THE STRUCTURE OF NATURAL AND SYNTHETIC ANTIGENS[1]

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WERE I to confine my remarks, in opening this discussion, to a literal interpretation of the subject or to definitely established facts, our committee's generous time allowance would be much too long. I must therefore risk your disapprobation, to fill the time allotted, by taking a broad view of the topic and by entering occasionally upon the realm of the speculative. As I have taken the bit in my teeth, let me also give credit here to all my colleagues in this field, and since you will have no difficulty in recognizing the contributions discussed, I may be permitted to save time by mentioning few names.

With regard to the structure of the natural antigens of the type of the animal proteins there is little that can be said. Denaturation effects a change in specificity, as does also reduction of disulfide linkages, but the latter effect may be due to a splitting of the molecule at the –S–S– linkage as well as to the conversion of these groups into –SH. Of greatest promise, however, has been the work of Bergmann on gelatin and Waldschmidt-Leitz on clupein, substances which are not antigens at all. Extension of these fundamental researches on the number and arrangement of the amino acids to more complete proteins will afford a direct approach to an understanding of the chemical basis of the specificity of proteins, the class of substances to which most antigens belong. Work along these lines should eventually clear up the riddle of species specificity and tell us why, and how, for example, horse serum albumin differs from human serum albumin. While it is probably the occurrence of definite groupings of amino acids which confers the property of serological specificity on these natural

[1] Opener's address, Joint Discussion on the Structure of Natural and Synthetic Antigens, Second International Congress for Microbiology, London, July 27, 1936.

[24] H. Poincaré in "The Foundations of Science." The Science Press, pp. 207, 209, 1929.

antigens, the completeness of their antigenic function would seem to depend upon the ordered recurrence of these groupings to build up a multivalent whole. It is likely that these groupings contain aromatic nuclei, and it is also probable that the ε-amino groups of the lysine component play a part.

In addition to the animal antigens there is another antigenic group of deep significance—the antigens elaborated by the bacterial cell. Comparatively little is yet known of the bacterial cellular proteins except that they represent exceedingly complex mixtures and that fractions isolated under conditions likely to reduce enzymatic and chemical changes to a minimum usually contain more or less nucleic acid, all or part of which may be combined as an ester. Most fractions also contain carbohydrate in excess of that ascribable to nucleic acid, to which I shall refer later. To attempt to classify these proteins as "albumins" or "globulins," or to speak of their precipitability by acetic acid as an indication of "denaturation," as has been done, is, I believe, premature and out of harmony with the accepted usages of these three terms. Little is known, as well, of the bacterial toxins, except that they appear to be proteins, or only slightly degraded proteins.

Until twelve or thirteen years ago it was assumed that only proteins could be carriers of the antigenic function, but the discovery that polysaccharides are the determinants of type specificity among the encapsulated bacteria greatly widened the scope of immunochemistry and cleared up several puzzling problems. Indeed, owing to the nature of the carbohydrates, the chemical basis of specificity in this group is far better understood to-day than among the proteins, but even here our knowledge is fragmentary. With the discovery of a labile acetyl group in the specific polysaccharide of Type I pneumococcus, many of the conflicting reports in the literature were reconciled and explained, and there seemed little more to be done than to work out the chemical structure of this unusual nitrogen-containing polygalacturonide. We have just been made aware, however, that the Types I, II and III pneumococcus specific polysaccharides are not even stable to heat, as has been supposed for so long. The use of absolute, quantitative methods of immunological analysis, of which I shall speak on another occasion and which were not available at the time of the earlier work, has brought out this thermolability very clearly, and the irreversible drop in viscosity of specific polysaccharide solutions when heated is an even more sensitive indicator of a change which is probably a depolymerization.

I may mention, also, that preliminary ultracentrifugal studies on the specific polysaccharide of Type II pneumococcus, in which electrical effects would be expected to be much less than with the Type I or Type III substance, indicate that the particle weight of even the unheated carbohydrate is relatively small. Moreover, when we consider that some thirty serological types of pneumococcus are known, each of which must be characterized by a chemically distinct specific polysaccharide, and that only the carbohydrates of Types I, II, III, IV and VIII have been studied and at least partially identified, we must admit that even the much worked over field of pneumococcus immunochemistry is woefully incomplete.

The question of the antigenicity of specific polysaccharides is an interesting and complicated one. It would appear that, given a suitable, undegraded preparation, and a suitable animal such as the mouse, or a suitable route of injection into other animals, as for example, the human skin, specific polysaccharides may function as antigens and directly stimulate the production of antibodies. Landsteiner has just shown, however, that even simple aromatic substances may share this property, which is perhaps due to their ability to combine with certain tissue proteins to form new antigens. Another recent development is the finding that protein fractions of meningococcus, streptococcus and tubercle bacillus, when injected into rabbits, quite regularly give rise not only to antibodies reacting with the bacterial protein, but also to antibodies which precipitate the somatic specific polysaccharides of these microorganisms. Since the mere mixture of a specific polysaccharide with protein does not suffice to render the carbohydrate antigenic for rabbits, it must be concluded that these protein fractions contain specific polysaccharide in chemical combination, and this has been further indicated in the case of streptococcus by actual chemical analyses. While the carbohydrate in synthetic carbohydrate-protein antigens has been shown to be immunologically active, these are the first instances in which such activity has been definitely indicated in a natural protein antigen. The question is also of importance in connection with the hunt for the elusive pneumococcus type specific antigen which has always been assumed to be a labile carbohydrate-protein complex.

There is one other phase in the development of polysaccharide chemistry which never fails to afford me a quiet chuckle. You will recall that the specific polysaccharide of Type III pneumococcus was found to be a polyaldobionic acid, that is, composed of units of glucuronic acid linked to glucose. Since then, it has been discovered that aldobionic acids form an important part of the hemicelluloses and plant gums, so that actually untold numbers of tons of these sugar derivatives exist. Thus, even though the woods were full of them, the first aldobionic acid was found in the capsular substance of a pathogenic microorganism.

Two other most important classes of natural anti-

gens are the filterable viruses and bacteriophage. The chemical study of these antigens is in its infancy, but will, I feel sure, occupy a prominent place on the agenda of the next international congress. At present the weight of evidence would appear to favor the view that 'phage and some of the plant viruses, at least, are not living agents but proteins capable of increase in some such autocatalytic fashion as Northrop and his collaborators have found in the case of certain enzymes. Tobacco mosaic virus, for example, could not be separated from a most unusual crystalline protein of particle size greater than that found for any other protein.

Whether or not to include lipins among the natural antigens is still uncertain. Too often the serological work on this subject has been without adequate chemical control and, in more than one instance in which isolation of the serologically active so-called lipin has been attempted, much of the lipin character has been lost during the process of purification. Although on chemical grounds lipins should possibly be capable of functioning antigenically as well as carbohydrates, for example, the few apparently authentic instances of such action may well be due to carbohydrate-lipin complexes such as have been found in the Forssman hapten and among the specific polysaccharides of the tubercle bacillus or to protein-lipin complexes such as have been reported in serum. Perhaps a more decisive answer to this question, also, may be available when the next congress convenes.

If you will permit me I shall define "synthetic antigens" as "chemically altered antigens." We may then include such derivatives as the halogenated and nitro-proteins, which have been so useful in showing the importance of aromatic amino acids in serological specificity, and the acylated and phenylureido-derivatives which have also indicated the participation of the free ε-amino groups of the lysine component.

Preeminent among the synthetic antigens, however, are the azoproteins, which Landsteiner has used so fruitfully in tracing the delicate changes in specificity brought about by coupling proteins with one or another diazotized aromatic amine. That the original species specificity may, on occasion, disappear entirely, and be replaced by a specificity characteristic of the entering aromatic amine is a truly remarkable finding, and its exploitation has given us much that we know of the chemical basis of serological specificity. It was shown that position isomerism in the entering radicals causes a change in specificity, that the presence of an acidic grouping sharpens the new specificity and decreases cross reactivity, and di-, tri- and tetrapeptides have been coupled to protein by the same reaction and the influence of the arrangement of the individual amino acids traced. In Avery's laboratory sugars were most ingeniously coupled with proteins by the diazo reaction, and it was shown that the rotation of a single carbon atom in the entering group through an angle of 180°, as in glucose and galactose, produced a radical change in specificity. Moreover, it was possible to "reconstitute," as it were, the Type III pneumococcus antigen by coupling the pneumococcus specific polysaccharide to protein and showing that the antibodies produced on injecting this synthetic antigen into rabbits agglutinated Type III pneumococcus and protected mice against many lethal doses of the microorganism.

Azoproteins have also been most serviceable in testing the old Buchner hypothesis of antibody formation. Serum containing antibodies to arsanilic acid azoprotein contained no more arsenic than did the corresponding normal serum, indicating the failure of the specific determinant of the antigen to participate in the specific function of the antibody. Azoproteins have also served as useful reagents in the study of the mechanism of the precipitin reaction since, under special conditions, they permit the separate determination of antigen and antibody in specific precipitates of which the azoprotein forms a part.

If one attempts to penetrate more deeply into the reason for the change of specificity with change of the entering or hapten or determinant group in synthetic antigens one encounters immediate difficulties, some of which are possibly due to the vagaries of antibody formation in rabbits. Perhaps the most nearly successful correlation is with the "force fields" surrounding the molecular groupings in question, but agreement with the theory is scarcely more frequent than the exceptions, and some modification at the very least, appears necessary. Certainly it is to be expected that changes in serological specificity may be ultimately referred to changes, however minute, in chemical structure, even if the altered force fields resulting from such changes are not the operative causes.

A discussion of the structure of natural and synthetic antigens would scarcely be complete without a word as to the relation of antigenic structure to the antibody response in animals. This phase of the subject is somewhat complicated by the growing list of differences between antibodies engendered, for example, in the rabbit and in the horse, to which may be added the most recent finding, based on ultracentrifugal studies, indicating differences in molecular dimensions. Even if one neglects these differences until they have received more thorough study, one may still risk one or two generalizations.

Structural considerations indicate that the groupings responsible for serological specificity are repeated many times in the protein antigens and several times,

at least, in the specific polysaccharides. This structural peculiarity, or multivalence, is not shared by lipins, and this may possibly be a factor contributing to the difficulty of demonstrating antigenic properties in lipins. The multivalence of the antigens is not necessarily confined to a single serologically active grouping, but may readily be a property of several such groupings, so that it is not surprising to find that a single, crystalline antigenic substance, such as egg albumin, may give rise to more than one antibody, or even a whole series of antibodies. And since there is every evidence that these antibodies are modified serum globulins they also afford the opportunity of recurrence of serologically reactive groupings, or multivalence, so that antibodies, even to a single crystalline antigen, may differ both as to the number and character of their reactive groupings. These relationships are reflected not only in the wide range of combining proportions between antigen and antibody—often a tenfold one—shown by quantitative studies on the precipitin and agglutination reactions, but also by qualitative and quantitative differences in the reaction between antigen and fractionally absorbed or precipitated antibodies.

In conclusion, our knowledge of the structure of antigens, while still fragmentary, has at least progressed to the extent that we possess some knowledge of their chemical character and some inkling of the chemical differences between antigens which may be expected to give rise to differences in serological specificity. We are also beginning to acquire precise data on other chemical substances which we have hitherto designated rather vaguely as antibodies, and there would even appear to be certain advantages in considering the reactions between the chemical substances known as antigens and antibodies as chemical reactions, complicated, it is true, but subject to the same laws as simpler chemical systems. Our young science of immunochemistry has thus demonstrated its utility and promise as a powerful aid in the solution of many of the most puzzling problems both in biology and in immunity to infectious disease.