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A Theory of the Structure and Process of Formation of Antibodies*

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I. Introduction

During the past four years I have been making an effort to understand and interpret serological phenomena in terms of molecular structure and molecular interactions. The field of immunology is so extensive and the experimental observations are so complex (and occasionally contradictory) that no one has found it possible to induce a theory of the structure of antibodies from the observational material. As an alternative method of attack we may propound and attempt to answer the following questions: What is the simplest structure which can be suggested, on the basis of the extensive information now available about intramolecular and intermolecular forces,[1] for a molecule with the properties observed for antibodies, and what is the simplest reasonable process of formation of such a molecule? Proceeding in this way, I have developed a detailed theory of the structure and process of formation of antibodies and the nature of serological reactions which is more definite and more widely applicable than earlier theories, and which is compatible with our present knowledge of the structure and properties of simple molecules as well as with most of the direct empirical information about antibodies. This theory is described and discussed below.

II. The Proposed Theory of the Structure and Process of Formation of Antibodies

When an antigen is injected into an animal some of its molecules are captured and held in the region of antibody production.[2] An antibody to this antigen is a molecule with a configuration which is complementary to that of a portion of the antigen molecule.[3] This complementariness gives rise to specific forces of appreciable strength between the antibody molecule and the antigen molecule; we may describe this as a bond between the two molecules. I assume, with Marrack, Heidelberger, and other investigators,[4] that the precipitate obtained in the precipitin reaction is a framework,[5] and that to be effective in forming the framework an antibody molecule must have two or more distinct regions with surface configuration complementary to that of the antigen. The rule of parsimony (the use of the minimum effort to achieve the result) suggests that there are only two such regions, that is, that the antibody molecules are at the most bivalent. The proposed theory is based on this reasonable assumption. It would, of course, be possible to expand the theory in such a way as to provide a mechanism for the formation of antibody molecules with valence higher than two; but this would make the theory considerably more complex, and it is likely that antibodies with valence higher than two occur only rarely, if at all.

Antibodies are similar in amino-acid composition to one or another of the fractions of serum globulin of the animal producing the serum. It is known that there exist antibodies of different classes, with different molecular weights—the molecular weights of rabbit antibody and of monkey antibody (to pneumococcus polysaccharide) are about 157,000, whereas those of pig, cow, and horse antibodies are about 930,000.[6] The following discussion is for antibodies with molecular weight about 160,000, and similar in constitution to the γ fraction of serum globulin[7]; the changes to be made to cause it to apply to antibodies of other classes are obvious.

* Some of the material in this paper was presented on April 17th, 1940, at The Rockefeller Institute for Medical Research and on April 23rd, 1940, at the meeting of the National Academy of Sciences, Washington, D. C.

(1) See, for a summary of this information, L. Pauling, "The Nature of the Chemical Bond and the Structure of Molecules and Crystals," Cornell University Press, Ithaca, New York, second edition, 1940.

(2) There is some evidence that this is the cells of the reticuloendothelial system: see Florence R. Sabin, *J. Exptl. Med.*, **70**, 67 (1939), and references quoted by her.

(3) The idea of complementary structures for antibody and antigen was suggested by (a) F. Breinl and F. Haurowitz, *Z. physiol. Chem.*, **192**, 45 (1930); (b) Stuart Mudd, *J. Immunol.*, **23**, 423 (1932); (c) J. Alexander, *Protoplasma*, **14**, 296 (1931), and has come to be rather generally accepted. There is some intimation of it in the early work of Ehrlich and of Bordet.

(4) J. R. Marrack, "The Chemistry of Antigens and Antibodies," Report No. 230 of the Medical Research Council, His Majesty's Stationery Office, London, 1938; M. Heidelberger, *Chem. Rev.*, **24**, 323 (1939), and earlier papers.

(5) The framework is sometimes called a "lattice" by immunochemists; the use of this word in immunology is to be discouraged because of the implication of regularity associated with it through its application in crystallography.

(6) E. A. Kabat and K. O. Pedersen, *Science*, **87**, 372 (1938); E. A. Kabat, *J. Exptl. Med.*, **69**, 103 (1939).

(7) A. Tiselius, *Biochem. J.*, **31**, 1464 (1937); *Trans. Faraday Soc.*, **33**, 524 (1937); T. Svedberg, *Ind. Eng. Chem.*, **30**, 113 (1938).

The effect of an antigen in determining the structure of an antibody molecule might involve the ordering of the amino-acid residues in the polypeptide chains in a way different from that in the normal globulin, as suggested by Breinl and Haurowitz[3a] and Mudd.[3b] I assume, however, that this is not so, but that *all antibody molecules contain the same polypeptide chains as normal globulin, and differ from normal globulin only in the configuration of the chain; that is, in the way that the chain is coiled in the molecule.* There is at present no direct evidence supporting this assumption. The assumption is made because, although I have found it impossible to formulate in detail a reasonable mechanism whereby the order of amino-acid residues in the chain would be determined by the antigen, a simple and reasonable mechanism, described below, can be advanced whereby the antigen causes the polypeptide chain to assume a configuration complementary to the antigen. The number of configurations accessible to the polypeptide chain is so great as to provide an explanation of the ability of an animal to form antibodies with considerable specificity for an apparently unlimited number of different antigens,[8] without the necessity of invoking also a variation in the amino-acid composition or amino-acid order.[9]

The Postulated Process of Formation of Antibodies.—Let us assume that the globulin molecule consists of a single polypeptide chain, containing several hundred amino-acid residues, and that the order of amino-acid residues is such that for the center of the chain one of the accessible configurations is much more stable than any other, whereas the two end parts of the chain are of such a nature that there exist for them many configurations with nearly the same energy. (This point is discussed in detail in Section IV.) Four steps in our postulated process of formation of a normal globulin molecule are illustrated on the left side of Fig. 1. At stage I the polypeptide chain has been synthesized, the amino-acid residues having been marshalled into the proper order, presumably with the aid of polypeptidases and protein templates, and the two ends of the chain, A and C, each containing perhaps two hundred residues, have been liberated with the unstable extended configuration. (The horizontal line in each drawing separates the region, below the line, in which the polypeptide chain is not able to change its configuration from the region, above the line, where this is possible.) Each of these chain ends then coils up into the most stable or one of the most stable of the accessible configurations (stage II) and is tied into this configuration by the formation of hydrogen bonds and other weak bonds between parts of the chain. The central part B of the chain is then liberated (stage III) and assumes its stable folded configuration (stage IV) to give the completed globulin molecule.

There are also indicated in Fig. 1 six stages in the process of formation of an antibody molecule. In stage I there are shown an antigen molecule held at a place of globulin production and a globulin molecule with its two ends A and C liberated with the extended configuration. At stage II each of the ends has assumed a stable coiled configuration. These stable configurations A′ and C′ are not, however, identical with those A and C assumed in the absence of the antigen. The atoms and groups which form the surface of the antigen will attract certain complementary parts of the globulin chain (a negatively-charged group, for example, attracting a positively-charged group) and repel other parts; as a result of these interactions the configurations A′ and C′ of the chain ends which are stable in the presence of the antigen and which are accordingly assumed in the presence of the antigen will be such that there is attraction between the coiled globulin chain ends and the antigen, due to their complementarity in structure. The configuration assumed by the chain end may be any one of a large number, depending upon which part of the surface of the antigen happens to exert its influence on the chain end and how large a region of the surface happens to be covered by it.

When the central part B of the globulin chain is liberated from the place of its synthesis (stage III), one of two processes may occur. If the forces of attraction between the antigen and the portions A′ and C′ are extremely strong, they will remain bonded to the antigen for an indefinite time, and nothing further of interest will happen. If the forces are somewhat weaker, however, one will in time break away—dissociate from the antigen (stage IV). Then the portion B of the chain will fold up to achieve its normal stable configuration

(8) See K. Landsteiner, "The Specificity of Serological Reactions," Charles C. Thomas, Springfield, Ill., 1936.

(9) It has been pointed out by A. Rothen and K. Landsteiner, *Science*, **90**, 65 (1939), that the possibility of different ways of folding the same polypeptide chain to obtain different antibodies is worth considering.

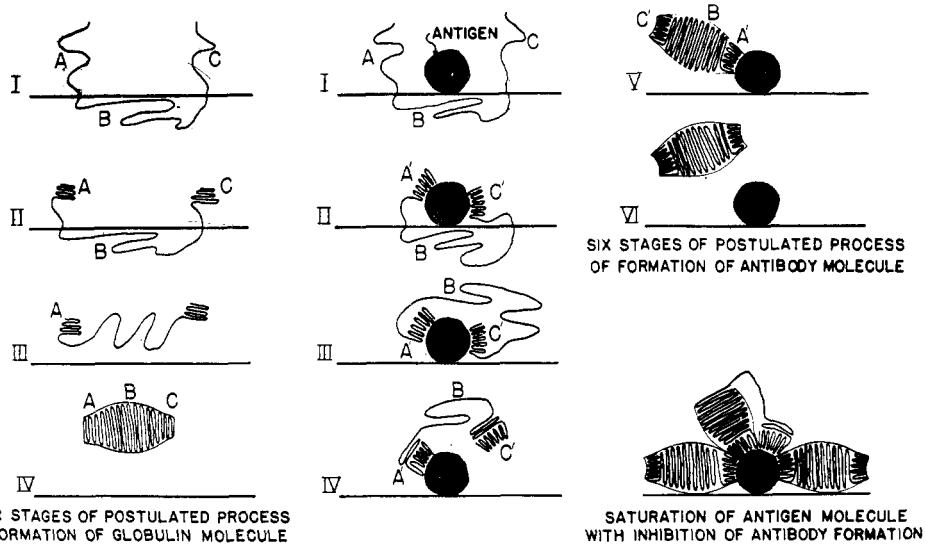

Fig. 1.—Diagrams representing four stages in the process of formation of a molecule of normal serum globulin (left side of figure) and six stages in the process of formation of an antibody molecule as the result of interaction of the globulin polypeptide chain with an antigen molecule. There is also shown (lower right) an antigen molecule surrounded by attached antibody molecules or parts of molecules and thus inhibited from further antibody formation.

(stage V), making a completed antibody molecule. In time this will dissociate from the antigen and float away (stage VI). It is possible that an auxiliary mechanism for freeing the active ends A′ and C′ from the antigen molecule comes into operation; this is discussed in Section VI.

The middle part of the antibody molecule thus produced would be like that of a normal globulin molecule, and the two ends would have configurations more or less complementary to parts of the surface of the antigen. These two active ends are effective in different directions, so that, after the antibody is completely formed, only one of them at a time can grasp a particular antigen molecule.

The antigen molecule, after its desertion by the newly-formed antibody molecule, may serve as the pattern for another, and continue to serve until its surface is covered by very strongly held antibodies or portions of antibodies or until the concentration of antibodies becomes so great that even with weak forces operating the antigen is combined with antibodies most of the time (as illustrated in Fig. 1), or until the antigen molecule is destroyed or escapes from the region of globulin formation.

III. Some Points of Comparison with Experiment

a. The Heterogeneity of Immune Sera.—The theory requires that the serum homologous to a given antigen be not homogeneous, but heterogeneous, containing antibody molecules of greatly varied configurations. Many of the antibody molecules will be bivalent, with two active ends with configuration complementary to portions of the surface of an antigen molecule. Great variety in this complementary configuration would be expected to result from the accidental approximation to one or another surface region, and further variety from variation in position of the antigen molecule relative to the point of liberation of the globulin chain end and from accidental coiling and linking of the chain end before it comes under the influence of the antigen. Some of the antibody molecules would be univalent, one of the chain ends having, because of its too great distance from the antigen, folded into a normal globulin configuration.

These predictions are verified by experimental results. It is well known that an immune serum to one antigen will, as a rule, react with a related heterologous antigen, and that after exhaustion with the latter there remains a fraction which will still react with the original antigen. Landsteiner and van der Scheer,[10] using as antigens azoproteins carrying various haptens containing the same active group, have shown that the antiserum for one antigen contains various fractions differing in the strength of their attraction for the haptens.

(10) K. Landsteiner and J. van der Scheer, *J. Exptl. Med.*, **63**, 325 (1936).

By the quantitative study of precipitin reactions Heidelberger and Kendall[11] reached the same conclusion, and showed in addition that even after prolonged immunization the antiserum studied (anti-egg albumin) contained much low-grade antibody, incapable by itself of forming a precipitate with the antigen, but with the property of being carried down in the precipitate formed with a more reactive fraction.[12]

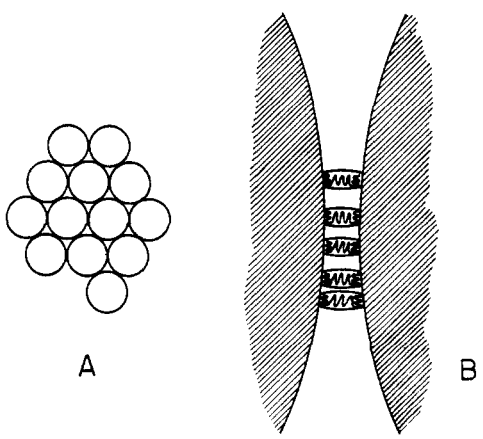

Fig. 2.—(A) Diagram representing agglutinated cells. (B) Diagram of the region of contact of two cells, showing the postulated structure and mode of action of agglutinin molecules.

b. The Bivalence of Antibodies and the Multivalence of Antigens.—Our theory is based on the idea that the precipitate formed in the precipitin reaction is a network of antibody and antigen molecules in which many or all of the antibody molecules grasp two antigen molecules apiece and the antigen molecules are grasped by several antibody molecules. The direct experimental evidence for this picture of the precipitate has been ably discussed by its propounders and supporters, Marrack and Heidelberger and Kendall, and need not be reviewed here. To the structural chemist it is clear that this picture of the precipitate must be correct. The great specificity of antibody–antigen interactions requires that a definite bond be formed between an antibody molecule and an antigen molecule. If antibodies or antigens were univalent, this would lead to complexes of one antigen molecule and one or more antibody molecules (or of one antibody molecule and one or more antigen molecules), and we know from experience with proteins that these aggregates would in general remain in solution. If both antibody and antigen are multivalent, however, the complex will grow to an aggregate of indefinite size, which is the precipitate.

This process is observed directly in the agglutination of cells. On the addition of an agglutinin to a cell suspension the cells are seen to clump together. It is obvious that the agglutinin molecules which are holding the cells together are bivalent[13]—each has two active ends, with configuration complementary to that of a portion of the surface of the cells; the agglutinin molecules hold the cells together at their regions of contact, as shown in Fig. 2.

It seems probable that all antibodies have this structure—that they are bivalent, with their two active regions oppositely directed. Heidelberger and his collaborators and Marrack have emphasized the multivalence of antibodies and antigens,[14] but limitation of the valence of antibodies to the maximum value two (ignoring the exceptional case of the attachment of two or more antigens or haptens to the same end region of an antibody) has not previously been made.

The maximum valence of an antigen molecule would be given by the ratio of its surface area to the area effectively occupied by one antibody molecule, if all regions of the antigen surface were active. In the special case that the antibody were able to combine only with one group (a hapten, say, with immunization effected by use of another antigen with the same hapten attached) the maximum valence of the antigen would be equal to the number of groups per molecule.

c. The Antibody–Antigen Molecular Ratio in Precipitates.—Our theory provides an immediate simple explanation of the observed antibody–antigen molecular ratios in precipitates. Under optimum conditions a precipitate will be formed in which all the valences of the antibody and antigen molecules are satisfied. An idealized repre-

(11) M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, **61**, 559 (1935); **62**, 467, 697 (1935).

(12) The older experimental results bearing on this question did not permit a clear distinction between antibody fractions differing in being complementary to different active groups in the antigen and fractions differing in the extent of their complementariness to the same group. The experiments are discussed in Marrack's monograph.

(13) Following Heidelberger and Kendall, I use the terminology of chemical valence theory in discussing the specific mutual attraction of antigen and antibody. The antibody–antigen "valence bonds" are not, of course, to be confused with ordinary covalent chemical bonds; they are due instead to the integrated weak forces discussed in Sec. IV.

(14) Professor Heidelberger has informed me that in their quantitative treatment of data on the precipitin reaction he and Dr. Kendall have found no incompatibility with this restriction; in their papers they discussed the general case of multivalence of antibody as well as of antigen.

sentation of a portion of such a precipitate is given in Fig. 3. The figure shows a part of a layer with each antigen molecule bonded to six surrounding antibody molecules; this structure represents the value $N = 12$ for the valence of the antigen, each antigen molecule being attached also to three antibody molecules above the layer represented and to three below. Each antibody molecule is bonded to two antigen molecules, one at each end. An ideal structure of the antibody–antigen precipitate for $N = 12$ may be described as having antigen molecules at the positions corresponding to closest packing, with the twelve antibody molecules which surround each antigen molecule lying along the lines connecting it with the twelve nearest antigen neighbors.

Similar ideal structures can be suggested for other values of the antigen valence. The antigen molecules might be arranged for $N = 8$ at the points of a body-centered cubic lattice, and for $N = 6$ at the points of a simple cubic lattice, with antibody molecules along the connecting lines. For $N = 4$ the antigen molecules, connected by antibody molecules, might lie at the points occupied by carbon atoms in diamond; or two such frameworks might interpenetrate, as in the cuprous oxide arrangement (copper and oxygen atoms being replaced by antibody and antigen molecules, respectively).

It is not to be inferred that the actual precipitates have the regularity of structure of these ideal arrangements. The nature of the process of antibody formation, involving the use of a portion of the antigen surface selected at random as the template for the molding of an active end of an antibody molecule, introduces so much irregularity in the framework that a regular structure analogous to that of a crystal is probably never formed. The precipitate is to be compared rather with a glass such as silica glass, in which each silicon atom is surrounded tetrahedrally by four oxygen atoms and each oxygen atom is bonded to two silicon atoms, but which lacks further orderliness of arrangement. Additional disorder is introduced in the precipitate by variation in the effective valence of the antigen molecules and by the inclusion of antibody molecules with only one active end.

The antibody-antigen molecular ratio R of a precipitate is given by the equation

$$R = N_{\text{eff.}}(\text{antigen})/N_{\text{eff.}}(\text{antibody}) \qquad (1)$$

in which $N_{\text{eff.}}(\text{antigen})$ and $N_{\text{eff.}}(\text{antibody})$ are the

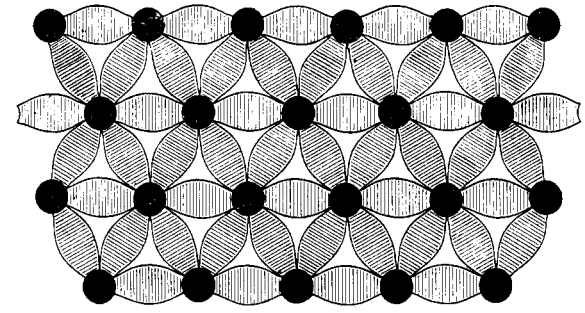

Fig. 3.—A portion of an ideal antibody–antigen framework. One plane of the structure corresponding to the value twelve for the valence of the antigen molecules is shown.

average effective valences of antigen and antibody molecules, respectively. The maximum value of $N_{\text{eff.}}(\text{antibody})$ is 2 (ignoring the exceptional possibility that two small haptens can attach themselves to the same combining region at one end of the antibody; steric repulsion of antigen molecules would usually prevent this occurrence), and under optimum conditions for formation of the most stable precipitate we may expect this maximum value to be closely approached. The antibody–antigen molecular ratio then becomes

$$R = N/2 \qquad (2)$$

in which N is $N_{\text{eff.}}(\text{antigen})$. Now a sphere can be brought into contact with twelve surrounding spheres equal to it in size; hence a spherical antigen molecule with molecular weight equal to that of the antibody (157,000) might have the valence $N = 12$, if all regions of the antigen surface were active and if the antibody molecules were spherical; the assumption of elongated antibody molecules would permit the valence to be somewhat larger. The value 12 of N corresponds to the value 6 for the ratio R. For larger antigens larger values of R would be expected, and for smaller ones smaller values. Even for antigens with molecular weight as small as 11,000 the predicted maximum value of R is 4 (for spherical antibodies) or larger. In fact, a simple calculation based on the packing of spheres[15] leads to the results given in Table I.[16] It is seen

(15) See L. Pauling, ref. 1, Sec. 48a.

(16) It may be noted that values of R calculated in the text change with molecular weight in about the same way as those calculated by W. C. Boyd and S. B. Hooker, *J. Gen. Physiol.*, **17**, 341 (1934), on the assumption that each antigen molecule is surrounded by a close-packed layer of (univalent) antibody molecules. The Boyd–Hooker values agree roughly with experiment (Marrack, *loc. cit.*, p. 161).

TABLE I

COÖRDINATION OF SPHERICAL ANTIBODY MOLECULES ABOUT SPHERICAL ANTIGEN MOLECULES

No. of antibody molecules about antigen	Minimum ratio of antigen radius to antibody radius	Minimum mol. wt. of antigen (antibody 160,000)	Maximum mol. ratio $\frac{\text{Antibody}}{\text{Antigen}}$ in satd. ppt.	Maximum mass ratio $\frac{\text{Antibody}}{\text{Antigen}}$
12	1.000	160,000	6	6
8	0.732	63,000	4	10
6	.414	11,000	3	44
4	.225	1,800	2	178

that our theory provides a simple explanation of the fact that for antigens of molecular weight equal to or less than that of the antibody the precipitate contains considerably more antibody than antigen. The values given in Table I are not to be considered as having rigorous quantitative significance. The calculated maximum molecular ratio would be larger for elongated antibody molecules than for spherical antibody molecules, and larger for non-spherical than for spherical antigen molecules, and, moreover, in many sera the antibodies might be complementary in the main only to certain surface regions of the antigen, the number of these determining the valence of the antigen. That this is so is indicated by the observation[17] that after long immunization of a rabbit with egg albumin serum was obtained giving a precipitate with a considerably larger molecular ratio than that for earlier bleedings.[18]

Observed values of R for precipitates formed in the equivalence zone (with amounts of antigen solution and serum so chosen that neither excess antibody nor excess antigen can be detected in the supernate) for antigens with molecular weights between about 4000 and 700,000 lie between about 2.5 for the smaller antigens and 15 for the larger ones.[19] It is seen that the values of R are somewhat less than the corresponding values from Table I, which indicates that not all of the surface regions of the antigens are effective.

(17) M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, **62**, 697 (1935).

(18) The discussion of the nature of this phenomenon of change in the serum on continued immunization must await the detailed treatment of intracellular antibody–antigen interactions. The phenomenon may involve the masking of the more effective surface regions of the injected antigen molecules by serum antibodies produced by earlier inoculations, leaving only the less effective regions available for template action.

(19) It is our restriction of the valence of the antibody to the maximum value two which leads to our explanation of the antibody–antigen ratios. The general observation of values of R considerably greater than 1 is not accounted for by a framework theory in which antibody and antigen molecules are both multivalent, unless some auxiliary postulate is invoked to make the effective valence of antigen considerably greater than that of antibody.

The data given in Table II are those of Heidelberger and his collaborators; the values reported by other investigators are similar in magnitude.

TABLE II

VALUES OF ANTIBODY–ANTIGEN MOLECULAR RATIOS FOR PRECIPITATES FROM RABBIT ANTISERA[a]

Antigen	Mol. wt.	R Equivalence zone	R Extreme antibody excess	R Antigen excess	R Soluble compound
Egg albumin	42,000	2.5–3	5	2	1
Dye egg albumin[b]	46,000	2.5–3	5	3/4	1/2
Serum albumin	67,000	3–4	6	2	1
Thyroglobulin	700,000	10–14	40	2	1

[a] The experimental values are those obtained by Heidelberger and collaborators, and quoted by M. Heidelberger, THIS JOURNAL, **60**, 242 (1938). [b] R-salt-azobiphenylazo egg albumin.

In a precipitate formed from a solution containing an excess of antibody not all of the antibody valences will be saturated. At the limit of antibody excess the precipitate will be a network of linear aggregates with a structure such as that represented in Fig. 4. Here each antigen molecule (with an occasional exception) is surrounded by N antibody molecules, only two of which bond it to neighboring antigen molecules. The padded strings formed in this way are tied together by an occasional cross-link to form the precipitate. The antibody-antigen molecular ratio is seen to be close to $N - 1$, which is one less than twice the value $N/2$ for the valence-saturated precipitate. The predicted relation between these ratios

$$R_{\text{antibody excess}} = 2R_{\text{equivalence zone}} - 1$$

is seen from the data in Table II to be verified approximately by experiment for the antigens other than thyroglobulin.

The discrepancy shown by thyroglobulin is, indeed, to be expected for an antigen with molecular weight greater than that of the antibody. The requirements of geometry are such that an arrangement in which each antigen is bonded equivalently by antibodies to more than twelve surrounding antigens is impossible. Hence for large antigen molecules the molecular ratio can exceed 6 in the valence-saturated precipitate only if two or more antibody molecules are shared between the same pair of antigen molecules, whereas in the antibody-excess region the entire surface of the large antigen may be covered by antibody molecules.

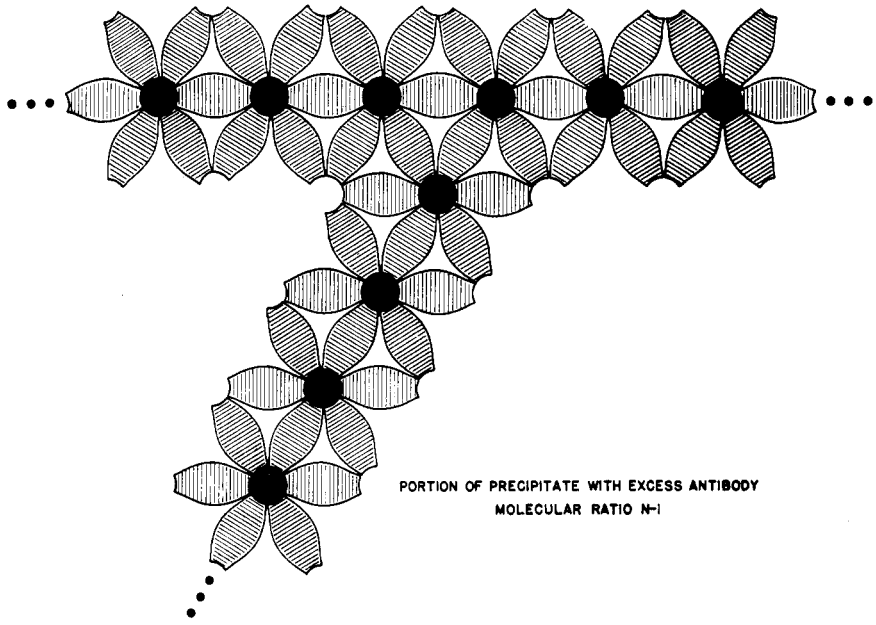

Fig. 4.—A portion of an antibody–antigen network formed in the region of antibody excess.

With antigen excess the precipitate formed will have the limiting structure shown in Fig. 5, in which (with an occasional exception) both antigen and antibody are bivalent, the molecular ratio approaching unity. The reported experimental values for this ratio (Table II) lie between 2 and $^3/_4$.

With great excess of antigen finite complexes which remain in solution are formed, with structures such as shown in Fig. 6. For these the molecular ratio varies between 1 and the minimum value $^1/_2$. It is observed that in general no precipitate forms in the region of great antigen excess, and Heidelberger and his collaborators have in fact assigned values 1 and $^1/_2$ to the molecular ratios for the complexes in solution.

Whereas precipitation is inhibited by antigen excess, it usually occurs even with great antibody excess, although soluble complexes with molecular ratio N and the structure shown in Fig. 7 are expected to exist. It seems probable that the difference in behavior of systems in the excess antigen region and excess antibody region is to be attributed to the fact that the molecular ratio for precipitate and soluble complex differs by a factor as great as two for the former case, and by only $N/(N-1)$ for the latter.

d. The Use of a Single Antigen Molecule as the Template for an Antibody Molecule.—There are two ways in which an antibody molecule with two opposed active regions complementary to the antigen might be produced. One is the way described in Section II. The other would involve the manufacture of the antibody molecule in its final configuration between two antigen molecules, one of which would serve as the pattern for one antibody end and the other for the second. No attempt to decide between these alternatives seems to have been made before; there exists

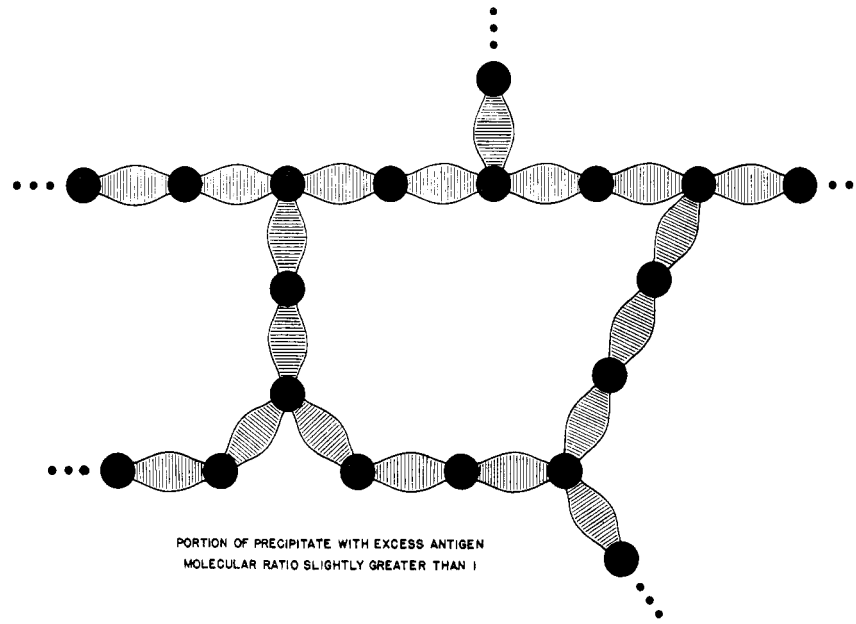

Fig. 5.—A portion of the network formed in the region of antigen excess.

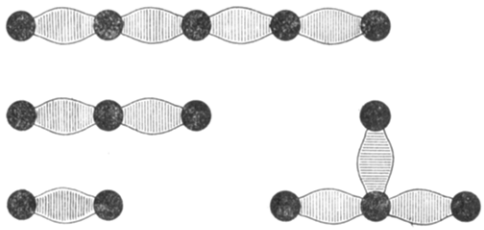

Fig. 6.—Representative soluble complexes formed with excess antigen.

evidence, however, some of which is mentioned below, to indicate that the first method of antibody production, involving only one antigen molecule, occurs predominantly. It is for this reason that I have developed the rather complicated theory described above, with the two end portions of the antibody forming first, one (or both) then separating from the antigen, and the central part of the antibody then assuming its shape and holding the active ends in position for attachment to two antigen molecules.

Fig. 7.—A soluble complex formed with excess antibody.

This theory requires that the formation of antibody be a reaction of the first order with respect to the antigen, whereas the other alternative would require it to be of the second order. There exists very little evidence as to whether on immunization with small amounts of antigen the antibody production is proportional to the amount of antigen injected or to its square. Some support for the one-antigen-molecule theory is provided by the experiments dealing with the injection of a mixture of antigens. If two antigen molecules were required for antibody formation, it would be expected that antibodies A′–B′, A′–C′, B′–C′, · · · complementary to two different antigens A and B, A and C, B and C, · · · as well as those A′–A′, B′–B′, C′–C′, · · · complementary to a single antigen would be formed. The available evidence speaks strongly against this. Thus Dean, Taylor, and Adair[20] have reported that the serum produced by immunization with a mixture of egg albumin and serum albumin contains distinct antibodies homologous to the two antigens, and that precipitation with one antigen leaves the amount of the heterologous antibody unaltered. An even more rigorous demonstration was furnished by Heidelberger and Kabat,[21] who, from the serum of a cow which had been injected with types I, II, and III pneumococci, isolated in succession, with the corresponding specific polysaccharide, the three anticarbohydrates, each in an apparently pure state and with no appreciable cross-reactivity as to pneumococcus type. In another striking experiment Hektoen and Boor[22] found that a serum obtained on injecting a rabbit with a mixture of 35 antigens reacted with 34 of the antigens, and that absorption with any one had in the main little effect on subsequent reaction with another. Since on the two-antigen-molecule theory the amount of antibodies A′–A′, B′–B′, · · · capable of causing precipitation with a single antigen would be small compared with the total amount of antibody (of the order of $1/n$, for n antigens—about 3% in this case), these qualitative observations provide significant evidence in favor of the alternative theory.

e. Criteria for Antigenic Power.—There has been extensive discussion of the question of what makes a substance an antigen, but no generally accepted conclusions have been reached. Our theory permits the formulation of the following reasonable criteria for antigenic activity:

1. The antigen molecule must contain active groups, capable of sufficiently strong interaction with the globulin chain to influence its configuration.

2. The configuration of the antigen molecule must be well-defined over surface regions large enough to give rise to an integrated antibody–antigen force sufficient to hold the molecules together.

3. The antigen molecule must be large enough to have two or more such surface regions, and in case that the antigenic activity depends upon a particular group the molecule must contain at least two of these groups. (This criterion applies to antibodies effective in the precipitin and agglutinin reactions and in anaphylaxis.)

These criteria are satisfied by substances known to have antigenic action. Many proteins, some carbohydrates with high molecular weight (bacterial polysaccharides, invertebrate glycogen[23]), and some lipids and carbohydrate–lipid complexes are antigenic. The simple chemical substances so far studied have been found to be inactive, except those which are capable of combining with proteins in the body. Non-antigenic

(20) H. R. Dean, G. L. Taylor and M. E. Adair, *J. Hyg.*, **35**, 69 (1935).

(21) M. Heidelberger and E. A. Kabat, *J. Exptl. Med.*, **67**, 181 (1938).

(22) L. Hektoen and A. K. Boor, *J. Infect. Diseases*, **48**, 588 (1931).

(23) D. H. Campbell, *Proc. Soc. Exptl. Biol. Med.*, **36**, 511 (1937); *J. Parasitol.*, **23**, 348 (1937).

substances have been reported to become antigenic when adsorbed on particles (Forssman antigen on kaolin[24]); in this case the particle with adsorbed hapten is to be considered the antigen "molecule" of our theory. I predict that relatively simple molecules containing two or more haptens will be found to be antigenic; experiments to test this prediction are now under way.

IV. A More Detailed Discussion of the Structure of Antibodies and Other Proteins

There has been gathered so far very little direct evidence regarding the detailed structure of protein molecules. Chemical information is compatible with the polypeptide-chain theory of protein structure, and this theory is also supported by the rather small amount of pertinent X-ray evidence.[25] It was pointed out some years ago[26] that the well-defined properties of native proteins require that their molecules have definite configurations, the polypeptide chain or chains in a molecule being coiled in a definite way and held in position by forces acting between parts of the chains. The phenomenon of denaturation involves the loss of configuration through the partial or complete uncoiling of the chains. Of the forces involved in the retention of the native configuration those described as hydrogen bonds are probably the most important. Our knowledge of the properties of the hydrogen bond has increased to such an extent during the past five years as to justify some speculation as to the nature of the stable configurations of protein molecules.

Hydrogen bonds can be formed by the peptide carbonyl and imino groups of a polypeptide chain, and also by the carboxy, amino, hydroxy, and other oxygen- and nitrogen-containing groups in the side chains of the amino-acid residues. In a stable configuration as many strong hydrogen bonds as possible will be present. One configuration in which all of the peptide carbonyl and imino groups are forming strong hydrogen bonds is that shown in Fig. 8. Here extended chains are bonded together to form a compact layer, with the side chains extending alternately above and below the plane of the layer (provided that the *levo* configuration is the only one represented by the amino-acid residues). This configuration has been assigned to β-keratin and other fibrous proteins by Astbury[27] on the basis of X-ray data. Although the structure has not been verified in detail by the analysis of the X-ray data, the agreement in the dimensions found experimentally for the pseudo-unit cell of β-keratin and those predicted from the complete structure determinations of glycine[28] and diketopiperazine[29] makes it very probable that the structure is essentially correct, with, however, the chains somewhat distorted from the completely extended configuration.[30]

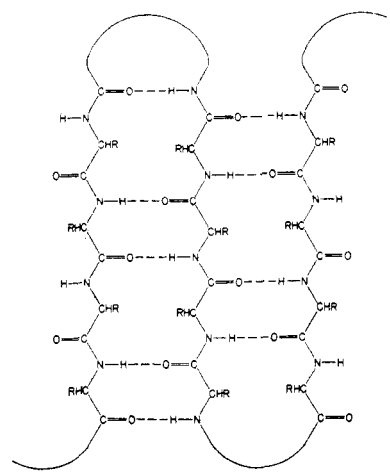

Fig. 8.—The folding of polypeptide chains into a layer held together by imino-carbonyl hydrogen bonds.

It is to be noted that the —NH—CO—CHR— sequence alternates in alternate lines in such a layer, so that a layer of finite size could be constructed by running a single polypeptide chain back and forth. A globular protein could then be made by building several such layers parallel to one another and in contact, like a stack of pancakes, the layers being held together by side-chain interactions as well as by the polypeptide chain itself. A protein molecule with, for example, roughly the shape and size of a cube 40 Å. on edge might contain four layers, each with about eight strings of about twelve residues each.

A few years ago I noticed, by studying molecular models, that a proline or hydroxyproline residue in the chain would interfere with the

(24) P. Gonzales and M. Armangué, *Compt. rend. soc. biol.*, **106**, 1006 (1931); K. Landsteiner and J. Jacobs, *Proc. Soc. Exptl. Biol. Med.*, **30**, 1055 (1933).

(25) A brief statement of the situation has been made by L. Pauling and C. Niemann, THIS JOURNAL, **61**, 1860 (1939); see also R. B. Corey, *Chem. Rev.*, **26**, 227 (1940).

(26) A. E. Mirsky and L. Pauling, *Proc. Nat. Acad. Sci.*, **22**, 439 (1936); H. Wu, *Chinese J. Physiol.*, **5**, 321 (1931).

(27) W. T. Astbury, *Trans. Faraday Soc.*, **29**, 193 (1933), and other papers.

(28) G. Albrecht and R. B. Corey, THIS JOURNAL, **61**, 1087 (1939).

(29) R. B. Corey, *ibid.*, **60**, 1598 (1938).

(30) R. B. Corey, ref. 25.

structure shown in Fig. 8 in such a way as to cause the chain to tend to turn through 180°; hence if these residues were suitably distributed along the chain during its synthesis the chain would tend to assume the configuration discussed above.

Layer structures other than this one might also be assumed, in which the chains are not extended. Some fibrous proteins, such as α-keratin, are known to have structures of this general type, but the nature of the folding of the chains has not yet been determined.

We have postulated the existence of an extremely large number of accessible configurations with nearly the same energy for the end parts of the globulin polypeptide chain. A layer structure, with variety in the type of folding in the layer, would not, it seems to me, give enough configurational possibilities to explain the great observed versatility of the antibody precursor in adjusting itself to the antigen, and I think that skew configurations must be invoked. But simple considerations show that it would be difficult for the chain to assume a skew configuration in which most of the peptide carbonyl and imino groups take part in forming hydrogen bonds, as they do in the layer structures; and in consequence the skew configurations would be much less stable than the layer configurations. The way out of this difficulty is provided by the postulate that *the end parts of the globulin polypeptide chains contain a very large proportion (perhaps one-third or one-half) of proline and hydroxyproline residues and other residues which prevent the assumption of a stable layer configuration.*

We may, indeed, anticipate that globular proteins may be divided into two main classes, comprising, respectively, those in which there is a layer structure and those in which the stable configuration of the chains is more complex; the latter may show the high proline and hydroxyproline content postulated for the globulin chain ends.

About one-third of the residues in gelatin are proline and hydroxyproline. We expect accordingly that there are many configurations with nearly the same energy accessible to a gelatin molecule, and that gelatin is not characterized by a single well-defined native molecular configuration.[31] Since the substance contains no strong antigenic groups, a definite configuration is a requisite for antigenic activity. These considerations thus provide a possible explanation of the well-known fact that gelatin is not effective as an antigen.[32]

Serological experiments with artificial conjugated antigens,[33] especially azoproteins, have provided results of great significance to the theory of antibody structure. Many of the arguments based on these results are presented in the books of Landsteiner and Marrack. The data obtained regarding cross-reactions of azoprotein sera with related azoproteins show that electrically charged groups (carboxyl, sulfonate, arsenate) interact strongly with homologous antibodies, and that somewhat weaker interactions are produced by hydrogen-bond-forming groups and groups with large electric dipole moments (hydroxy, nitro). The principal action of a weak group such as alkyl, phenyl or halogen is steric; this is shown clearly by the strong cross-reactions between similar chloro and methyl haptens. The data on specificity of antibodies with respect to haptens indicate strongly that the hapten group fits into a pocket in the antibody, and that the fit is a close one. It should not be concluded that all antibody–antigen bonds are of just this type; for example, the fitting of an antibody group into a pocket in the antigen may also be often of importance. Extensive work will be needed to determine the detailed nature of the antibody structures complementary to particular haptens and antigens.

V. Further Comparison of the Theory with Experiment. Possible Experimental Tests of Predictions

a. Methods of Determining the Valence of Antibodies.—The following methods may be proposed to determine the valence of antibodies. First, let a serum be produced by injection of an azoprotein of the following type: its hapten is to be sufficiently strong (that is, to interact sufficiently strongly with the homologous antibody) that one hapten group forms a satisfactory antibody–antigen bond, and the number of hapten groups per molecule is to be small enough so that in the main only one group will be present in the area serving as a pattern for an antibody end.

(31) Collagen has a definite fiber structure, as shown by X-ray photographs. A possible atomic arrangement has been suggested by W. T. Astbury and F. O. Bell, *Nature*, **145**, 421 (1940).

(32) Gelatin with haptens attached is antigenic (for references see Landsteiner, *loc. cit.*, p. 102, and C. R. Harington, *J. Chem. Soc.*, 119 (1940)). The presumption is that the haptens interact with the antibody so effectively that one hapten forms the antigen–antibody bond, and the lack of definite configuration of the gelatin is of no significance.

(33) K. Landsteiner and H. Lampl, *Biochem. Z.*, **86**, 343 (1918); K. Landsteiner, *ibid.*, **104**, 280 (1920).

The same hapten is then attached to another protein, and this azoprotein is precipitated with the serum. If it be assumed that the precipitate is valence-saturated, the ratio of hapten groups to antibody molecules in the precipitate gives the average valence of the antibody.

Data of essentially this sort, obtained with arsanilic acid as the hapten and the rabbit as the experimental animal, have been published by Haurowitz and his collaborators[34] in a paper reporting many interesting experiments. The equivalent weight of antibody per arsanilic acid residue was found to vary from 23,000 for antigens with very many attached haptens to 51,000 for those with only a few. The expected value for saturation of all haptens by bivalent antibody molecules is one-half the antibody molecular weight, that is, about 79,000. The low experimental values are probably due to failure of some haptens to combine with antibodies. In particular, if two haptens are attached to the same tyrosine or histidine residue steric interactions of antibodies may permit only one of the haptens to be effective.[35]

The bivalence of antibodies and our postulate that only one antigen molecule is involved in the formation of an antibody molecule require that a precipitin-effective antihapten be produced only if the injected antigen contain at least two hapten groups. Pertinent data have been obtained on this point by Haurowitz and his collaborators, who found that effective antihapten precipitin serum was produced by an azoprotein, made from arsanilic acid and horse globulin, containing 0.24% arsenic (4.8 haptens per average molecule of molecular weight 157,000), and a trace by one containing 0.13% arsenic (2.6 haptens per molecule).

b. The Possible Antigenic Activity of Simple Substances.—The criteria given above for antigenic activity would be satisfied by a substance of relatively low molecular weight in which several hapten groups (such as several arsanilic acid residues) are present in the molecule. A substance of this sort would be expected also to show the precipitin reaction with its own serum or with serum homologous to an azoprotein containing the same hapten, and to be capable also of producing anaphylaxis.

A substance with only two hapten groups per molecule might be expected not to give a precipitate with the homologous antibody, but rather to form long strings with antibody and antigen molecules alternating. These strings would remain in solution, and would confer on the solution the property of pronounced birefringence of flow. If, however, there were in one end of some of the antibody molecules complementary regions for two hapten groups, making these molecules effectively trivalent, the strings would be tied together and a precipitate would be formed. It is probably significant in this connection that Landsteiner and van der Scheer[36] have observed both the precipitin reaction and anaphylactic shock (in guinea pigs sensitized with the corresponding azoproteins) with azo dyes, such as resorcinoldiazo-*p*-suberanilic acid, $(OH)_2C_6H_2(NNC_6H_4NHCO(CH_2)_6COOH)_2$, formed by coupling two anilic acid molecules with resorcinol. Landsteiner himself has explained these observations as resulting from the low solubility of the dyes, but the explanation advanced above seems more probable.

c. Experiments with Two or More Different Haptens in the Same Antigen.—We would predict that if there were used as an antigen a molecule to which several hapten groups A and several different hapten groups B were attached, the serum obtained would contain three kinds of bivalent antibodies, A′–A′, B′–B′, and A′–B′ (as well as the univalent antibodies A′– and B′–). Our picture of the process of formation of antibodies permits little or no correlation between the two chain ends of the globulin in the selection of surface regions of the antigen molecule to serve as templates, except that the two regions must not overlap. We accordingly predict that for n_A and n_B groups A and B, respectively, per antigen molecule the numbers of bivalent antibody molecules of different kinds in the serum would be

$$N_{A'-A'} = \alpha^2 n_A(n_A - 1) \quad (3a)$$

$$N_{A'-B'} = 2\alpha\beta n_A n_B \quad (3b)$$

$$N_{B'-B'} = \beta^2 n_B(n_B - 1) \quad (3c)$$

(34) F. Haurowitz, F. Kraus and F. Marx, *Z. physiol. Chem.*, **245**, 23 (1936).

(35) From the data discussed above, which in our opinion indicate that two hapten groups combine with a bivalent antibody molecule, Haurowitz drew the different conclusion that one arsenic-containing group in the antigen combines with one antibody molecule. He reached this result by assuming 100,000 (rather than 157,000) for the molecular weight of the antibody and by assuming that the active group in the antigen consists of two haptens attached to a tyrosine or histidine residue. It is, of course, likely that this occurs in antigens with high arsenic content, but it seems probable that the haptens are mainly attached to separate residues in the antigens containing only a few haptens per molecule.

(36) K. Landsteiner and J. van der Scheer, *Proc. Soc. Exptl. Biol. Med.*, **29**, 747 (1932); *J. Exptl. Med.*, **56**, 399 (1932).

in which α and β are coefficients which give the probabilities that the groups serve as templates. It is seen that *the amount of A'–B' antibodies is predicted to be equal to or slightly greater than twice the geometric mean of the amounts of A'–A' and B'–B'*. The only data permitting a quantitative test of this relation which have come to my attention are those obtained by Haurowitz and collaborators (*loc. cit.*) by use of azoproteins made from arsanilic acid. A serum produced by injecting rabbits with an antigen made from arsanilic acid and horse globulin was found to contain antibodies to the arsanilic hapten (precipitating an azoprotein made from arsanilic acid and rabbit globulin), others to horse globulin itself, and still others to the homologous antigen, these last presumably being complementary in structure both to the hapten and to the active groups of horse globulin. The quantitative results obtained are the following: 10 ml. of immune serum gave 17–18 mg. of precipitate with the maximal precipitating amount of azoprotein from arsanilic acid and rabbit globulin, and 8 mg. of precipitate with horse globulin, these amounts being independent of the order of the two precipitations. After exhaustion with these two antigens the serum gave 17 mg. of precipitate with the homologous antigen. If we assume that the conditions of each precipitation were such that only suitable bivalent antibody molecules were incorporated in the precipitate, the equations above would require the third precipitate to weigh about 24 mg.; the experiment accordingly provides some support for the theory. The quantitative discrepancy may possibly be due to the incorporation of some effectively univalent antibody molecules in the first two precipitates.

The qualitative experimental results which have been reported are in part compatible and in part incompatible with the theory. The most interesting of the experiments are those of Landsteiner and van der Scheer,[37] who prepared azoproteins containing two different kinds of haptens and studied the antibodies produced by them. In some cases only one of the haptens was effective in antibody formation. With the azoprotein made from 3-amino-5-succinylaminobenzoyl-*p*-aminophenylarsenic acid, however, a serum was obtained which would combine not only with the homologous antigen but also with azoproteins formed either from *m*-aminosuccinanilic acid or from *p*-aminophenylarsenic acid. After the serum was exhausted by interaction with stromata coupled with either one of these two simple haptens, it reacted as strongly (as measured by the estimated amount of precipitate) with the azoprotein containing the other simple hapten as before exhaustion. From this experiment and others the investigators concluded that there appeared to be present in the sera, if any, only small amounts of antibodies with two combining groups capable of interaction with the two different haptens in the antigen. It seems possible that the conclusion is not justified by the data, and, with the kind coöperation of Dr. Landsteiner, we are continuing this investigation.

d. The Antigenic Activity of Antibodies.— Our picture of an antibody molecule requires that the configuration of its middle portion be the same as that of normal serum globulin. Hence antibodies should have antigenic activity, with essentially complete cross-reactions with normal globulin. This is in agreement with experiment; Landsteiner and Prasek[38] found that precipitins which precipitated the serum of an animal precipitated also the agglutinins in the serum, and Eisler[39] showed that a precipitin to horse serum would precipitate tetanus antitoxin. On the other hand, according to our picture the active end regions of the antibody molecules would not have effective antigenic power, since their configurations would be different from molecule to molecule (depending on the accidentally selected template region and accidental way of coiling), and an antibody complementary to one antibody end would as a rule not combine with another. The antibody ends would hence in the main be left free in the precipitate formed by an antibody and its precipitin, as well as in that formed by an antibody and the precipitin to normal globulin. In agreement with this, Smith and Marrack[40] found that a precipitate formed by a precipitin and a serum containing diphtheria antitoxin has the power of combining with diphtheria toxin; and similar results have also been obtained recently by Heidelberger and Treffers[41] in the case of specific precipitates formed by pneumococcus antibody with homologous antiserum.

(37) K. Landsteiner and J. van der Scheer, *J. Exptl. Med.*, **67**, 709 (1938).

(38) K. Landsteiner and E. Prasek, *Z. Immunitäts*, **10**, 68 (1911).

(39) M. Eisler, *Zentr. Bakt. Parasitenk. Infekt.*, **84**, 46 (1920).

(40) F. C. Smith and J. Marrack, *Brit. J. Exptl. Path.*, **11**, 494 (1930).

(41) Personal communication of unpublished material by Professor M. Heidelberger and Dr. H. P. Treffers.

e. Factors Affecting the Rate of Antibody Production and the Specificity of Antibodies.—In order that an antibody be effective, the surface region of the antigen covered by an antibody end must be large enough so that the integrated attractive forces constitute an antibody–antigen bond of significant strength. With a bond of average strength the antibody molecule will be dissociated from its antigen template, perhaps with the aid of the auxiliary mechanism mentioned in Section VI, until an equilibrium or steady-state concentration is built up in the serum. Now if the antigen surface contains a large number of strong groups, capable of interacting strongly with complementary structures in the antibody, the antibody–antigen bond may be so strong that the antibody is not able to separate itself from the antigen, and only a small antibody concentration can be built up in the serum. We hence conclude that an *antigen containing weak groups will in general be a good antigen, whereas one containing many strong groups will be a poor antigen, with respect to antibody production.*

This prediction, which at first thought seems paradoxical, is in fact borne out by experiment. Thus a bland protein such as egg albumin is a good antigen, as are also conjugated proteins with weak groups attached. An azoprotein with many strong groups attached (arsenic acid, sulfonic acid, nitro, etc.; the azo group itself is a rather strong group, capable of forming hydrogen bonds) is a poor antigen; in order to obtain serum to such haptens an azoprotein containing a limited number of the groups must be used. I am told by Dr. Landsteiner that this observation was made in the early days of the study of azoproteins.[42] Pertinent data have been reported in recent years by Haurowitz and his collaborators (*loc. cit.*), who found the optimum arsenic content for the production of antihapten by azoprotein made from arsanilic acid to be between 0.5 and 1.0%; very little antibody is produced by antigens with over 2% arsenic, although strong precipitin reaction is shown by azoprotein with an arsenic content as great as 10%.

A second deduction, relating to specificity, can also be made. To achieve a sufficiently strong antibody–antigen bond with an antigen containing only weak groups a large surface region of the antigen must come into play, whereas with an antigen containing strong groups only a small region (in the limit one group) is needed. Hence *antibodies to antigens containing strong groups show low specificity, and those to antigens containing weak groups show high specificity.* This prediction is substantiated by many observations. Egg albumin, hemoglobin, and similar proteins give highly specific sera, whereas azoproteins produce sera which are less specific, strong cross-reactions being observed among various proteins with the same hapten attached. This shows, indeed, that a single hapten group gives a sufficiently strong bond to hold antibody and antigen together. In such a case the approximation of the antibody to a strong hapten is very close, and great specificity is shown with regard to the hapten itself, this specificity being the greater the stronger the hapten. Many examples of these effects are to be found in Landsteiner's work.

f. The Effect of Denaturing Agents.—We made the fundamental postulate that the end parts of the polypeptide chains of the globulin molecule are characterized by having a very large number of accessible configurations with nearly the same energy, whereas there is only one stable configuration for the central part. It is accordingly probable that the end configurations, giving characteristic properties to the antibodies, would be destroyed before the central part of the molecule is affected and, moreover, that the sensitivity to denaturing agents or conditions of antibodies to different antigens would be different. The available meager experimental information seems to be compatible with these ideas.[43]

Some remarks may be made regarding the difference in behavior of antibodies and antigens in the presence of denaturing agents. An antigen molecule may undergo a considerable change in configuration without losing completely its power of reacting with the homologous serum; if some of the surface regions remain essentially unchanged after partial denaturation of the protein, the antibody molecules complementary to these regions will retain the power of combining with them, whereas the antibody molecules complementary to the regions which have been greatly changed by denaturation will no longer be effective. In particular some native proteins may be built of superimposed layers, as described in Section IV. The antigenic regions on top of the top layer and on the bottom of the bottom layer would still be effective after the partial denatura-

(42) See K. Landsteiner and H. Lampl, *loc. cit.*

(43) See Marrack, *loc. cit.*, pp. 48–53.

tion of the molecule by the unleafing of the layers, whereas the antigenic regions at the sides of the original molecule would in large part lose their effectiveness by this unleafing. The observation by Rothen and Landsteiner[44] that egg albumin spread into surface films 10 Å. thick retains the ability to combine with anti-egg-albumin rabbit serum is most simply explained by the assumptions that the native egg albumin molecule has the layer structure suggested above and that the process of surface denaturation of this molecule involves the unleafing of the layers without the loss of their structure.

As mentioned above, it is probable that for most antibodies the end regions are affected by denaturing agents more easily than the central region, and that the first step in denaturation of an antibody involves these end regions and leads to loss of their specific properties. It has been shown by Danielli, Danielli and Marrack[45] that the reactivity of antibodies is destroyed by surface denaturation.[46]

An interesting possible method of producing antibodies from serum or globulin solution outside of the animal is suggested by the theory. The globulin would be treated with a denaturing agent or condition sufficiently strong to cause the chain ends to uncoil; after which this agent or condition would be removed slowly while antigen or hapten is present in the solution in considerable concentration. The chain ends would then coil up to assume the configurations stable under these conditions, which would be configurations complementary to those of the antigen or hapten.

Many of the experiments suggested above are being undertaken in our Laboratories, with the collaboration of Dr. Dan Campbell.

VI. Processes Auxiliary to Antibody Formation

It seems not unlikely that certain processes auxiliary to antibody formation occur. The reported increase in globulin (aside from the antibody fraction) after immunization suggests the operation of a mechanism whereby the presence of antigen molecules accelerates the synthesis of the globulin polypeptide chains. There is little basis for suggesting possible mechanisms for this process at present.

The occurrence of the anamnestic reaction—the renewed production of antibodies to an antigen caused by injection of a second antigen—may be explained by the assumption that following the synthesis of an antibody a mechanism comes into operation in the cell to facilitate the removal of the antibody from the antigen, perhaps by changing the hydrogen-ion or salt concentration or dielectric constant. This would assist in removing antibodies not only from the second antigen but also from those molecules of the first antigen which had remained, covered with homologous antibody attached too firmly for spontaneous removal, in the cell. The evidence indicates that the anamnestic reaction is not in general strong. At a time after inoculation with typhoid bacillus or erythrocytes long enough that the corresponding agglutinins are no longer detectable in the serum injection of another antigen gives rise to the presence of these agglutinins in amounts detectable by the very sensitive agglutination test; but Kabat and Heidelberger[47] found that the amount of additional antibody to serum albumin produced by injection of egg albumin or typhoid toxin was too small to be detected by their method of analysis.

The mechanism for catching the antibody molecule and holding it in the region of globulin synthesis may be closely related to that of antibody production—possibly a partially liberated globulin chain which forms a bond or two bonds with an antigen molecule directly above it is prevented from freeing its central part from the cell wall, and so serves as an anchor.

The renewed production of antibody in the serum after bleeding is to be attributed to the presence of trapped antigen molecules in the cells. The greater duration of active than of passive immunization may be attributed to this or to the presence of complexes of antigen and surrounding antibodies, the outer ends of which could combine with additional antigen.

Acknowledgments.—My interest in immunology was awakened by conversations with Dr. Karl Landsteiner; I am glad to express my gratitude to him, and to acknowledge my indebtedness to him for ideas as well as for facts. I wish also to thank Professors Michael

(44) A. Rothen and K. Landsteiner, *Science*, **90**, 65 (1939).

(45) J. F. Danielli, M. Danielli and J. R. Marrack, *British J. Expll. Path.*, **19**, 393 (1938).

(46) Rothen and Landsteiner (*loc. cit.*) have pointed out that from these facts regarding surface denaturation the conclusion can be drawn that "the specific reactivity of antibodies is to a large extent dependent upon structures different from those which mainly determine the specificity of antigens."

(47) E. A. Kabat and M. Heidelberger, *J. Exptl. Med.*, **66**, 229 (1937).

Heidelberger and Dan Campbell for advice and assistance.

Summary

It is assumed that antibodies differ from normal serum globulin only in the way in which the two end parts of the globulin polypeptide chain are coiled, these parts, as a result of their amino-acid composition and order, having accessible a very great many configurations with nearly the same stability; under the influence of an antigen molecule they assume configurations complementary to surface regions of the antigen, thus forming two active ends. After the freeing of one end and the liberation of the central part of the chain this part of the chain folds up to form the central part of the antibody molecule, with two oppositely-directed ends able to attach themselves to two antigen molecules.

Among the points of comparison of the theory and experiment are the following: the heterogeneity of sera, the bivalence of antibodies and multivalence of antigens, the framework structure and molecular ratio of antibody–antigen precipitates, the use of a single antigen molecule as template for an antibody molecule, criteria for antigenic activity, the behavior of antigens containing two different haptens, the antigenic activity of antibodies, factors affecting the rate of antibody production and the specificity of antibodies, and the effect of denaturing agents. It is shown that most of the reported experimental results are compatible with the theory. Some new experiments suggested by the theory are mentioned.

PASADENA, CALIFORNIA RECEIVED JUNE 25, 1940

[CONTRIBUTION FROM THE AVERY LABORATORY OF CHEMISTRY OF THE UNIVERSITY OF NEBRASKA]

The Thermal Decomposition of Benzoyl Peroxide

BY DENTON JACOBS BROWN

In a recent article, Walker and Wild[1] accept the idea that the acyl peroxide decomposes directly to the dialkyl and carbon dioxide, or reacts with other substances. The object of this work was to make a series of determinations of the rate of decomposition of benzoyl peroxide in order to secure a better insight into the mechanism of the reaction.

Calculated amounts of benzoyl peroxide were diluted with dried benzene to a definite volume in a platinum still or Kjeldahl flask or bomb tubes and placed in a thermostat kept at 80 ± 0.1°. At definite intervals samples were removed, cooled quickly to 20° and a measured portion titrated iodometrically for the undecomposed peroxide. In case of the small bomb tubes, to test the effect of surface, the volume was measured when the original portion was added to the tube.

In the observations for the initial changes of concentration, sufficient benzoyl peroxide was added to the hot benzene in the platinum still so that when all had dissolved the temperature was 80 ± 0.5°. For an example we have included a concentration equivalent to that of tenth molar at 20°. The first portion, 10 ml., was measured hot as soon as the peroxide had dissolved. Of course if a median time were used the initial change for fifteen minutes would be less.

(1) Walker and Wild, *J. Chem. Soc.*, 1132 (1937).

TABLE I

t, min.	A	$(C - x)$ cc. B	0.956 N/10 C	$Na_2S_2O_3$ D
0	19.55	20.10	20.45	20.10
15	19.20	19.80	20.15	19.70
30	18.60	19.10	19.45	18.95
45	17.95	18.30	18.80	18.40
60	17.25	17.60	18.20	17.85

Following this "induction period" a series of observations were made to determine the order of the reaction.

The data for the least concentrated solutions were the least accurate. For this reason we will include two parallel series for the most dilute

TABLE II

t, min.	$C-x$ (a)	(b)	C_c	K_1	K_2
30	0.0236	0.0230	(0.0233)		
90	.0214	.0208	.0211	0.00165	0.0730
150	.0194	.0195	.0193	163	768
210	.0175	.0175	.0175	166	831
270	.0155		.0159	175	929
300		.0150	.0152		
360	.0142		.0138	154	853
390		.0129	.0132		
450	.0135		.0120	133	760
480		.0122	.0116		