

Report of Dr. Goebel.On the nature of pneumococcus antibody and its union with hapten.

The older concepts regarding the non-protein nature of antibodies, and the hypothesis of Buchner that antigen itself formed a part of the antibody complex must now be regarded merely as evolutionary steps in an attempt to understand the true nature of these biologically active substances. During the past decade these older theories have gradually given way to accumulated chemical evidence which at the present time clearly points toward the fact that antibodies are in reality modified serum globulins.

The ideas of the French School of Immunologists sponsored by Bordet accounted for the interaction of antigen and antibody by the phenomenon of adsorption. This concept, however, failed to explain the specificity of immunological reactions. More recently the specificity of antibody production has been explained independently by Mudd in this country and by Breinl and Horowitz in Germany. In their opinion, the normal course of serum globulin synthesis is altered in the animal body when antigen reaches the site at which the synthesis takes place. Under the influence of the antigen, the serum globulin is altered in a way characteristic for the foreign stimulus. When the modified globulin eventually encounters the foreign antigen either in the circulation, or in vitro, interaction of the two is possible.

From the following interesting analogy - the results of certain experiments carried out in our laboratories - it seems not unlikely that the synthesizing function of a cell may be specifically oriented by a given stimulus. When the "R" form of Pneumococcus is grown in the presence of a "specific activator" derived from a cell-free filtrate of the "S" organism, the former is induced to resynthesize the capsular polysaccharide. If the

nature of the activator is changed, however, the "R" organism can be made to synthesize a polysaccharide quite different in its type-specificity from that which it produced when stimulated by the original activator. Thus it is seen that bacterial cells, under the same general environmental conditions may, by means of specific stimuli, be directed to synthesize products which, though chemically related, are biologically specific.

In regard to the non-protein nature of antibodies there are even today certain immunologists who maintain that solutions of antibodies may be secured which give no chemical tests for proteins. It must be pointed out, however, that the sensitivity of immunological reactions far exceeds the limit of sensitivity of chemical tests for proteins. Until weighable quantities of non-protein antibodies can be secured, the evidence for this point of view remains inconclusive.

An analogous situation has existed in the attempts to define the chemical nature of enzymes. It has been the method of the European investigators to purify enzymes by adsorption on colloids. Solutions have thus been secured which become increasingly lower in nitrogen content without loss of enzymatic activity. This group of investigators has failed, however, to secure by these methods protein-free enzymes, nor have they obtained substances the chemical composition and biological potency of which bear any constant relationship to one another. However, a new approach to the problem of enzyme chemistry has made possible the preparation of crystalline proteins bearing the biologically active component of crude enzyme mixtures. These crystalline proteins show both constant physical properties and biological activity on subsequent crystallization.

The success of Northrop and his associates in isolating from mixtures of proteins a crystalline derivative of unique biological and

chemical properties has served as a stimulus to us in attempting to isolate an immune protein from antipneumococcus serum. It is conceivable that this protein might have certain properties which would differentiate it from the accompanying inert serum proteins. The nature of serum globulins, however, is a problem of great complexity. Fortunately the investigations of the Carlsberg laboratories have afforded an insight into the chemical nature of globulin complexes as they occur in normal horse serum.

From extensive studies on the proteins of normal horse serum, Sorensen came to the conclusion that the globulins occur not as mixtures but as labile compounds of eu- and pseudo globulin. He found that these compounds are easily dissociable and that by means of fractional precipitation and dialysis the constituents can be partially separated as protein fractions which become, with each subsequent fractionation, increasingly richer either in eu- or pseudo globulin, as the case may be. Although globulins have never been obtained in a state of purity, it has been possible to dissociate the complexes of serum globulins to such an extent that fractions have been obtained of which either eu- or pseudo globulin is the predominant constituent.

We have made use of this principle of the dissociation of the serum globulins in the separation of the antibodies in Type I Pneumococcus anti-serum from the accompanying inert globulins with which the immune bodies are associated. The water insoluble globulin fraction of antipneumococcus serum, described by Felton and commonly used in the therapeutic treatment of lobar pneumonia, has been separated into an inert euglobulin fraction, and a pseudo globulin fraction containing practically all of the antibodies present in the original mixture of the two serum proteins.

When finally dissociated and separated from inert euglobulin, the

water soluble immune globulin is considerably more potent in antibody content than the parent substance. Half a centimeter of a solution of the purified antibody containing as little as 0.005 mg. of protein is effective in protecting mice against 500,000 minimal lethal doses of virulent pneumococci of the homologous type.

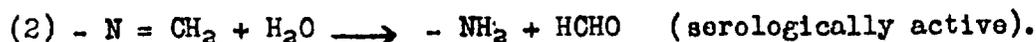
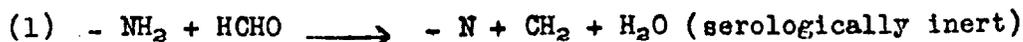
In addition to increased biological potency, the immune globulin has certain chemical properties which distinguish it from normal serum globulin. Determinations of the basic amino acid distribution of normal and immune globulin reveal no striking difference in the chemical units which constitute the two proteins. However, the immune globulin has a very alkaline isoelectric point (pH 7.6) whereas that of normal serum globulin lies in the more acid range (pH 5.2). Thus the most distinguishing property of the immune protein, its alkaline isoelectric point, is one which can be accounted for only by a relative increase in the number of basic amino groups in the protein molecule.

Efforts have been made to crystallize the immune globulin, but thus far without convincing results. Solubility measurements have indicated that the antibody in its present state of purity is not a chemical entity, despite many repeated fractional precipitations. It may be for this reason that attempts to crystallize the antibody have thus far been unsuccessful.

In view of its unusual basic properties it seems not unlikely that the amino groups of the antibody protein may play some role in the union of the antibody molecule with its type-specific haptene - the capsular polysaccharide of Type I Pneumococcus. In collaboration with Dr. Chow, it was subsequently found that when the amino groups of the antibody were acetylated with ketene gas, under conditions which eliminated any

possibility of protein denaturation, the resultant acetylated antibody protein failed not only to precipitate the type-specific capsular polysaccharide, but to agglutinate suspensions of Type I pneumococci, and to protect mice against infection with homologous virulent Type I pneumococci as well.

The basic amino groups of the antibody protein have also been covered by the grouping = CH<sub>2</sub>, with a resulting loss in serological activity. When the grouping = CH<sub>2</sub> is replaced by two hydrogen atoms, however, the amino groups are restored and the immunological properties of the antibody protein are fully regained. The chemical reactions which are involved are as follows:



Reaction 1 goes to completion if an aqueous solution of pneumococcus antibody is treated with formaldehyde at 0°C. and at pH 8.5. The excess formaldehyde is removed by dialysis, and the solution of the resulting "formalized" protein is adjusted to pH 7.5. The protein derivative is perfectly stable under these conditions and shows no serological activity whatsoever. If the aqueous solution of the formalized antibody is adjusted to pH 5.0, however, reaction 2 takes place rapidly. When the reaction mixture is dialysed and the pH again adjusted to 7.5, the immunological properties of the protein are fully restored. From these experiments it appears that the presence of basic amino groups is of utmost importance in determining the biological activity of the antibody protein.

Studies carried out in this laboratory on the chemical nature of the type-specific polysaccharides derived from encapsulated microorganisms have revealed the interesting fact that all the specific carbohydrates

thus far investigated contain uronic acids as constituents of the polysaccharide molecule. The invariable occurrence in these bacterial products of glucuronic acid or its isomers suggests that the highly polar carboxyl group of the uronic acid and its stereochemical relationship to other groups in the molecule determines the biological specificity of these substances and actually enters into chemical combination with the basic amino group of the homologous antibody when these two substances are brought together either in vivo or in vitro. Thus, if the carboxyl group of the Type I specific carbohydrate could be covered by an ester grouping, the derivative should no longer react with its homologous antibody. The polysaccharide was therefore treated with an ethereal solution of diazo methane, and the methyl ester separated from the reaction mixture. Solutions of the methyl ester of the polysaccharide gave no serological reaction when added to homologous antibody. When the methyl groups were removed by gentle alkaline hydrolysis the carbohydrate again reacted specifically with antibody in dilutions as high as 1 part in 4 million. Quantitative determinations of the methoxyl content of the carbohydrate before and after hydrolysis showed that the methoxyl derivative contained one methoxyl group per carboxyl group. The reversible changes in immunological specificity brought about by reversible alterations in chemical structure indicate that at least two of the molecular groupings involved in antigen-antibody union are the electro-positive amino group of the immune protein and the electro-negative carboxyl group of the specific polysaccharide.

This concept alone does not explain the specificity of the reaction. There is, however, certain evidence to support the view that the specificity of such serological reactions is governed by the arrangement in space of the polar groups of the reactive carbohydrate. It has been

shown that antigens prepared from the diazophenol glycosides of glucose and galactose react only in their homologous antisera. In respect to the number and nature of their polar groups, these two glycosides are identical. Since the reactive groups are in each instance the same, the mechanism underlying the union of both glycosides with their homologous antibodies must likewise be the same. The specificity of this reaction therefore can be accounted for only by known differences in the spatial arrangement of the polar groups of the fourth carbon atom of each glycoside.

Since the spatial arrangement of identical polar groups in a carbohydrate suffices to determine specificity, it therefore seems justifiable to assume in the case of the antibody that the spatial arrangement of polar groups in immune protein may likewise determine its specific capacity to react with a given hapten.

In view of these considerations it is believed that the general mechanism underlying the union of antibody and carbohydrate involves the interaction of polar groups of opposite charge. In the case of the Pneumococcus Type I hapten and its specific antibody, it is a certain amino group of the latter, and the carboxyl group of the polysaccharide which interact to form the immune precipitate. It is suggested, furthermore, that the specificity of this reaction is determined by the stereochemical relationship of the dominant polar groups in the reacting molecules, whether they be antigen or antibody. If the spatial pattern of the polar groups of both antigen and antibody is of exactly the correct order, then union occurs. If, however, this relationship is disturbed by artificial means, as has been experimentally demonstrated by covering the dominant polar group of either antigen or antibody with a chemical radical, the pattern is destroyed, and union between antigen and antibody fails to take place. When the original constitution of the acting substances is restored, however, serological specificity is once more regained.

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