A CONCEPTION OF IMMUNOLOGICAL SPECIFICITY[1]

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During the last few years a considerable amount of experimental evidence has accumulated which indicates the importance of chemical structure in determining the specificity of both natural and synthetic antigens. This work has been summarized by Marrack (1934), Landsteiner (1936) and Morgan (1936), and it is therefore not proposed to discuss in detail the investigations which have contributed to our present-day conception of this aspect of immunity reactions. Contributions which seem essential for understanding the ideas developed in this communication will, however, receive mention.

The most exact knowledge we possess of the specific antigenic function of well-defined chemical structures has been obtained largely through the investigations of Landsteiner & van der Scheer and of Avery & Goebel. These workers have used artificially prepared antigens in which the chemical structure of the serologically important part of the antigenic complex was fully known. In this way it has been possible to ascertain the influence on the specificity of an antibody, of the addition to, or of the withdrawal from, an antigen of a definite chemical structure, such as a carboxyl or an acetyl group. As a result of these investigations it soon became evident that in some instances extremely small differences in chemical constitution were accurately reflected in the specificity of the corresponding antibody. For example, it was possible to distinguish the isomeric laevo-, dextro-, and meso-tartaric acids by the use of immune sera which had been produced by means of artificially prepared antigens built up from the optically active and meso-tartaric acids. Similarly it was possible to distinguish the α- and β-glycosides of glucose by serological methods. A closer examination of the experimental results, however, reveals the frequency with which serological cross-reactions occur between antigens and antibodies engendered by antigens which are similarly constituted, but which are not identical. Unfortunately, in most of the experiments in which a simple artificial antigen was employed, no attempt was made to ascertain whether more than one kind of antibody molecule was present in the corresponding immune serum, as, for example, an antibody molecule which reflected a difference in antigen structure and another which carried a specific serological property corresponding to that portion of the antigenic structure which was common to both antigens.

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The early experiments of Wells & Osborne (1913) and the more recent investigations of Hooker & Boyd (1933, 1934, 1936) have produced evidence which indicates that two different proteins can contain a common antigenic structure, and the latter authors consider that the experimental facts are in agreement with the conception that determinant groups of diverse specificity can exist in a single molecule of an antigenic (protein) substance and that each group can give rise to a corresponding antibody.

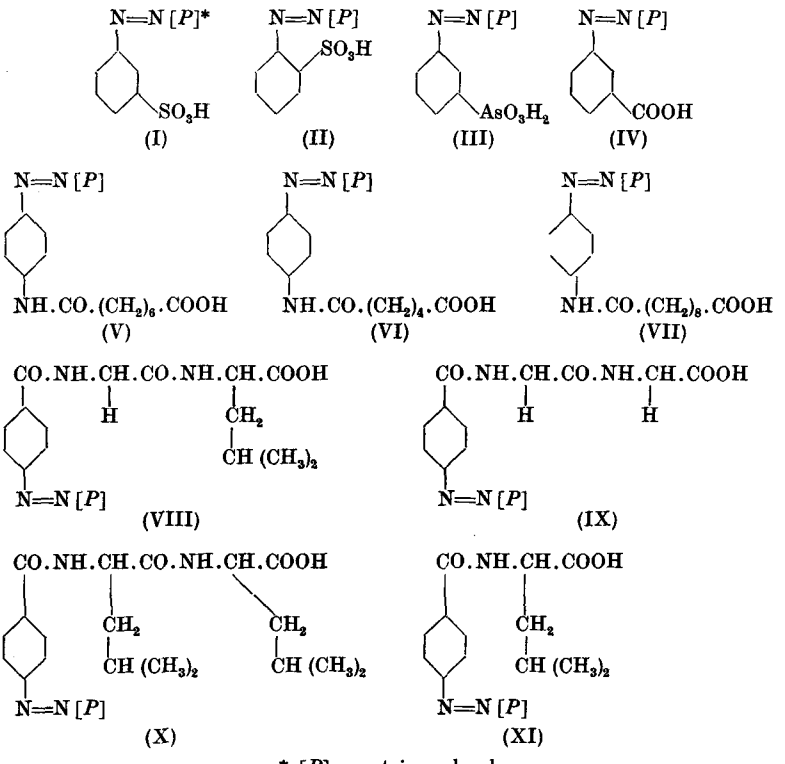
Furth & Landsteiner (1929) were unable to demonstrate in the specific precipitable polysaccharide of the "0" antigen of certain members of the Salmonella group the existence of separable serologically reactive components, corresponding to the specific antibody fractions that had been revealed by absorption tests. Later Burnet (1934), on the basis of the phage-inhibiting activity of bacterial extracts prepared from certain well-recognized strains of Flexner's dysentery bacillus, concluded that the specific antigen of each of the strains investigated is composed of immunologically similar molecules and not of a mixture of antigens as had been hitherto postulated according to the mosaic conception of antigenic structure originally brought forward by Durham (1901). Further evidence in support of the idea that a pure antigenic substance is able to engender more than one kind of antibody molecule was obtained by Meyer & Morgan (1935). These workers showed that the pure specific bacterial polysaccharide that had been isolated from the "smooth" form of *Bact. shiga* possesses the property of neutralizing "Shiga" heterophile antibody in addition to its well-known property of combining with the homologous agglutinins and precipitins. The fact that this chemically homogeneous specific polysaccharide hapten is able to form a specific combination with two different kinds of antibody led them to suggest that a pure bacterial polysaccharide may contain in each of its molecules at least two kinds of determinant antigenic structure of diverse specificity. Later Morgan (1936*a*) showed that a heterophile immune substance in addition to "Shiga" agglutinins and precipitins was engendered in rabbits as the result of immunization with an artificial antigen built up from the "Shiga" specific polysaccharide and horse serum globulin. As a result of these investigations, it seems possible that in certain cases an antigen, by virtue of a number of antigenic structures contained in its molecule, can elicit more than one type of antibody.

In most of the foregoing investigations the serologically active substances employed were naturally occurring complex protein or polysaccharide molecules, the detailed chemical structure of which was almost completely unknown. For this reason it was impossible for the various workers to decide definitely whether certain specific structures on the antigen molecule were responsible for the production of the different kinds of immune body or whether antibody molecules which differed somewhat in their immunological specificity could arise as a result of the antigenic capacity of a single chemical structure.

Quite recently, however, Landsteiner & van der Scheer (1936) have examined the relationship of certain cross-reactions given by anti-azoprotein

immune sera to the chemical structure of the determinant portion of the azo-protein antigen employed.

For example, it was shown that when an immune serum produced against an azo-protein antigen, which had been prepared from *m*-aminobenzene sulphonic acid (I), was absorbed with *o*-aminosulphonic acid (II), *m*-amino-phenylarsenic acid (III) or *m*-aminobenzoic acid (IV) combined with sheep red-cell stroma to form an insoluble azo-stroma antigen, the serum, which originally reacted with all these substances, could be made in turn non-reactive for each of the absorbing antigens. Similar observations were made with an immune serum prepared by means of the azo-protein antigen derived from *p*-aminosuberanilic acid (V) and absorbed with azo-stroma prepared

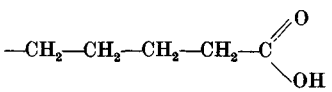


* [*P*] = protein molecule.

from *p*-aminoadipanilic acid (VI) or *p*-aminosebacanilic acid (VII), and finally it was found that an immune serum prepared against the azo-protein derivative of aminobenzoyl glycyl-leucine (VIII) would likewise yield a serum, after absorption with azo-antigens prepared from glycyl-glycine (IX), *d*, *l*-leucyl-*d*, *l*-leucine (X) or *d*, *l*-leucine (XI) and stroma, which was non-reactive for the absorbing antigens. As a result of these experimental observations Landsteiner & van der Scheer concluded that as there are not several specific groupings in the antigens used for immunization that are identical with the groupings

which are contained in the positively reacting heterologous antigens, to which the antibodies engendered could be specifically related, it seemed probable that antibodies formed in response to one antigen, although capable of reacting with a certain antigenic structure are nevertheless not entirely uniform but vary in specificity to some extent. These observations would seem to suggest that the separation from an immune serum of antibody molecules which possess a different immunological specificity cannot be accepted as proof for the existence of immunologically distinct groups in the immunizing antigen. Landsteiner & van der Scheer have also shown that the reactions of an immune serum with the heterologous antigens are generally inhibited more strongly by the simple substances corresponding to the homologous antigen than by the heterologous substance. Furthermore, immune sera which had been absorbed with heterologous antigens were still found to be inhibited in their reaction with the homologous antigen by the simple substances corresponding to the heterologous antigen that had been used for absorption. It would appear, therefore, that the antibodies remaining after absorption still possessed a combining affinity for the antigen used for absorption but failed to yield a precipitate of an antigen-antibody complex.

It may be argued, however, that there exist in some of the antigens used certain chemical structures that are common to all the antigens contained in each of the groups mentioned. For example, the chain



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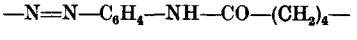
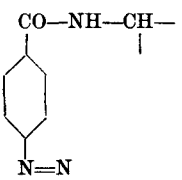


are common to all members of the second group of antigens, whereas the groupings



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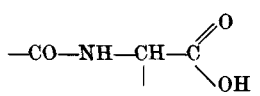

are found to be present in the glycyl-leucine, glycyl-glycine, leucyl-leucine, and leucine antigens (VIII–XI) of the third group. It may be, therefore, that these common structures, which are found to occur in each of the groups of antigens considered, give rise to either primary or secondary or minor specific receptors on the antibody molecules which would in consequence possess common receptor sites and thus give rise to overlapping specificity.

During a study of the cross-reactions which occur between antipneumococcus (type II) serum and various gums, Marrack (1936) has examined the experimental results he has obtained on the basis of two conceptions of antigen-antibody relationship.

The first view supposes that an antigen A contains two or more distinct antigenic structures a, b, c, ..., and that the antigen B contains structures $a, p, q, \ldots$. The antigen B, by virtue of the a antigenic determinant, will remove antibodies to the a structure in anti-A serum.

The second hypothesis assumes that antigen A contains a determinant group a^1 and that antigen B contains a determinant group a^2 which is similar to but not identical with a^1. The antibody molecules formed in response to the group a^1 differ in their ability to combine with the antigenic structure a^2. Only those which are suited to a^2 will be removed by the antigen B. Marrack finds that his experimental data fit the second hypothesis.

In view of the somewhat conflicting evidence as to the relationship that exists between an antigen and its corresponding immune serum, it seemed of some importance to try to construct a working hypothesis which would enable the antigen-antibody relationship to be expressed in more or less definite terms and which would at the same time account for many of the experimental results.

The numerous papers of Landsteiner and of Avery have given many examples of antigen-antibody reaction which show that certain chemical groups present in the antigen, owing to their special physical and chemical properties, play a dominant part in determining antibody specificity and that they give rise to immune globulin molecules with which they are able to combine specifically. The results of these investigations have also demonstrated that minor antigenic structures can co-exist in the same antigen molecule and that in certain serological reactions the minor antigenic structure is most probably the cause of the observed cross-reactions with the heterologous immune sera which have been induced by similarly but not identically constituted antigens. For example, Goebel *et al.* (1932) showed that the artificial antigens composed of the α- and of the β-glycosides of glucose, combined with protein, each give rise to an immune serum which accurately reflects, in the specific serological reactions, the difference in the spatial arrangement of the groups attached to the terminal carbon atom of the glucose molecule. The specificity of the reaction between the α-antigen and the corresponding immune serum is, however, not strictly specific for it is found that the β-antigen also reacts with the α-immune serum. Similarly the β-immune serum gives cross-reactions with the α-antigen. The specific reactions of the α- and β-antigens with their homologous serum must be due to the differences in the chemical structure, which consists of the different spatial arrangement of the α- and β-glycoside linkages only. The cross-reactions, on the other hand, must presumably be due to a chemical structure that is common to both antigens, that is, to the identical spatial arrangement of the polar hydroxyl groups that are attached to the remaining five carbon atoms in each glycoside.

Landsteiner & van der Scheer (1925) first suggested, as a result of their investigations into the antigens of red blood corpuscles, that an antibody may react with several substances which although not identical are chemically

similar. More recently Heidelberger & Kendall (1934) have expressed similar views in terms of dominant and minor reactive groupings on the antigen and antibody molecules, to explain the precipitin reaction given by crystalline egg albumin with an immune serum prepared against *R*-salt-azo-benzidine-azo-crystalline egg albumin.

In view of the important part played by certain chemical groups in determining the ultimate specificity of an antibody, it seems reasonable to suppose that the dominant antigenic structures in antigens will induce the formation of corresponding immune receptors on *all* the antibody molecules that are formed, whereas the less active antigenic structures that co-exist in the same antigen molecule will cause their own receptor sites to appear less readily on the antibody molecules. Whether or not all the antibody molecules that are formed possess specific receptors for the whole of the specific antigenic structures in the antigen must depend on the relative antigenic capacity of the individual chemical structures of the antigen.

If, for example, we designate the antigenic structures of a specific bacterial polysaccharide as *A*, *B*, and *C*, and we consider that these structures are arranged in order of their capacity to engender specific receptors on immune globulin molecules, *A* being considered the dominant antigenic determinant, then the structures will give rise to three main types of antibody molecule which will possess receptors corresponding to the following antigenic structures:

a ab abc.

The active structures *A*, *B*, and *C* in the scheme correspond to the more generally used term "determinant group" but it should be emphasized that such antigenic structures need not be constituted in a similar manner. For example, the *A* structure may represent an antigenic capacity resulting from the influence of a single highly polar chemical group such as an acetyl or carboxyl group, whereas the antigenicity of the *B* or *C* structure may be due to the combined influence of the intramolecular forces arising from the spatial distribution of a number of different, less active groupings or indeed from a varied mosaic of polar fields resulting from the chemical structure of the whole of the remaining polysaccharide surface.

It is considered improbable that immune globulin molecules possessing a single kind of receptor for a minor antigenic structure such as *b* or *c*, will be formed because the dominant antigenic character of the structure *A* will ensure that all the antibody molecules will possess receptors for this structure. These ideas imply that *b* receptors must always appear on antibody molecules which possess *a* receptors and that *c* receptors will only form part of an antibody surface which already has receptors for the more potent antigenic structures *a* and *b*.

It will be seen that these simple ideas on antigen-antibody relationship differ in an important way from the views developed by Burnet (1934). Burnet believes that from antigenic areas *A*, *B*, *C* and *D*, antibodies corre-

sponding to the active areas will arise in a random manner and suggests, as an example, that in six consecutive antibody molecules we might expect to find corresponding active areas distributed as follows:

aaa abcd aad bc bbd b.

According to our ideas, the antigenic structures, *A*, *B*, *C* and *D*, each possess their own inherent capacity to engender a corresponding receptor on an antibody molecule, but the antigenic efficiency of the structure alone determines the frequency with which the corresponding immune receptor will appear on the antibody molecule. This means that during immunization as many different kinds of antibody molecules may be formed as there are antigenic structures in the antigen. However, they need not all be produced simultaneously and it is suggested that immune globulin molecules, which contain receptors for all of the antigenic structures in a complex antigen, will be formed relatively slowly but will steadily increase in number during immunization. Obviously, antigenic molecules might exist that possess two structures of approximately equal antigenic efficiency. In such a case the simplest antibody molecule would most probably possess receptors for both of the antigenic structures. Similarly one might expect that certain *simple* artificial carbohydrate-protein antigens which possess a strongly polar group as the dominant antigenic determinant would give rise to extremely specific immune sera, especially if the immune serum was collected early during the course of immunization. These ideas are in complete accord with the results of Goebel's recent work on the artificial carbohydrate-protein antigens that have been prepared by combining the diazonium derivatives of the *p*-aminobenzyl glycosides of glucose and glucuronic acid with protein. It has been shown (Goebel, 1936) that the immune serum to either antigen fails to react with the remaining antigen; the sera are therefore strictly specific. It would appear that this is due to the very dominant antigenic character of the strongly polar carboxyl group in such a simple carbohydrate structure. It will be remembered that of the bacterial polysaccharides so far investigated the most acidic is the pneumococcus type III polysaccharide which, however, has been found to contain at least one hexose molecule associated with each uronic acid molecule in the polysaccharide structure. Prolonged immunization with the artificial glucuronic acid antigen would most probably give rise to a proportion of immune globulin molecules that would react with a minor common antigenic structure in the other antigen and give rise to serological cross-reactions.

There are other aspects of the problem of immunological specificity which cannot be overlooked and which most probably play an important part in determining the ultimate specificity of antigens and antibodies. I refer to the influence on the immunological specificity of the antigenic determinant structures, of the co-existing chemical groupings in the molecule which surround these active structures but which are not generally considered to

play an essential part in determining the specificity of the antigen molecule. Numerous investigations have shown that strongly polar groups, such as carboxyl or acetyl groups, have a much greater determinative influence on the specificity of the resulting antibody than non-polar groups. It is moreover established that polar groups such as acetyl and carboxyl groups have a large dipole moment which is not constant for the individual grouping but which exhibits a definite although slight variation in value according to the distorting influence of the surrounding molecular structure on the electron orbits of the atoms in the group under consideration. It would appear therefore, that when considering, especially in relation to its serological reactivity, the forms which a complex molecule can assume, it is necessary to consider the effect produced on the configuration by the mutual interaction of polar groupings. It seems reasonable to suppose, therefore, that a given immunologically active polar group in an antigen will have the electrical field of force around the grouping altered and in consequence presumably the corresponding immunological specificity of the determinant structure modified, according to the nature and polarity of the surrounding structures. In this manner it is conceivable that the same antigenically active determinant structure will, when present in differently constituted molecules, give rise to receptor sites on antibody molecules which differ slightly but significantly in specificity. These ideas therefore imply that a given specific determinant group, such as a carboxyl or an acetyl group, will possess an artificially induced and modified specificity according to the mutual influence of the co-existing chemical structures in the antigen molecule. This conception of specificity receives some indirect support from the recent work of Erlenmeyer & Berger (1932) and Berger (1936). These workers have had considerable success in obtaining experimental evidence in support of the view that molecules which possess a similar electrical field of force—in the sense of containing identical electronic systems around dissimilar nuclei—will possess a common immunological specificity.

Furthermore, any modification of the immunological specificity of an antigenic structure must also be reflected in the specificity of the homologous antibody molecule and, if we accept the view of Heidelberger & Kendall (1934), of Marrack (1934), of Chow and Goebel (1935), and of Haurowitz & Breinl (1933) that the specific combination of an antigen with its homologous antibody involves polar groups of opposite charge, then the specificity of a receptor group or site on the corresponding antibody molecule will likewise be influenced by the nature, the polarity and the stereochemical configuration of the surrounding receptors. It is, therefore, suggested that the specificity of a given antibody receptor, which has been formed in response to the antigenic action of a definite polar group, will be modified further according to the influence of the co-existing and simultaneously induced minor specific receptors on the antibody molecule.

If a dominant determinant group, A, is present in more than one antigen it cannot be expected to induce the formation of identical antibody molecules

when animals are immunized with the different antigens, since, not only is the ultimate immunological specificity of the *A* determinant somewhat different in each type of antigen molecule, but the engendered receptors are also exposed to the modifying influences of the immunologically active secondary and minor receptors which have been formed on the same immune molecule as a direct result of the antigenicity of co-existing structures on each of the different antigen molecules. Ideas of this nature might readily explain the serological cross-reactions observed by Marrack between pneumococcus (type II) antibody and its homologous polysaccharide, gum acacia and cherry gum. Each of these polysaccharide substances contains glucuronic acid as the immunologically important antigenic structure. However, the antibody molecules in the pneumococcus type II serum which react with the type II pneumococcus polysaccharide, the gum acacia and the cherry gum are not identical but nevertheless possess certain serological properties in common. It seems probable that the immunological specificity of the antigenically dominant glucuronic acid determinant group which is common to all three polysaccharides, is modified to some extent in each polysaccharide owing to the influence of the different surrounding chemical structures in each type of polysaccharide molecule, which may or may not themselves possess a direct specific immunological function.

If the type specific antigen of the pneumococcus (type II) is made up of antigenic structures which can be represented as

$$A \quad B \quad \text{and} \quad C$$

and we consider the *A* structure as representing the dominant glucuronic acid molecule and *B* and *C* as secondary and minor antigenic structures then the antibody molecules can be represented as

$$a \quad ab \quad abc.$$

Furthermore, if the serologically reactive structures of the polysaccharides, cherry gum and gum acacia, are represented as

$$A \quad B \quad \text{and} \quad F \quad \text{and} \quad A \quad G \quad \text{and} \quad H$$

respectively, then although the *A* structures are *chemically* identical in all antigens the *immunological* specificity of all three differ somewhat owing to the presence of different neighbouring structures which, as has already been suggested, modify the antigenic specificity of the *A* (glucuronic acid) structure. A more accurate representation of the serological specificity of cherry gum and gum acacia may therefore be

$$A^1 \quad B^1 \quad \text{and} \quad E \quad \text{and} \quad A^2 \quad G \quad \text{and} \quad H$$

respectively.

If the antigenic structure of cherry gum is found to be closely related to that of the pneumococcus (type II) it is conceivable that this similarity may arise in two ways. First, the structures surrounding the glucuronic acid

portion of the cherry gum molecule are of such a nature that they exert only a slight modifying influence on the specificity of the uronic acid molecule so that the receptors induced by the pneumococcus uronic acid structure fit very closely the glucuronic acid determinant group in the cherry gum molecule. This means that the dominant antigenic structure of each polysaccharide is immunologically very similar and that therefore the uronic acid portion of the cherry gum molecule is capable of firm combination with the glucuronic acid receptors on the pneumococcus (type II) antibody molecules. Secondly, a similarity can arise if both antigen molecules possess a common or very similar secondary antigenic structure which reinforces the weak combining affinity already established between the chemically identical but immunologically dissimilar dominant antigenic glucuronic acid structures and their corresponding receptor sites of the antibody molecules.

It is clear therefore that if the antigenic structure of cherry gum is more closely related to that of the pneumococcus (type II) polysaccharide than is that of gum acacia, the dominant immunological structure A^1 will most probably be less altered from the original glucuronic acid specificity *as it exists* in the pneumococcus (type II) polysaccharide, than that of the A^2 structure in gum acacia. Thus, whereas the whole of the antibody molecules would readily be removed from a pneumococcus (type II) immune serum by the homologous polysaccharide we should expect the heterologous polysaccharides, cherry gum and gum acacia, to show a weaker combining affinity for the type II antibody, since steric influences which are not appreciable in the homologous antigen-antibody reaction may, for reasons already given, assume considerable importance in the heterologous reactions. Indeed, it is well known that owing to the short range of polar forces the spatial arrangement of the polar groups on a molecular surface has an important influence on the strength with which two oppositely charged polar groups are held together by these forces and thus we realize that relatively minor changes in the polar reactivity of the specific configuration of the antigenic determinant structures of the polysaccharide molecule, due to the intensity of locally induced electrostatic fields, most probably have a direct and important influence on the combining affinity of antigen and antibody.

According to this hypothesis of antigen-antibody relationship the removal of a portion only of the antibody molecules from a pneumococcus (type II) serum with gum acacia or cherry gum and the removal of the remaining antibody by treatment with the homologous polysaccharide can be readily understood, since it appears probable that the combining affinity of the glucuronic acid configuration in either of the gum molecules for the pneumococcus (type II) antibody is considerably weakened, and will be weakest in the polysaccharide that possesses heterologous chemical groupings which will induce the most profound influence on the specificity of the essential glucuronic acid structure. Similarly the possession by the heterologous polysaccharide of different minor antigenic structures would tend to make the combining affinity

of the polysaccharide with the pneumococcus (type II) antibody weaker, particularly when antibody molecules possessing receptors for the whole of the antigenic structures of the pneumococcus (type II) polysaccharide are involved.

These ideas suggest that the avidity of combination of these polysaccharides with pneumococcus (type II) immune serum can be determined by the amount of glucuronic acid necessary to inhibit the combination. Accordingly, we should expect the homologous reaction to require a high concentration while the amount required to inhibit the combination of cherry gum with the antibody would be much less and finally the inhibition of combination of gum acacia with pneumococcus (type II) antibody would require the least amount of glucuronic acid.

The hypothesis, which has been outlined in this communication, is able to account for the well-established observation that, in spite of the dominant part played by certain structures in an antigen molecule, it is the nature and the molecular configuration of the antigenic molecule as a whole that ultimately determines the complete immunological specificity of the homologous antibody molecules. Similarly Landsteiner & van der Scheer's observation that the reaction of an immune serum with an heterologous antigen that will react with the serum is generally inhibited more strongly by the simple substance corresponding to the homologous antigen than by the heterologous substance becomes readily envisaged in terms of the hypothesis outlined, since an immune serum containing antibody molecules with receptor sites for an antigenic structure A, B, C will obviously show a stronger combining affinity for this structure, even when it exists on a simple molecule, than for any heterologous antigenic structure of the type $A^1 XY$.

It is not suggested that the factors which have been discussed are the only ones that play a part in determining the combining capacity of antibody for antigen. Marrack (1934) and, independently, Haurowitz (1936) have already produced evidence that the number as well as the nature of the specific receptors on an antigen and an antibody molecule determines to a considerable extent the firmness and the ultimate size of the antigen-antibody complex which is formed. Furthermore, the position of the various receptors in an immune globulin molecule would be yet a further aspect of the antigen-antibody relationship to be considered. Are dominant receptors essentially surface structures and do secondary or minor receptors reside largely, although not exclusively, in the more central parts of the antibody molecule?

If the ideas set forth in this paper are even approximately true, then it is obvious that the number as well as the nature and the position of the determinant antigenic structures on an antigen and of the receptor sites on an antibody molecule must play a very important part in antigen-antibody reaction.

The conception of antigen-antibody relationship which has been developed here may be considered by some as not too well founded on experimental data, and for that reason alone, somewhat premature. It is hoped, nevertheless,

that some of the ideas discussed will prove a stimulus to others working in this field and that ultimately an orderly conception of the relationship that must exist between an antigen and its antibody will emerge.

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