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(Continued)

### THE IMMUNOCHEMICAL ASPECTS OF ALLERGY\*†

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PERHAPS the title chosen is somewhat presumptuous since so little is known of the basic mechanisms responsible for allergy responses. However, I cannot help but feel that many of the underlying causes of hypersensitivity reactions in man are intimately associated with phenomena of immunity and that an immunochemical approach to the problem of allergy is of fundamental importance. This view is held not only by immunologists, but also by many allergists who, as a group, are more interested in and have a better understanding of fundamental immunology than most other medical groups. In the following presentation I will consider first some of the more or less accepted aspects of immunochemistry and then proceed to a discussion of the possible significance of these aspects to allergy. Obviously the projection of immunochemical data to allergic reactions in man must be made with considerable caution, since the immunochemist has limited himself almost entirely to laboratory animals, but the knowledge that has been obtained will permit certain first approximations and furnish a starting point for discussion and future research.

Since immunochemistry is essentially a study of antigen-antibody reactions, we are immediately confronted with the problem of defining these two reactants which often leads to abstractly expressing one in terms of the other. In fact, no clear concise definition is possible for either at the present time, so that it becomes necessary to describe the properties of each to the point where a general concept is developed. Originally the term antigen was used to designate those substances which had the ability to stimulate antibody formation, but the meaning has been broadened until now it is used to designate any immunologically active substance. We, therefore, have two categories of antigens: the complete or functional antigens which have the power of inciting antibody formation, and the incomplete or haptenic antigens which do not have this power but which will react specifically with antibodies.

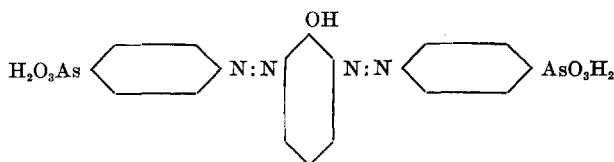
Functional antigenicity is not explicable in terms of chemical or physical properties, although one might suppose that it would be possible to prepare such

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a list and thereby be able to discover antigens without recourse to animal experimentation. In the first place, one must consider that antigenicity is relative to the animal which is being injected and an animal will not form antibodies against its own normal proteins or even against some of the proteins of closely related species. Furthermore, the antibody response varies from one species of animal to another, and it is well known that rats and guinea pigs are poor producers of precipitins as contrasted with such animals as rabbits and horses. This also brings up the question of the method of testing for antigenicity since one method may fail entirely while another method would indicate a high degree of antigenicity as, for example, the use of the precipitin reaction in comparison with anaphylaxis tests in the guinea pig. However, out of the mass of experimental observations there emerges a few properties which are fairly characteristic of functional antigens. For example, it appears that the minimal molecular weight is around 10,000, and there is some evidence that antigenicity may be roughly proportional to molecular weight. Although Landsteiner and others have succeeded in sensitizing guinea pigs to very simple organic compounds such as picryl chloride, it is entirely probable that these substances first combined with the animal's own proteins to form an antigenic complex. One can also state that, in general, functional antigens are proteins, but there is now no doubt that at least a few of the polysaccharides can also incite antibody formation. For example, this is true of the polysaccharides of certain parasitic helminths and the pneumococcus capsular polysaccharides. A third property which is usually mentioned, but which certainly needs further investigation, is that functional antigenicity is contingent upon digestibility.

The incomplete or haptenic antigens are also difficult to characterize clearly since they could theoretically have practically any chemical composition or structure. Although such substances will not stimulate antibody formation when injected alone, it is possible to obtain antibodies specific for the hapten by the injection of a hapten-protein conjugate, where the protein carrier may be derived from almost any source, including the protein from the animal, to be used for immunization. Once such antibodies are formed, all of the classical antigen-antibody reactions can be obtained with a simple homologous haptenic antigen, providing the molecule of antigen has at least two haptenic groups in its structure. Thus, antibodies obtained by the injection of *p*-arsanic-phenyl-azo-ovalbumin will give all of the serologic and hypersensitive reactions when tested with a simple dye antigen containing two arsanilic acid groups (a divalent antigen) such as the following compound:



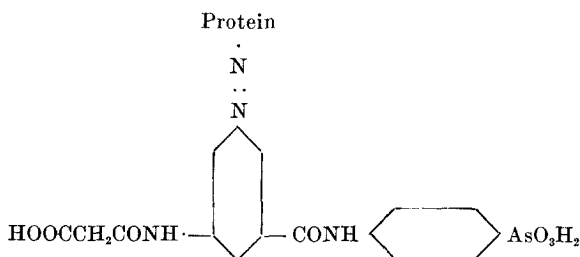
If only one arsanilic acid group is present (a monovalent antigen), the antigen combines with the antibody but no visible reaction takes place. This combina-

tion can be detected only when the subsequent addition of an antigen of known reactivity fails to give a reaction. Thus, we have a type of simple molecular structure which is referred to as an inhibiting antigen.

Needless to say, these concepts of the three different types of antigenic substances are important to the allergist since he is constantly dealing with them, either as excitants of allergic responses or the induction of a state of hypersensitivity, and the molecular structure becomes of great importance.

The characterization of classical antibodies is now relatively simple. Thus antibody is limited not only chemically to serum proteins, but also physically to a particular type having a characteristic surface charge. Although antibodies have been isolated in highly purified form, very little study has been made of their chemical composition and structure. In fact, the characterization of antibodies is based almost entirely upon their physical properties. Thus, when an electrical current is passed through a sample of serum it can be seen after a suitable interval that the protein components migrate toward the electrodes at different velocities, and under proper conditions four different components can be detected (1 albumin and 3 globulins,  $\alpha$ ,  $\beta$ ,  $\gamma$ ). Antibodies, regardless of their other properties, are always found in, or closely associated with, the slowest moving fraction which is usually referred to as the gamma globulin. This gamma component is usually increased during immunization and reduced if the antibody is removed from immune serum by precipitation with homologous antigen. Furthermore, certain techniques permit the isolation of any single component of the serum which leaves no doubt as to the association of antibody with the gamma component. The next most common property of antibodies is their molecular weight, which falls into two general classes: 160,000 or 1,000,000. Most of the molecular weight studies of the antibodies of human beings have given values of 160,000, although some recent investigations indicate that a heavy component has been isolated which contains not only antibodies against a variety of antigens, but also a considerable proportion of the hemagglutinins. It has been known for considerable time that horses produce two such molecular weight classes of antibodies: a heavy component (1,000,000) which is produced in response to injection of a suspension of pneumococcus cells and a light component (160,000) which is produced in response to injection of a soluble toxoid antigen.

The most characteristic property of antigens and antibodies is their specific affinity for each other. Thanks to the efforts of Landsteiner and many others we now have a fairly clear picture of the basis for this immunological specificity, at least as far as antigenic structure is concerned. We know, for example, that when a protein is modified by treatment with nitric acid, which introduces nitro groups into the protein molecule, that antibodies formed against this antigen will react with any protein that has undergone similar modification or even with simple chemical substances containing nitro groups. We also know that there is a limit to the size of the group which is involved in the determination of specificity. For example, when the following dihaptenic group



is attached to a protein, antibodies are formed either against the arsonilic acid group or against the succinanilic acid group, but not against both simultaneously, and when simple polypeptides are attached to a protein, most of the specificity is conferred by the terminal or distal amino acid. It is apparent, therefore, that the area of an antigen which is involved as a determinant is relatively small, which, of course, suggests that the area of the combining site on the antibody molecule must also be relatively small. Regardless of the fact that the size of the antigenic determinant may be relatively small, the specificity is easily changed by very small rearrangements of the various atoms in the structure. In fact, the spatial configuration seems to be the important factor in determining specificity so that cross reactions will be obtained between structures having similar spatial structures. (The degree of cross reaction will depend upon the similarity of the structures involved.) It becomes apparent then that cross reactions between antigens which may be chemically different is brought about by the presence of relatively small chemically identical or similar groups. When complex mixtures are used as antigens, such as extracts of animal or plant tissues, all degrees of cross reactions may be expected; in fact, one wonders why more cross reactions are not observed in immunologic reactions. The actual number of specific groups which must be present on an antigen molecule before it can be detected by the formation of specific antibodies is not definitely known. However, using the formation of precipitins in rabbits, it has been found that at least ten groups of arsonilic acid per molecule of protein are necessary for precipitation but that an average of only one arsonilic acid group per molecule of protein is necessary in order to produce antibodies against arsonilic acid. It is possible, of course, that the valence of the antigen which is necessary for the production of hypersensitive reactions may be much lower and that antigen-antibody reactions which do not result in precipitation may still produce a hypersensitivity reaction. There have been reports of anaphylaxis with a simple univalent antigen. The amount of antigen and antibody which is necessary to bring about observable reactions depends upon the character of the two reactants. Thus, there may be more than a thousandfold difference in the amount of various substances required to produce a detectable amount of precipitin in rabbit serum or a hypersensitive state in guinea pigs. This variation probably depends, (1) upon the actual stimulation of antibody formation, (2) upon the degree or intensity of the antigen-antibody reaction, and (3) upon the method used for detection of the reaction.

Approximately 0.002 Gm. of either rabbit or guinea pig antibody protein is sufficient to sensitize normal guinea pigs passively to a state of hypersensitivity which will give fatal anaphylactic shock. Although not much is known of the quantities of reagin in the circulation or amounts necessary to produce local or general allergic reactions, it may be assumed that they will be small.

It is generally agreed that the native antigens are multivalent, although there is still some argument as to the number of combining sites on the antibody molecule, which brings up the interesting question of the possible heterogeneity of antibodies. It is becoming increasingly evident from theoretic considerations that an immune serum to a chemically pure antigen should contain a variety of antibodies to this antigen, and it is an experimental fact that antibodies do not have the same reaction capacity. For example, Loveless has shown that apparently reagin has some of the properties of classical antibodies, but it is well known that in many respects it is very different, and Miller and myself have suggested that the failure of reagin to give serologic reactions is due to the univalence of the reagin molecule. Another anomalous type of antibody is the so-called Rh "blocking" antibody.

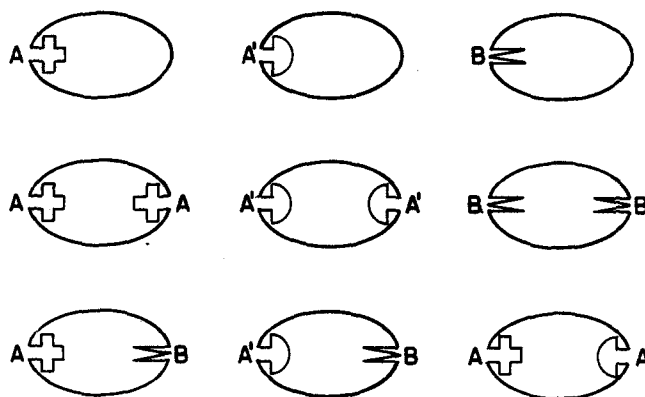


Fig. 1.—Schematic representation of possible antibody molecules in a simple antiserum, illustrating heterogeneity with respect to valence, specificity, and combining strength.

One can easily conceive of two types of heterogeneity: that which involves the number of combining sites on the antibody molecule and that which has to do with specificity (the specific portion of the antigen molecule toward which the antibody combining site is directed). Fig. 1 gives a diagrammatic representation of a condition in which two specificities are involved, *A* and *B*, and an antibody molecule having either one or two valences. Whereas there is some evidence that univalent antibody molecules are present in immune or hypersensitive serums, there is no good evidence at present that a single antibody molecule will contain combining sites for more than a single type of specificity. A third type of heterogeneity is also shown in Fig. 1 as *A'*, which attempts to show a situation where the specificity is similar to *A*, but the reaction with *A* will be weak due to a lack of precise fit. Another type of heterogeneity, which

is also conceptional, but which seems logical from the previous discussion of the basis of specificity of antigen, is diagrammatically represented in Fig. 2. There is no reason to suppose that antibodies will be produced to only one portion of an antigen molecule so that antiserum will contain a whole "spectrum" of antibody specificities of which only a fraction of the antibodies will have specificities directed against any given part of the antigen. Thus an immune serum prepared against arsanilic-azo-protein will contain antibodies against both the arsanilic acid group and against the carrier. Obviously, antibody configurations directed against  $H$  will react very little, if at all, with the protein carrier,  $C$ , and portions which are directed against small areas of both the hapten and the carrier,  $C-H$ , will react only weakly with either. It can be shown experimentally by precipitin reactions that antibodies have different combining strengths for a given antigenic structure, but it is often difficult to distinguish between "weak" antibodies and univalent antibodies.

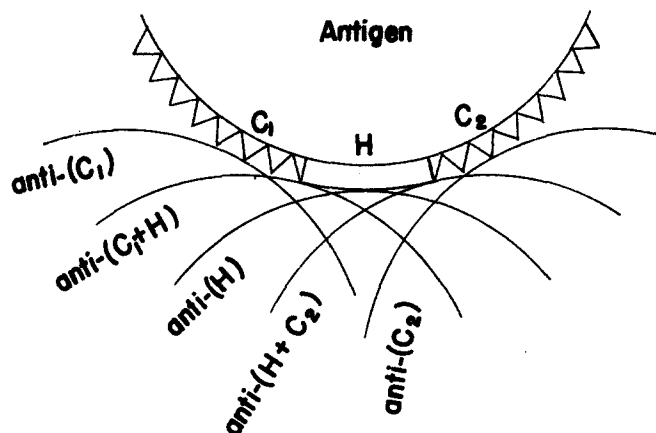


Fig. 2.—Diagram representing heterogeneity with respect to homology. There is shown a portion of the surface of a conjugated protein antigen consisting of hapten,  $H$ , and adjacent regions of the carrier,  $C_1$  and  $C_2$ , on opposite portions of the hapten. Of the large number of possible combining sites which may be considered to fall in a continuous series with respect to their homology, there are shown only five, of which one, anti- $H$ , is directed solely against the hapten, two, anti- $C_1$  and anti- $C_2$ , are directed solely against the carrier, and two, anti- $(C_1 + H)$  and anti- $(H + C_2)$ , are directed against adjacent portions of the carrier and hapten simultaneously.

With these observations in mind let us consider briefly some of the established as well as theoretic aspects of antibody formation. First, since antibodies are protein, it is often assumed that they must be formed in sites where other serum proteins are formed. However, the possibility arises that the antibody configuration is not imposed during the early synthesis of the protein molecule but rather during a later stage of formation of the final globulin structure. There is evidence that many proteins may be constructed from relatively large "building blocks" of polypeptides. It may also be that the site of formation may vary with the antigen and that localization of antigens may result in antibody formation under conditions which do not result in an antibody structure similar to that which is produced in some other site or condition. An-

other consideration is that of specificity. Briefly, three theories have been proposed to account for the role of the antigen in determining the specificity of the antibody. One theory assumes that the antibody globulin is a *new* protein and that the antigen determines the manner and order in which the amino acids are incorporated into the polypeptide chain. A second theory assumes that the order of amino acids and chemical composition is the same in all globulins but that the folding of the polypeptide chain to form the final configuration is influenced by the antigen which acts as a pattern or template so that a mirror image will be impressed upon the surface of the final globulin. Both of the foregoing theories require the presence of antigen and assume that antigen directly influences the globulin configuration. In order to explain the apparent persistence of antibody formation long after no antigen can be detected in an animal, a third theory has been proposed which suggests that the antigenic pattern is impressed on the enzyme system responsible for the synthesis of globulin. It is of interest to note again the idea that an antigen must be digestible in order to induce antibody formation. If this is true, antigenic molecules may be "fragmented" by digestion and each fragment then behaves as a functional antigen, providing it is in suitable environment. Thus, apparent nonantibody gamma globulin, which is increased along with antibody (precipitating) globulin during immunization, is actually antibody directed against a portion of the antigen molecule, but which in the native protein used for test antigen is not exposed to the antibody. Such antigenic fragments might also be relatively resistant to further digestion and difficult to detect chemically. Fragmentation might also account for the peculiar reactions sometimes encountered in food allergies, when skin tests to an intact protein antigen is negative and symptoms are obtained only after ingestion of the food and sufficient time has elapsed to allow for partial digestion and absorption. Obviously, until more is known about the formation and structure of serum proteins, ideas of antibody formation must remain in the realm of speculation.

One of the most important immunochemical problems and one which is of particular interest to the allergist is the chemical and physiologic effects of antigen-antibody reactions. Since the two reactants may be separated and recovered in an unchanged condition, we know that, in the test tube at least, no marked permanent chemical or physical change occurs and when antigen and antibody (or reagin) are combined and injected either intravenously or into the skin, that no systemic or local reaction occurs. Regardless of which is injected first, several hours must elapse before injection of the second reagent will elicit a physiologic response. Aside from the lysis of cells (and only a very few are susceptible to lysis), immunity reactions are often explained on the basis of increased phagocytosis because, in general, if antibody does not lyse a bacterium it is apparently unharmed and will grow and multiply when inoculated into suitable media. However, if the antigen is a part of a cell structure (for example, an erythrocyte), the intrinsic integrity of the membrane is changed and if complement is present, lysis may occur. The lysis of cells is about the only evidence we have that some change must occur in either the

antigen or antibody, or perhaps both, when they combine. It is generally assumed that time is required for suitable absorption by cells and that hypersensitivity reactions are initiated by antigen-antibody reactions at cell surfaces. The question then arises as to the sequence of reactions which follow the initial combination. The fact that an antigen-antibody reaction results in a change of the cell membrane in lytic reactions suggests that some very subtle alteration may take place in cell surfaces, even if lysis or death of the cell is not evident. Such possible effects as reorientation of molecules or redistribution of charges have been given little study by immunologists and cellular physiologists but might yield very significant clues as to the mechanisms of hypersensitive reactions. For example, it is conceivable that changes occur in the permeability of cells or that potentials are produced by certain cells, particularly if a reaction takes place at a nerve cell surface. This, of course, brings up the question as to whether an antigen-antibody reaction is directly responsible for hypersensitive reactions or merely initiates a chain of reactions which finally produces the allergic response. Regardless of the mass of experimental work that has been directed toward placing the final responsibility upon histamine, there is still only very indirect evidence that histamine is the important agent in shock reactions. The so-called antihistaminic drugs produce a variety of complicated physiologic responses and, although they are valuable in the treatment of certain allergies and experimentally reduce histamine effects, there is not sufficient evidence to place histamine in such a fundamental role. There are, of course, many drugs and physical agents which cause smooth muscle contraction and also a variety of substances which will prevent the contraction of smooth muscle. These reactions and effects should be considered from a quantitative as well as qualitative viewpoint.

I should like to point out the possibility of the role of nerve excitation in allergic responses. For example, certain types of hypersensitive responses, such as skin tests, anaphylactic shock, and particularly the contraction of a strip of intestine from a sensitized guinea pig, are known to take place very rapidly. In the case of the guinea pig intestine the contraction occurs almost instantly, in spite of the fact that the smooth muscle is surrounded by a membrane which is essentially impermeable to the test antigen. Under such conditions the antigen probably never reaches the smooth muscle although contraction is not only produced, but it can be shown that desensitization has occurred. It is also possible to show that an isolated smooth muscle strip from an animal which has been sensitized to a large number of antigens will respond to the successive addition of each respective antigen. The strips not only respond specifically to each antigen, but also desensitize specifically to each, and the mechanism will show no signs of nonspecific desensitization or fatigue as long as good physiologic conditions are maintained. Thus, one is faced with explaining either the presence of a relatively large amount of humoral material, of which only a small amount is released with each specific antigen-antibody reaction and which is quickly dissipated, or devising some other mechanism. It seems to me that the possibility of a purely neurogenic mechanism should be more seriously considered.

Perhaps this paper is too brief to give an adequate foundation for any immunochemical concept of hypersensitivity, but an attempt has been made to give some of the current chemical concepts of serologically active antibodies and antigens and to offer some speculations on their formation, structure, and reactions. I feel that an immunologic approach to the problem of hypersensitivity is of great importance and, although some allergic manifestations may have other mechanisms, many such reactions indicate a close relationship to fundamental immunologic phenomena.

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