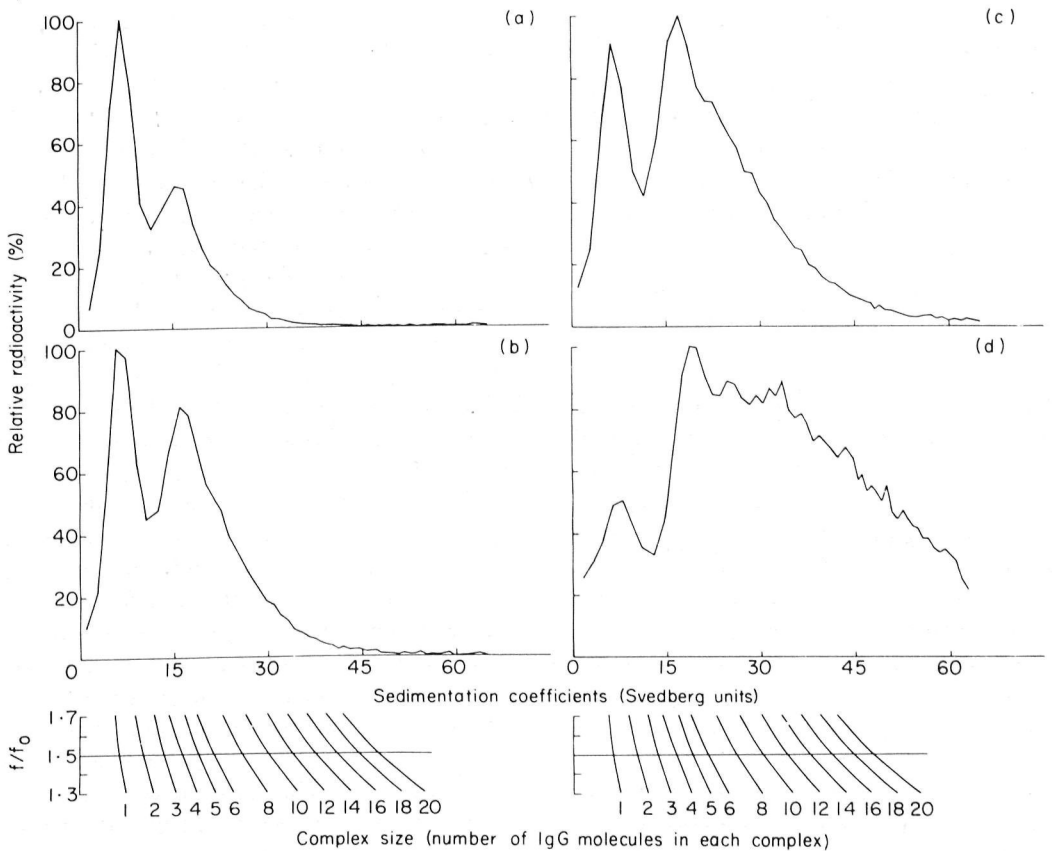


Fig. 4. *Experimental immune complex distributions.* Immune complexes were made by mixing I^{125} -labelled human IgG and rabbit IgG directed against the Fc-portion of human IgG in different portions. All samples are antigen excess samples, approaching equivalence from a to d. The samples were centrifuged by zonal centrifugation, and results were computer-treated to appear as shown with calculated sedimentation coefficients as abscissa. The ordinate shows the relative amounts of human IgG (as based on radioactive measurements). Inserted as an extra axe is a scale that shows where complexes of defined composition are most likely to be found. The numbers give the number of IgG molecules in a complex (see ref. 44).

complexes have been studied by a number of groups using different kinds of gradient centrifugation (11, 13, 17). Also samples from animals with experimental immune complex diseases have been studied (5). In rheumatoid arthritis it seems that the IgG complexes present are smaller than those found *in vitro*. Typically, IgG complexes in rheumatoid arthritis are complexes containing two or three IgG molecules. one obvious reason is that larger complexes are likely to be those complexes that are first removed by phagocytosing cells *in vivo*, and the complexes which

can be seen in this case will be complexes that are unable to trigger phagocytosis and therefore remain in the circulation. Another reason could be that the "antigenic" IgG component in rheumatoid arthritis has one or two active determinants only and that the IgG involved form highly special cyclical aggregates that are unable to grow further because all "epitopes" are used in the cyclic complex (28).

Heat treatment of IgG leads to large aggregates that are interesting because they can bind Clq and trigger phagocytosis (18).

Though these aggregates can simulate immune complexes in several experimental methods, they are formed by a completely different mechanism. Centrifugal studies have revealed that aggregated IgG form a very broad distribution of aggregates. Most of these are very large, containing 10 to 30 IgG molecules (35).

PRECIPITIN REACTIONS, THE ZONING PHENOMENON

Type O/d interactions *in vitro* are frequently precipitating. Precipitin reactions are peculiar types of reactions in that two kinds of otherwise soluble macromolecules form a precipitate by non-covalent interactions. No enzymic activities are involved, and the molecules are not denatured in the classical sense of this word. Moreover, precipitin reactions exhibit a zoning phenomenon, the hallmark of immunochemical interactions.

The zoning phenomenon is defined by a precipitin curve, typically made by adding increasing antigen concentrations to the same antibody concentration. In antibody excess all the added antigen will be precipitated. At a certain antigen concentration a maximum of precipitate is seen, and at this maximum (the equivalence point or zone) no, or at least very little, antigen and antibody remain soluble. With further increasing antigen concentrations less and less precipitate is produced, whereas the amounts of soluble antigen-antibody complexes increase. In this antigen excess zone a point will finally be reached where precipitate is no longer produced, and all antigen-antibody complexes are perfectly soluble.

To discuss the zoning phenomenon a little further, the complexes in the reaction scheme will be divided in three major groups. Antibody excess complexes are those with an antigen-antibody ratio below 1, and are typically formed in the antibody excess zone (Fig. 5). For simplicity these complexes will be taken as those in the lower diagonal of the reaction scheme with the exception of the

complex AgAb that has an antigen-antibody ratio of 1.0. Similarly, the top row of complexes in the reaction scheme can be regarded as antigen excess complexes as they have an antigen-antibody ratio above 1.0, and they are typically dominating in antigen excess. The remaining complexes will be regarded as an intermediary type, not being especially antigen-rich nor antibody-rich. The amounts of complexes in each group at different total antigen-antibody ratios can be calculated as shown in Fig. 6 to show the principal shift in complex type throughout the range of a precipitin curve. It appears from this Figure that the zoning tendency of antigen-antibody interactions is likely to be explainable as the result of formation of different complexes at different antigen-antibody ratios. The antibody-rich complexes are most likely at the lowest antigen-antibody ratios, and their appearance in the calculated Fig. 6 shows a clear zoning, and we have suggested that the zoning of these complexes forms the basis of the zoning that can be seen experimentally. The intermediary type of complexes also shows a calculated zoning. However, this zoning has maximum at a rather high total antigen-antibody ratio, not comparable to the rather low value seen experimentally (14, 15, 43).

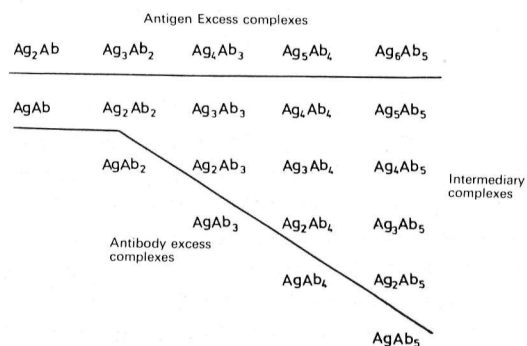


Fig. 5. Theoretical grouping of immune complexes. The theoretically possible immune complexes are divided into three different groups. At the top are the antigen excess complexes with an internal Ag/Ab ratio above 1.0. At the lower diagonal are the antibody excess complexes with the lowest possible Ag/Ab ratio. In between is the group of intermediary complexes.

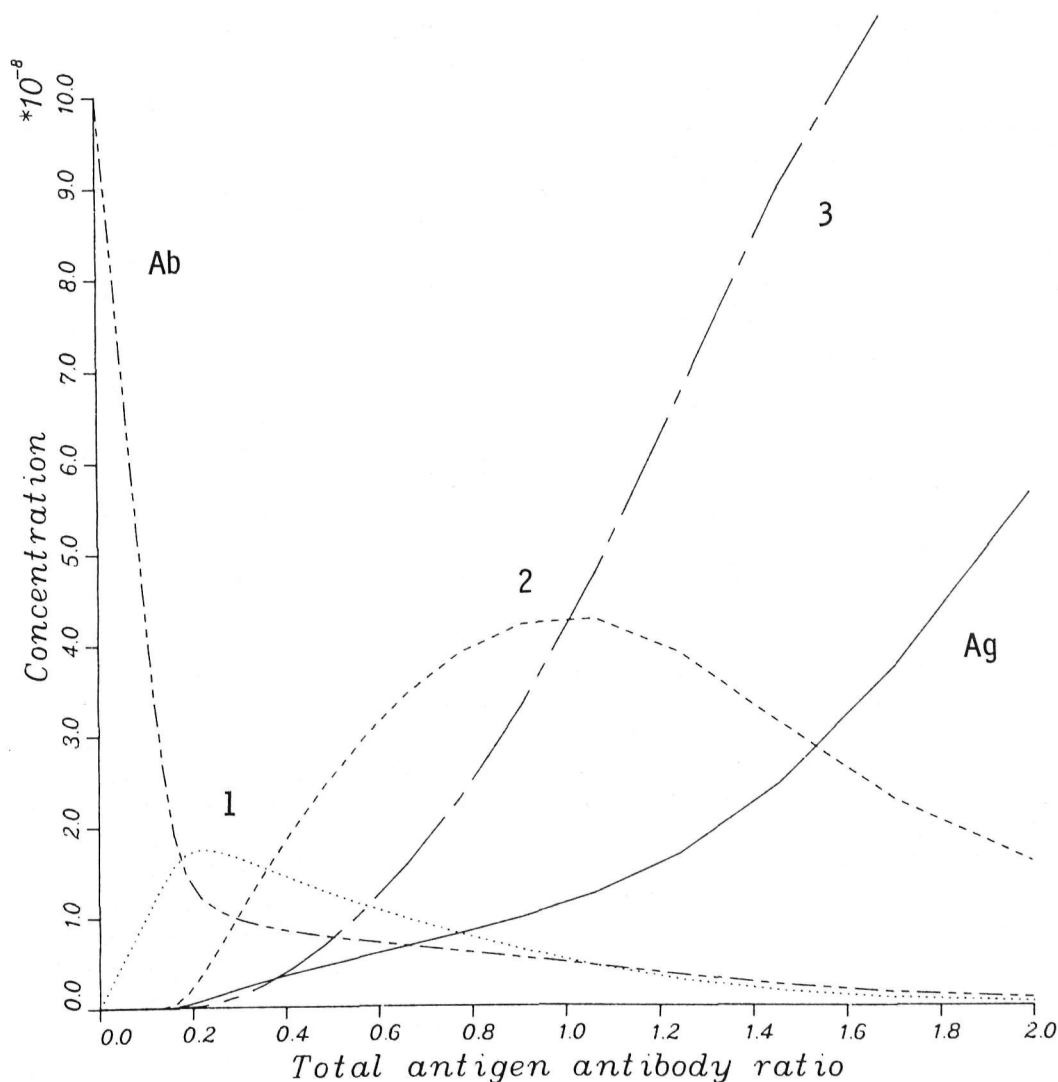


Fig. 6. Calculated complex distributions at different Ag/Ab ratios. All possible immune complexes are divided into three groups as shown in Fig. 5. The distribution of immune complexes at different Ag/Ab ratios has been calculated, and the values given in this figure. Ab, free antibody; Ag, free antigen; 1, antibody excess complexes; 2, intermediary complexes; 3, antigen excess complexes. The ordinate is a concentration axis (mol/l). The total antibody concentration was taken as 0.1 μ M. The association constant was 10^8 (1/mol). The complex concentrations were calculated by the method of Steensgaard & Frich (43).

Fig. 6 also contains calculated curves showing the theoretical amounts of free antibody and free antigen at different antigen-antibody ratios. These curves show that the present theoretical treatment accounts for the absence of free antigen in the very heavy antibody excess zone, and it also predicts that

the concentration of free antibody decreases rather drastically in the antibody excess zone as the concentration of added antigen is increased. Thus we can conclude that the zoning phenomenon is explainable by the formation of complexes of different composition at different antigen-antibody ratios.

Apart from the zoning phenomenon, precipitin reactions are characterized by their ability to evoke a complete precipitation of antigen before and at equivalence. The latter feature of precipitin reactions has proved far more difficult to explain than the first.

The classical lattice or framework hypothesis of precipitin reactions states that the antigen and its antibodies together form very large complexes exclusively by bonds between the determinants and their combining sites (epitope-paratope interactions), and these very large aggregates turn insoluble when they have reached a certain size. It has recently been shown that $F(ab')_2$ fragments are less able to precipitate antigen than equimolar amounts of intact IgG, suggesting that formation of precipitating immune complexes involves non-epitope-paratope interactions (15, 24, 25). A complete explanation of precipitin reactions should therefore comprise two stages, namely a first stage of immune complex formation as seen above, and a second stage where some complexes confluence forming insoluble immune complex aggregates. The latter processes are likely to be initiated by the antibody excess complexes, but the second stage must coprecipitate substantial amounts of intermediary complexes to account for the complete precipitation near equivalence (43).

IMPACT OF ANTIBODY HETEROGENEITY

An antibody population *in vivo* or an antibody preparation *in vitro* is a mixture of antibody molecules of the same class and with specificity towards the same antigen, but varying with respect to affinity towards the antigenic determinants in question. Antibody affinity heterogeneity has a profound impact on antigen-antibody interactions. Under circumstances where antibody molecules are abundant, the antigen molecules will select the antibody molecules with the highest affinity for interactions, and this will appear experimentally as if the antibody preparation had a higher average affinity than it should have. In

contrast, if the antigen molecules are in excess, the antibody preparation will exhibit a lower affinity because now all antibody molecules in the preparation, including those with lowest affinity, will participate in the interactions.

To evaluate the impacts of antibody affinity heterogeneity on typical experimental situations we have extended our computer simulation model to cover antibody heterogeneity also (45). It is assumed that the heterogeneity of an antibody preparation can be described by a continuous distribution function, related to a normal Gaussian or a Sipsian distribution function (33). By use of this approach one can calculate the theoretical outcome of type O/d interactions, and moreover the calculated values can be given graphically as they would appear experimentally.

The calculated data are presented in commonly used plot systems for evaluation of association constants, etc. The graph systems used are (cf. Fig. 7):

1. The Langmuir adsorption isotherm. r is the degree of saturation of antibody binding sites. $[Ag]$ is the calculated equilibrium concentration of free antigen. The upper limit of r is 2.0, because one antibody molecule at most can bind two antigenic determinants.

2. The reciprocal Langmuir plot with axes $1/r$ and $1/[Ag]$. The slope is $1/K$, where K is the association constant.

3. The $1/b$ versus $1/[Ag]$ plot is related to the previous plot type. The slope is a function of the association constant, and the y-intercept is the reciprocal total antibody site concentration (26, 34, 36).

4. $r/[Ag]$ versus r is the Scatcard plot (30).

5. $\log(r/(2-r))$ versus $\log[Ag]$ is the Sips plot (33). This is of special interest in the present context, because deviation of the slope from the value 1 is supposed to reflect the degree of antibody heterogeneity.

A calculated example is shown in Fig. 7. The antibody affinity function is defined by the distribution function given at the top right of the figure. Each figure section contains four curves. The fully drawn curve gives the

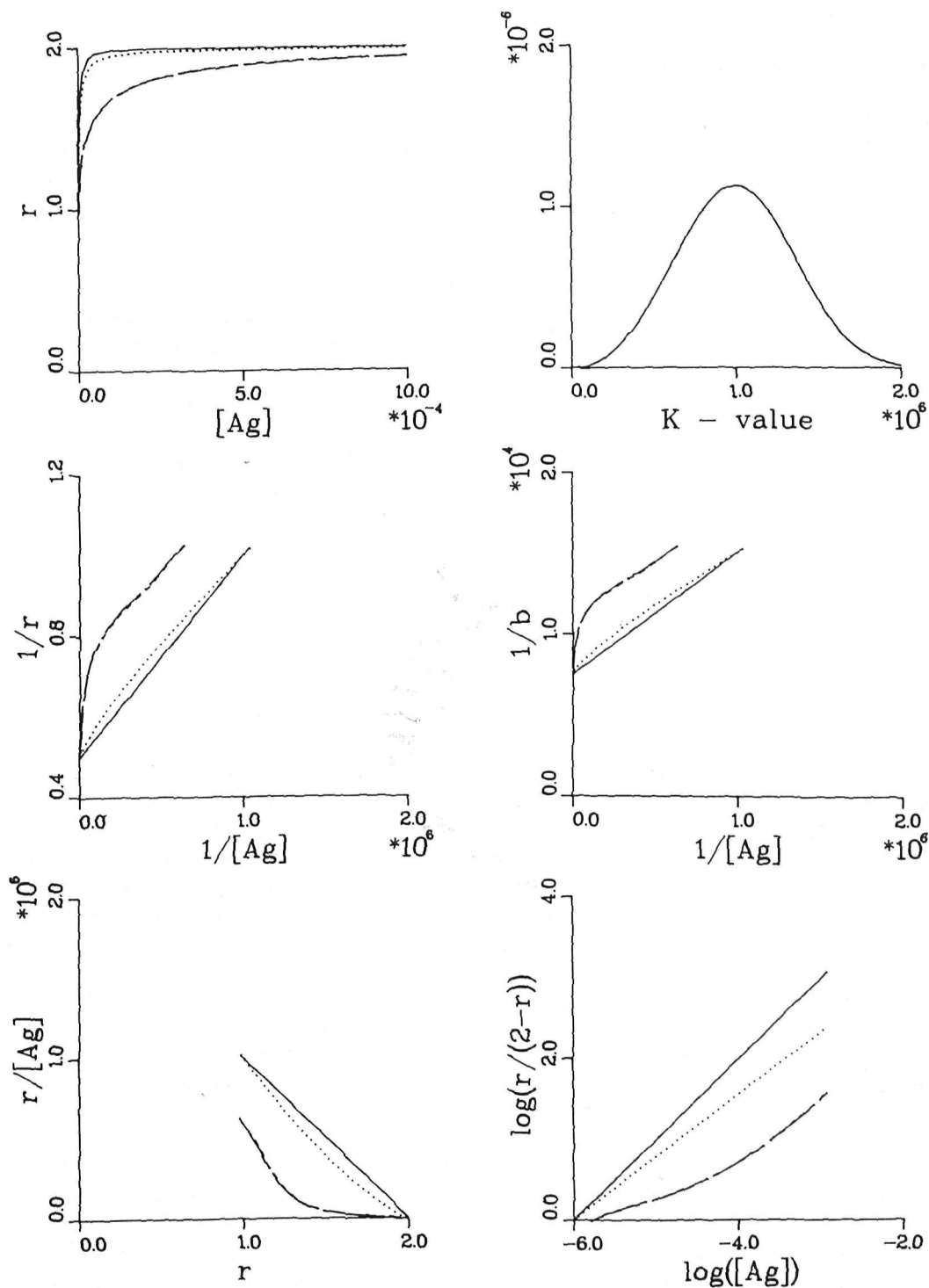


Fig. 7. Calculated results of antigen-antibody interactions. From upper left to bottom right the figures show: (1) the direct Langmuir plot, (2) the antibody affinity distribution used, (3) the reciprocal Langmuir plot, (4) the Steward plot, (5) the Scatchard plot, and (6) the Sips plot. r , the fraction of used antibody binding sites (maximum = 2); $[Ag]$, the concentration of free antigen; b , the concentration of bound antigen; K , the association constant. Four different systems are given in each graph: Haptenic interactions (type I) without antibody affinity heterogeneity (—), haptenic interactions including antibody heterogeneity (.....), type O/d interactions without heterogeneity (-----), and type O/d interactions with antibody affinity heterogeneity (-.-.-). For further details see text. The curves of type O/d interactions with and without heterogeneity have been calculated independently, but overlap in the cases shown here. The figure has been calculated as described in (45).

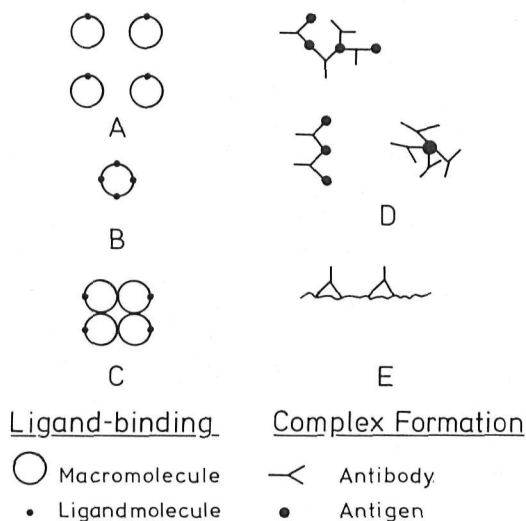


Fig. 8. Schematic illustration of the difference between immune complex formation (type O/d) and ligand binding process. In ligand binding processes a macromolecule can bind one or more small molecules (the ligand), or an aggregate of macromolecules can bind a number of ligand molecules. Every ligand molecule is bound by one, and only one, bond. In immune complexes antigens are linked by antibodies. The number of antibody binding sites that are used to form the complex may exceed the number of bound antigens.

calculated values for a homogeneous antibody preparation reacting with a monovalent hapten, the ideal case. The dotted curve (.....) gives calculated values for the same reaction but for a heterogeneous antibody preparation. The dashed curve (——) shows the results calculated for a 6-valent antigen reacting with a homogeneous antibody preparation, and finally the chain dashed curve (— · — ·) gives the calculated values for the reaction between the 6-valent antigen and a heterogeneous antibody preparation.

These calculated graphs illustrate very well some of the inherent difficulties in studying antigen-antibody interactions. For the reaction of a monovalent hapten heterogeneity of the antibody preparation a rather slight deviation from linearity is apparent, and only the Sips plot-technique yields a clear demonstration of antibody heterogeneity. For the reactions that involve a 6-valent antigen, genuine immune complexes occur as described in the section of

type O/d interactions. Formation of genuine immune complexes tends to complicate the reactions leading to very clearly expressed deviations from the supposed linearity of all the curves in all the plot systems investigated. The main reason for this is again that formation of genuine immune complexes means that the number of bonds needed to create a complex exceeds the number of bound antigen molecules as illustrated in Fig. 8. The deviation from simple linearity in the plot systems in Fig. 7 is so large that it hides the effects of heterogeneity, and in the examples shown here there is little difference between the lines of complex formation with and without heterogeneity. Complex formation may be taken as the main cause of deviation from expected linearity.

CONCLUDING REMARKS

Formation of immune complexes (type O/d interactions) is a unique form of biological binding reactions, differing in characteristics from other antigen-antibody interactions and from ligand binding processes (cf. Fig. 8). The distribution of the different immune complexes formed depends on the absolute concentrations of antigen and antibody and their mutual affinity. Though many different immune complexes are theoretically possible, relatively simple rules for immune complex formation can be formulated. Foremost, immune complex formation seems to be self-limiting. Theoretical considerations as well as experimental observations indicate strongly that the smallest immune complexes are formed in the largest amounts, and that the amounts of immune complexes decrease with increasing complex size. The only exception is that in heavy antibody excess, complexes of the type $AgAb_f$, where f is the antigenic valence, may be prevalent. Lattice- or framework-like immune complexes, which are often depicted in immunological texts and which comprise dozens of antigen and antibody molecules, are very unlikely products of antigen-antibody interactions. Type O/d interactions, moreover,

show a peculiar concentration dependence unknown in ligand binding reactions and simpler antigen-antibody interactions (42, 45).

Immune complex formation as such is the first stage only in the physiological removal of antigens. Some of the complexes formed in stage one can directly, or mediated through the complement system, trigger their own phagocytosis. Many of the effector functions of immunoglobulins are believed to reside in the Fc-portion, which raises the question as to how the Fc-portion is being changed, if changed at all, upon reaction with the proper antigen. Numerous studies have been performed to cast some light over this problem but the results have been unclear and not infrequently conflicting (9, 22, 23). It has been suggested that the mere fixation of a number of antibody molecules to the same antigen is sufficient to signal the initiation of biological removal processes. However, it should not be forgotten that the existence of precipitating antibodies implies that parts of the antibody molecule, upon interaction with antigen in proper ratios, can shift from a perfectly soluble to an insoluble state (stage two of precipitin reactions). Thus, an allosteric change in the antibody molecule upon reaction with antigen is not altogether improbable.

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