Immunology, 1975, **29**, 571.

Computer Simulation of Immunochemical Interactions

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(*Received 3rd December* 1974; *accepted for publication 20th February* 1975)

Summary. A computer model for simulation of the interactions between a macromolecular antigen and its corresponding IgG has been developed. The model takes all possible immune complexes into account, and it calculates the most probable immune complex distribution patterns on the basis of basic thermodynamic principles from the valences and initial concentrations of antigen and antibody, respectively, together with an association constant assumed to be common to all mutual interactions. In antigen excess small antigen-rich complexes are predicted. At or near equivalence a rich variety of relatively small complexes is predicted, while in antibody excess complexes of the type $AgAb_n$ are found to be the most probable. By further assuming that the precipitate consists of antibody excess complexes, a precipitin curve can be calculated. The agreement between calculated results and experimentally obtained data is found to be good. It is of special interest that this theory implies that the outcome of immunochemical interactions depend equally well on the concentrations of antigen and of antibody.

INTRODUCTION

Computer simulation of biological processes may serve several purposes. First, it provides a direct and precise way of checking an existing model. Secondly, it can be used to evaluate the relative importance of different parameters used in a model. Thirdly, and probably most important, attempts to construct computer models may help to improve or to sharpen the terminology of the concepts in question, because computer models can work only with precisely defined parameters.

The present paper will describe the principles of a simple computer model for simulation of antigen–antibody interactions. The model is based on the assumption that the outcome of a reaction between an f-valent macromolecular antigen and a bivalent antibody is determined by the concentrations of antigen and of antibody respectively, together with one association constant common to all mutual interactions in the system. The model predicts immune complex distribution patterns which are in accordance with present knowledge, and it can predict a theoretical precipitin curve which contains the most characteristic features of experimental precipitin curves.

MATERIALS AND METHODS

Precipitin curves were made by mixing equal volumes (400 μl) of a solution of human

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serum albumin (Amersham) and a solution of rabbit anti-human serum albumin IgG (lot 013, Dakopatts A/S, Copenhagen). The concentrations of antigen and of antibody used in the individual experiments are given in the legend to Fig. 4. After mixing the tubes were placed in a waterbath at 37° for 1 hour and subsequently in a refrigerator at 4° for 48 hours. The precipitates were washed three times in 2 ml of borate buffer at 1° (pH 8·3) and dried overnight in the refrigerator at 4°. Finally the precipitates were redissolved in 800 μl of a KCl–HCl solution (pH = 2·2, i = 0·058). Extinction was measured at 280 nm.

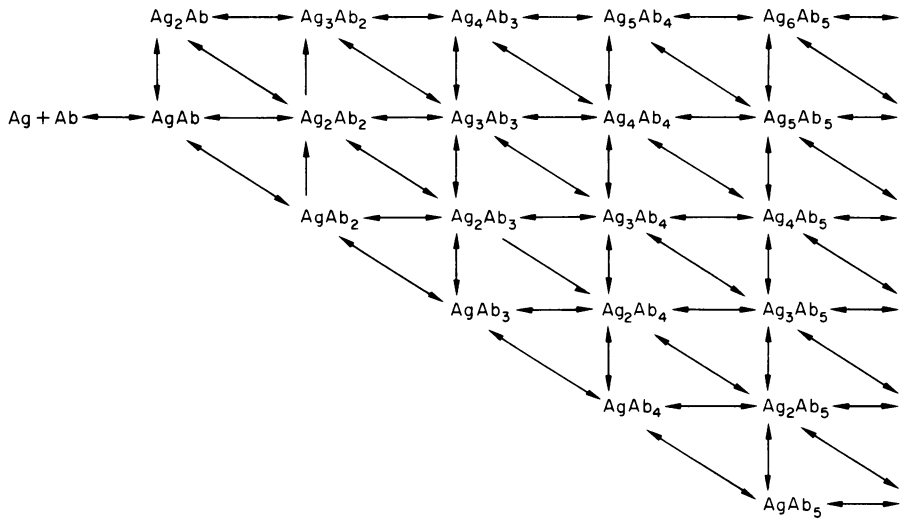

FIG. 1. Reaction scheme for the interaction between a macromolecular antigen and a corresponding IgG. The antigen is denoted by Ag, the antibody by Ab. Vertical arrows reflect growth of a complex by addition of one antigen molecule, diagonal arrows addition of one antibody molecule, and horizontal arrows addition of the simplest complex AgAb. Reproduced from Steensgaard and Funding (1974).

THEORETICAL CONSIDERATIONS

The simulation model is based on the following assumptions. The antigen possesses f identical antigenic determinants, and the antibody has two identical antigen-binding sites. The same association constant applies to all processes, and the activity of a compound is given by its concentration. As in Goldberg's treatment (Goldberg, 1952), cyclical complexes are excluded.

The antigen–antibody complexes (Ag_nAb_m) which can be formed during interactions between an f-valent antigen (Ag) and its bivalent antibodies (Ab) can be summarized in the triangular reaction scheme shown in Fig. 1 as described previously by Steensgaard and Funding (1974). By extension to the right of this scheme all possible antigen–antibody complexes can be included.

The individual processes in this scheme can be described by simple second order kinetical expressions. Hence, each arrow represents an association–dissociation process which imply one of the following expressions:

$$\frac{d[Ag_nAb_m]}{dt} = k_+[Ag][Ag_{n-1}Ab_m] - k_-[Ag_nAb_m] \qquad (1)$$

$$\frac{d[Ag_nAb_m]}{dt} = k_+[Ab][Ag_nAb_{m-1}] - k_-[Ag_nAb_m] \quad (2)$$

$$\frac{d[Ag_nAb_m]}{dt} = k_+[AgAb][Ag_{n-1}Ab_{m-1}] - k_-[Ag_nAb_m] \quad (3)$$

where square brackets denote the actual concentration of the complex in question, k_+ an association velocity constant, and k_- a dissociation velocity constant. The corresponding association constant is given by:

$$K = k_+/k_- \quad (4)$$

A computer program to perform the necessary calculations was written in the programming language PASCAL (Wirth, 1971). The main features of the program are as follows. It contains a sufficiently large matrix comprising the concentrations of each of the possible complexes as outlined in the reaction scheme. It calculates changes in concentration of each complex in short periods of time by use of equations (1), (2) and (3), and following each short period all complex concentrations are adjusted accordingly.

It has proved practical to use K^{-1} as unit of concentration, because this makes the differential equations (1), (2) and (3) dimensionless. The initial concentrations of antigen and antibody are given as input data in units of K^{-1}.

At the beginning of the calculations the concentration of each complex in the matrix is set equal to nought. In the first cycle some AgAb is formed. In the second cycle more AgAb is formed, and some AgAb reacts further forming the complexes Ag_2Ab, Ag_2Ab_2 and $AgAb_2$. In the third and following cycles these complexes react further forming still more complicated complexes according to the actual concentrations and velocity constants. The calculations are stopped when the concentrations of all complexes after several iterations have reached a constant value. These final concentrations of all complexes represent a state of equilibrium where all complexes are in mutual equilibrium.

RESULTS AND DISCUSSION

CALCULATED DISTRIBUTIONS OF COMPLEXES AT DIFFERENT Ag/Ab RATIOS

Table 1 shows four calculated distributions of different complexes obtained by the simulation technique at different initial Ag/Ab ratios. Seemingly, three patterns may be distinguished: (1) in antigen excess, the complex Ag_2Ab dominates, but smaller amounts of other antigen-rich complexes are formed too; (2) at or near equivalence, a rich variety of different complexes is formed; (3) in antibody excess, complexes of the type $AgAb_n$ are formed.

The complexes, shown in Table 1, may be regarded as the thermodynamically most probable complexes under conditions defined solely by the initial concentrations and valences of antigen and antibody, together with a given value of the association constant. It is interesting to note that the general pattern of theoretical complex distributions in principle does agree with the results of experimental studies on albumin–anti-albumin systems. Singer and Campbell (1955), using an analytical ultracentrifuge, found the complex Ag_2Ab, to be dominating in antigen excess. Larger complexes in decreasing antigen excess have been found by density gradient centrifugation (Cochrane and Koffler, 1973; Steensgaard and Funding, 1974).

TABLE 1

CALCULATED COMPLEX DISTRIBUTION AT DIFFERENT Ag/Ab RATIOS

Initial Ag/Ab ratio	Ag_xAb	Ag_xAb_2	Ag_xAb_3	Ag_xAb_4	Ag_xAb_5	Ag_xAb_6	Ag_xAb_7	Ag_xAb_8
20	9·00	0·465	—	—	—	—	—	—
	0·050	—	—	—	—	—	—	—
		—	—	—	—	—	—	—
			—	—	—	—	—	—
				—	—	—	—	—
					—	—	—	—
							—	—
								—
2	2·51	1·13	0·504	0·226	0·101	0·045	0·020	0·009
	0·448	0·201	0·090	0·040	0·018	0·008	0·004	0·002
		0·036	0·016	0·007	0·003	0·001	0·001	—
			0·003	0·001	0·001	—	—	—
				—	—	—	—	—
					—	—	—	—
							—	—
								—
0·5	0·163	0·060	0·022	0·008	0·003	0·001	—	—
	0·366	0·134	0·049	0·018	0·007	0·002	0·001	—
		0·303	0·111	0·041	0·015	0·005	0·002	0·001
			0·250	0·092	0·034	0·012	0·005	0·002
				0·206	0·076	0·028	0·010	0·004
					0·170	0·062	0·023	0·008
							0·051	0·019
								0·042
0·05	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
		0·001	—	—	—	—	—	—
			0·007	—	—	—	—	—
				0·054	—	—	—	—
					0·412	—	—	—
							—	—
								0·001

The values in the table are concentrations in units of K^{-1}. The positions in the table corresponds to the reaction scheme shown in Fig. 1. Hence, column 1 contains the complexes with 1 antibody molecule (Ag_2Ab and $AgAb$). Column 2 contains the complexes with two antibody molecules (Ag_3Ab_2, Ag_2Ab_2 and $AgAb_2$) etc. The initial antibody concentration was taken as 10 K^{-1}, and the antigenic valence as 5.

A THEORY FOR PRECIPITIN REACTIONS

The formation of a precipitate is a characteristic feature of interactions between IgG and a macromolecular antigen *in vitro*. The molecular mechanisms involved in the formation of a precipitate are still largely unknown. Formation of molecular frameworks or lattices is a widely used explanation (Day, 1972; Talmage and Cann, 1961). However, formation of such large molecular aggregates would require formation of intermediates like Ag_4Ab_{16}, and complexes of that size are not among the thermodynamically most probable complexes predicted by the simulation model, neither can they be detected by density gradient centrifugation (Steensgaard and Funding, 1974).

An alternative to the lattice hypothesis is to assume that some of the antibody-rich complexes, such as those typically predicted in antibody excess, are scarcely soluble for physical-chemical reasons, and that the precipitate simply consists of such complexes.

To study if this hypothesis could explain the classical features of a precipitin curve it

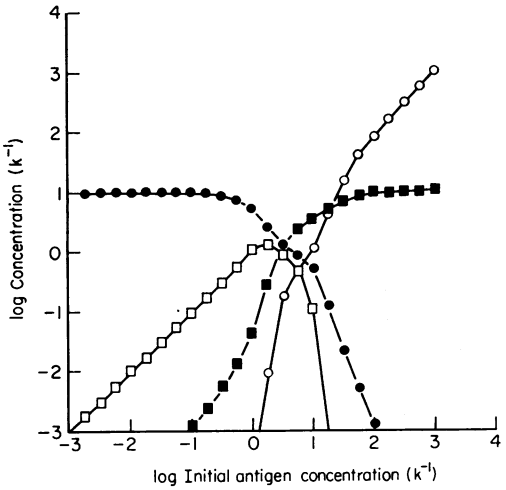

FIG. 2. Theoretical precipitin curve with constant antibody concentration (10 K^{-1}) and increasing antigen concentration. All concentrations are in units of K^{-1}. (○) Free Ag. (●) Free Ab. (■) Soluble complexes. (□) Precipitate.

was, as a first approximation, assumed that predicted complexes containing four or more antibody molecules per antigen molecule were insoluble, whilst the others were soluble. The simulation program was instructed to sum up insoluble and soluble complexes accordingly, giving rise to the theoretical precipitin curve in Fig. 2. It appears from this figure that the amount of precipitate at the lowest antigen concentrations is determined by the amount of available antigen. With increasing antigen concentrations still more

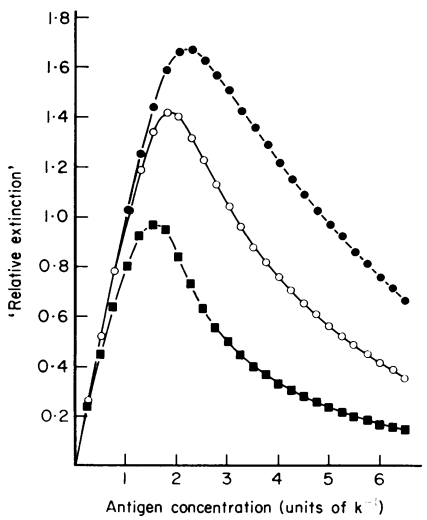

FIG. 3. Theoretical precipitin curves. Concentrations are in units of K^{-1}. 'Relative extinction' has been calculated from the extinction coefficients of human serum albumin ($E_1^{1\ per\ cent}_{cm}$ = 6·0) and of rabbit IgG ($E_1^{1\ per\ cent}_{cm}$ = 15·0), assuming complete solubilization of the calculated precipitate. The upper curve represents a distinction between soluble and insoluble immune complexes, based on the assumption that a complex is insoluble if the Ag/Ab ratio is 1/3 or less (●). In the middle curve this distinction was taken as 1/4 (○), and in the lowest curve as 1/5 (■).

precipitate is predicted. At initial Ag/Ab ratios close to 1, the theoretical precipitin curve reaches a maximum. Further increase in antigen concentration lowers the amount of predicted precipitate, while the formation of small soluble complexes (mainly Ag_2Ab) is favoured. It is interesting to note that Palmiter and Aladjem (1968) have found that the precipitin curves of several experimental systems show linear extremes in double logarithmic plots. It is our opinion that the theoretical precipitin curve in Fig. 2 contains those features which may be regarded as distinctive of precipitating antigen–antibody systems.

Experimental precipitin curves are often obtained by redissolving the precipitate and measuring the extinction at 280 nm. As the extinction coefficients of human serum albumin and of rabbit IgG are known (Sober, 1970), graphs showing the relative extinction of redissolved precipitate versus a linear antigen concentration can be made. Fig. 3 shows three precipitin curves calculated in this way and representing distinctions between soluble and insoluble complexes at Ag/Ab ratios of 1/3, 1/4 and 1/5, respectively. It appears from this figure that different distinctions between soluble and insoluble complexes will affect the size of the calculated precipitin curve as well as the shape of the curve. Experimental precipitin curves obtained for different antigens may differ from system to system. However, the similarity in shape of the shown calculated precipitin curve (Fig. 3, curve 1/5) and the shape of experimental precipitin curves of a fibrinogen–anti-fibrinogen system (Fritz, Lassiter and Day, 1967) is striking.

COMPARISON WITH GOLDBERG'S THEORY

The main difference between the concepts expressed in the present simulation model and those expressed in Goldberg's classical theory (Goldberg, 1952) is that the simulation model uses a second order association constant as a measure of the affinity between the antigen and its antibody whereas Goldberg's theory implies a value, p, referring to the extent of reaction and defined as the fraction of reacted antigenic sites. An explicit relationship between these two variables can be formulated only for heavy antigen excess conditions (Singer and Campbell, 1953), and for this reason an exact number-to-number comparison between predictions from the two theories cannot be made. However, in spite of these differences there are striking similarities between the predictions of the two theories. Goldberg's theory predicts Ag_2Ab to be the dominating aggregate in antigen excess, and a rich variety of complexes at or near equivalence (Talmage and Cann, 1961). The same general picture is predicted by the simulation model as shown in Table 1. In heavy antibody excess Goldberg's theory predicts the aggregate $AgAb_f$ to be dominating, whereas the simulation model predicts this and some closely related complexes to be dominating.

The advantages of the simulation model then are the following. It uses well-known thermodynamic variables only, and it provides a simple explanation of precipitin curves. Moreover, it is designed to be an open system easily modifiable to study the effects of new and improved concepts in this field.

DEPENDENCY OF ANTIBODY CONCENTRATION

The underlying assumption of the simulation model that antigen–antibody interactions can be described by second order thermodynamical expressions implies that the outcome

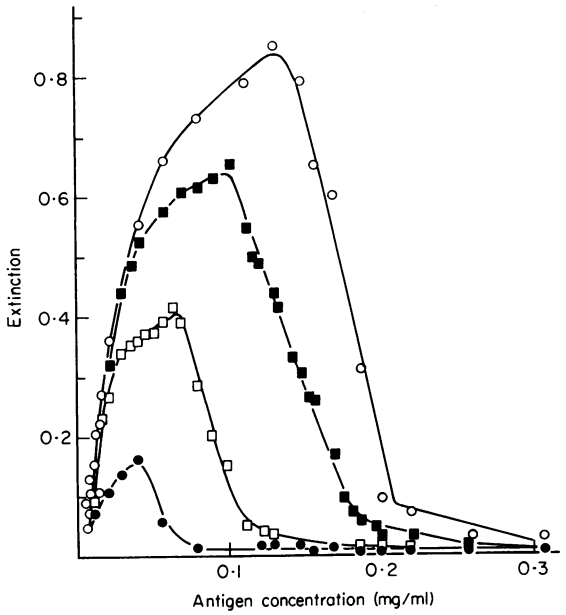

FIG. 4. Experimental precipitin curves, obtained from a human serum albumin–rabbit anti-human serum albumin IgG system. Extinction means the extinction at 280 nm of the redissolved precipitate. Antibody concentration of: (○) 2 mg/ml; (■) 1·43 mg/ml; (□) 1·0 mg/ml; (●) 0·67 mg/ml, respectively.

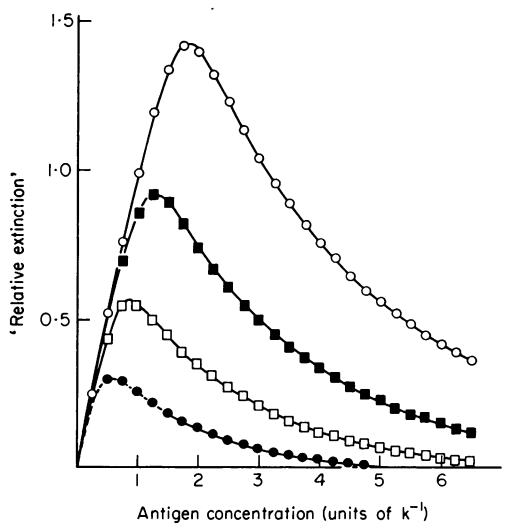

FIG. 5. Theoretical precipitin curves. 'Relative extinction' has been calculated from the extinction coefficients of human serum albumin ($E_1^{\frac{1}{2}}{}_{cm}^{per\ cent} = 6{\cdot}0$) and of rabbit IgG ($E_1^{\frac{1}{2}}{}_{cm}^{per\ cent} = 15{\cdot}0$), assuming complete solubilization of the calculated precipitate. The four curves have been calculated for four antibody concentrations of: (○) 2 mg/ml; (■) 1·43 mg/ml; (□) 1·0 mg/ml; (●) 0·67 mg/ml, respectively.

of an immunochemical interaction should depend equally on the absolute concentrations of antigen and of antibody.

To study this, four precipitin curves were made experimentally (Fig. 4), and concomitantly four theoretical precipitin curves were calculated (Fig. 5). Comparison between these figures shows that the experimental and the calculated precipitin curves depend in the same way on antibody concentration. In both figures, the points of maximum precipitation are changed in the same manner with decreasing antibody concentrations, and in both cases the areas below the precipitin curves parallel the decreasing antibody concentration. Thus, these comparisons provide further support for the validity of the simulation model.

GENERAL DISCUSSION

Two aspects of the calculated results may deserve special interest. First, large soluble immune complexes of a type which may cause glomerulonephritis according to Cochrane and Koffler (1973) are predicted mainly in low antigen excess, and they are predicted mainly under conditions where complexes of a supposed insoluble type are formed also. Secondly, the outcome of immunochemical interactions seems to be highly dependent on the concentrations employed, and hence these calculated results stress the importance of the use of the same antibody concentrations throughout series of serological measurements.

The present work also points out two aspects of immunochemical interactions which should be studied in detail in future works.

First, antibodies against an antigen are generally believed to consist of a heterogeneous population of molecules. To incorporate the concept of antibody heterogeneity it is necessary to define this concept in physical-chemical terms. This is hardly possible from available knowledge, but evidently such knowledge should comprise the amounts, the specificities and the affinities of antibodies against each individual antigenic determinant. It is hoped that attempts to account for antibody heterogeneity in computer simulations may lead to a precise definition of this concept.

It should, however, not be forgotten that the present simulation model where all antigenic determinants are treated as if they were identical is in accordance with the concept of multifunctional antibodies described by Richards and Konigsberg (1973).

Secondly, the present simulation model gives a description of those features of antigen–antibody interactions which are believed to be common to systems involving macromolecular antigens and their corresponding immunoglobulins. However, as precipitin curves representative of different systems are somewhat different in shape, and as it is known that the molecular properties of the antigen may influence the precipitin pattern (Siskind, 1966), it is clear that some additional parameters describing solubility properties should be introduced into the simulation model in order to make it completely system-specific.

The development of the simulation model for antigen–antibody interactions may be taken, also, as an attempt to study the extent to which the outcome of immunochemical interactions can be deduced from basic thermodynamic considerations. In this sense the present simulation model rests on a minimum of requirements; namely that one association constant applies to all mutual interactions, and that the precipitate consists of the most probable complexes in antibody excess. Then, from knowledge of concentrations and valences of antigen as well as antibody, complex distribution patterns and precipitin

curves may be calculated. The good agreement between calculated results and results obtained experimentally, as discussed in the previous sections, leads to the conclusion that the main features of interactions between a macromolecular antigen and a corresponding IgG are deducible from these very basic and very general thermodynamic considerations.

The simulation model has several potential applications. It can be used to study the importance of parameters already involved in the model, and of other new parameters which may be introduced to study the consequences of new concepts. The possibility of using the simulation model in an expanded version for numerical fittings of parameters on the basis of experimental data exists, and, finally, it may be useful in the planning of experiments designed to clarify some of the unsettled problems in molecular immunology.

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