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IMMUNOLOGY AS A TOOL IN BIOLOGICAL RESEARCH[1]

IMMUNOCHEMICAL APPROACHES TO BIOLOGICAL PROBLEMS

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I VENTURE to address this gathering of geneticists and zoologists with an exhilaration engendered by a sense of the daring involved in an excursion into well-explored fields of knowledge remote from those into which my own work has extended. I trust, however, you will forgive this excursion or incursion, as it is intended more to remind you of progress already made in your fields along immunochemical lines, rather than to suggest the adoption of wholly foreign techniques and ideas.

However, before reviewing these applications, it might be well to describe again in modern chemical terms some of the concepts fundamental to immunology.

Knowledge of antigens, or the substances stimulating immune responses in animals, has been greatly extended in recent years. Thanks to chemical fractionations, the ultracentrifuge, the Tiselius electrophoresis apparatus and other powerful tools, one may no longer consider horse serum, for example, or an animal or bacterial cell, as "an antigen," but must recognize it as a collection of antigens,

[1] Four papers from a symposium scheduled to be presented by the Genetics Society of America at the annual meeting of the American Association for the Advancement of Science, which was cancelled at the request of the Office of Defense Transportation, December, 1942.

each with distinct properties and potencies. Many immunological observations were and are difficult to interpret because this complexity was not taken into account. It is also apparent that many antigens are proteins and that most proteins are antigenic. Much work has been done showing that denaturation as well as introduction of the most varied chemical groupings at almost any point of substitution results in a definite change in immunological specificity.

Now most of you will remember that some time ago, in Avery's laboratory, we found that type specificity among the encapsulated bacteria depended upon another kind of antigen. This type specificity was due to a peculiar group of polysaccharides resistant to the usual sugar-splitting enzymes. The specific polysaccharide of each pneumococcus type, for example, was different from those of other types, and could be characterized by its distinctive physical and chemical properties. The sugars from types II and III pneumococcus were obtained free from nitrogen, and were the first instances in which immune specificity had been rigorously demonstrated in a class of substances other than proteins.

With this brief discussion of specificity as a basis, what can be said about the requisite conditions for antigenicity? It is obvious that we must have a complex structure and large molecules, and one of the important things seems to be the repetition of structural units. This is a highly probable consequence of the modern views of protein structure. We also know that the specific carbohydrate of type III pneumococcus, for instance, is made up of many cellobiuronic acid units. Some multiple of this unit must function as the immunologically reactive grouping, for when the carbohydrate is partially broken down by mild hydrolysis the fragments of two or more units still react in anti-pneumococcus type III horse serum. Therefore we may assume that in order to function fully as an antigen a substance of large molecular size must be of such nature as to allow repetition of cer-

tain structural units. Possibly for this reason ordinary lipids do not appear to have a clear-cut antigenic function.

I think Dr. Landsteiner would add that any simple chemical substance may also function as an antigen especially if the chemical properties are such as to allow its combination with protein to form new antigens. Complex structure is not necessary, therefore, if a number of molecules of a smaller entity can combine to form part of a larger structure.

With regard to antibodies, the immune substances engendered in animals as a result of the antigenic stimulus, we are in a position to be equally definite. Use of new quantitative chemical microanalytical methods made it possible to measure antibodies in sera in actual weight units. One could, for the first time, express antibodies in terms of specific nitrogen per cubic centimeter of serum, because after precipitation with a slight excess of antigen non-specific material could be washed out. Since the amount of nitrogen in the added antigen is known this may be subtracted and the residual nitrogen in the washed precipitate is due to the antibodies. Highly purified antibody solutions obtained as a consequence of information gained by these new methods were examined in the ultracentrifuge and electrophoresis apparatus and were shown to have the properties of typical serum proteins.

Buchner's hypothesis that antibody contained fragments of antigen was proposed at a time when the actual nature of antibodies was not understood. This hypothesis never appealed to the chemist because in a number of instances like repels like, rather than attracts. In 1932 Breinl and Haurowitz proposed a theory that antibodies are formed by a modification of the normal process of serum globulin synthesis as a result of penetration of antigen or specific portions of the antigen to the site of globulin synthesis. The disturbance so brought about influences the course of that synthesis in a sense characteristic of the antigen so that when the modified globulin appears in the circulation and again encounters the antigen

interaction is possible. This not very clear picture was later expressed in somewhat more definite form by Mudd. An extension of this hypothesis has recently been made by Pauling which is even more graphic and reasonable but as devoid of experimental basis as the Breinl and Haurowitz theory. The Pauling hypothesis carried a second idea—that if one could take normal globulin, denature it and fold it up again in the presence of antigen, artificial production of antibodies might be accomplished. Pauling now believes he has been successful in this, but such details of his experiments as have been published do not include complete controls. Burnet has recently proposed the origin of antibodies through modification by antigen of intracellular proteases which provide the framework for synthesis of partial replicas of themselves (globulins or antibodies). This would provide for antibody formation after destruction of antigen and for progressive changes in antibodies with successive immunizations.

These theories of antibody formation have been given a physiological basis in recent years by Dr. Florence Sabin as a result of experimental work with a red protein dye. Dr. Sabin has observed macrophages in the omentum and cells of the reticulo-endothelial system and found that, at a certain stage of development, surface layers which form folds waving back and forth finally disappeared as if they were being extruded from these cells. She believes this to be the source of serum globulins and that the presence of an antigen (for example, the red protein dye) results in the specific modification of these globulins into the appropriate antibody.

Now for a few applications to genetics and biology:

Nuttall's pioneer work on the mapping of biological relationships through the study of the interaction of animal sera with antibodies formed when these sera are injected into a standard animal such as the rabbit was most fruitful and has been extended by numerous workers. The immunochemist has shown that interpretation of the com-

plex findings is often simplified if a single protein is used, rather than serum, which we know to be a complex mixture of albumin, at least three globulins, complement with its four components, and other minor substances, all or most of which may function as antigens and cause overlapping or zone effects in reactions with antisera. Nor is it certain that precipitation in different antisera is always due to the same antigen when such a mixture is used.

An extreme instance of the simplification wrought by the use of pure, crystalline proteins was the demonstration by Landsteiner and myself of the non-identity or identity of the oxyhemoglobins of various species by a physical-chemical (solubility) method as well as by the serological technique. By use of the quantitative precipitin method, in which the amount of antibody nitrogen precipitated by a single purified antigen is measured, information as to species relationships may be gained that is unobtainable by qualitative measurements. In this way Stokinger and I were able to show the close relationship, but lack of identity, of sheep and bovine thyroglobulins, and to demonstrate that even this organ-specific globulin hormone possessed a species-specificity entirely independent of that of the corresponding serum globulins. With the same quantitative method Treffers, Moore and I were able to give a plausible explanation for the differences shown by normal horse γ-globulin and antipneumococcus horse γ-globulin in rabbit antisera to the antibody (horse).

It is not always necessary, however, nor is it necessarily an advantage, to study the immunological behavior of single antigens, as Irwin and his collaborators have shown in their intricate but clearly defined studies of the numerous gene-linked antigens of avian and mammalian red cells. Another fruitful immunological approach to genetic problems has been made by Tyler in his studies of the agglutination of sperm by egg substances of *Arbacia*.

The discovery of the specific polysaccharides of pneumococcus in Avery's laboratory and the recognition that these sugar derivatives are the determinants of type-

specificity in this and other groups of pathogenic microorganisms have led to far-reaching results, most of which are beyond the scope of this lecture. However, Griffith's initially almost unbelievable discovery that one pneumococcus type could be converted into another has important implications, not only in carbohydrate chemistry and bacteriology, but in general biology and genetics as well. As many of you know, pneumococci of Type I, for example, may be degraded to a form devoid of type-specificity and then converted, theoretically, at least, back to the original type, or into any one of some forty-odd other types. This was originally accomplished by growing the degraded cells in the presence of a heat-killed suspension of pneumococci of the type into which the living cells were to be converted. Studies by Avery, Dawson and Alloway showed, however, that certain extracts of type-specific pneumococci contained a substance or substances responsible for this conversion, and that the type-specific carbohydrates themselves were not the determining factors. Thus, any pneumococcus cell is potentially able either to synthesize, one at a time, nearly fifty different specific polysaccharides or may be so influenced by a series of substances that such varied syntheses become possible. The immunochemist must leave it to the geneticist to decide whether or not these processes are true mutations, but I am happy to say that Avery is continuing the study of the transforming principle, and the eventual elucidation of its nature is certain to throw light on this and many other questions.

These are merely a few of the instances in which immunological and immunochemical methods have provided an insight into biological mechanisms. To give a more complete summary would carry me far beyond the allotted time, but I hope you will recall other examples which I would have liked to mention. More important, however, I hope that those of you who may have required this reminder of the possibilities of these powerful tools will consider them as aids in the solution of present and future biological problems.