Victor A. Najjar, editor

immunity and virus infection

Surveys basic research in these two fields emphasizing the immunology and epidemiology of poliomyelitis as influenced by killed and live virus vaccine

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IMMUNITY AND VIRUS INFECTION

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Preface

This book embodies a collection of papers presented at a symposium that was held on May 1 and 2, 1958, at Vanderbilt University School of Medicine and organized by the Department of Microbiology. At that time, Sir Macfarlane Burnet was the Flexner Lecturer of the year and an official guest of the department. Because of his well-known contributions in immunology and virology, it was deemed appropriate to organize a symposium dealing primarily with these two subjects. Indeed, the great advance made in this area of research in recent years "hath trifled former knowings" (*Macbeth*) and rendered the publication of these contributions all the more timely and necessary.

In this symposium a special effort was made to bring together two interdependent but widely separated disciplines. Consequently, contributions originating in basic research, as well as in biological and clinical investigations, pertaining to the mechanism of immunity and the study of virus infection were included. It is hoped that this book will serve to acquaint the biologist, the clinician, and the clinical investigator with the basic facts and principles and bring to those in basic research the clinical and biological problems that have so often furnished the stimulus to fundamental research.

The section on immunity encompasses theoretical considerations of antibody formation based on clonal selection at the cellular level, and

on the role of antigen at the molecular level. New concepts pertaining to immunological reactions, the allergic state and immunologic diseases are also discussed. A good part of this section is devoted to a discussion of the genesis of fever in infection, the properdin system, and the phenomenon of immunological tolerance. The latter may well be of far-reaching significance and of potential practical value in human tissue transplants. The comprehensive review of the methods for separation and purification of antibody should be of great practical value.

The section on virology incorporates a discussion of virus infections that spans the field of plant, bacterial, and animal viruses including influenza and poliomyelitis. It was the elegant demonstration of the infectivity of tobacco mosaic virus nucleic acid that pointed the way to the very recent finding that the nucleic acid of poliomyelitis virus is likewise infectious. In like manner, the extensive work on the transmission of bacterial traits by bacteriophage has emphasized its possible role in human disease. This section also includes a discussion of the methods of purification of plant and animal viruses.

It should now be apparent that the object in each section of this volume is to provide the reader with a continuum of information, from the basic elements of the problem to its medical application. Both sections finally are brought to focus on the important problem of the immunology and epidemiology of poliomyelitis as influenced by killed and live virus vaccine.

I want at this point to express my gratitude to the National Foundation for Infantile Paralysis, Inc. for sponsoring this symposium and making it possible to assemble the participants. I am also indebted to Sir Macfarlane Burnet, Dr. Wendell M. Stanley, Dr. Ernest W. Goodpasture, and Dr. Alwin M. Pappenheimer for acting as moderators in the symposium.

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Nashville, Tennessee
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The Theories of Antibody Production

When Pauling put forward his theory of the structure and formation of antibodies in 1940, he prefaced it by a remark that the field was so extensive that the induction of a comprehensive theory directly from the observational material was impossible. Instead he proposed to attempt to find the simplest structure which could be suggested on current knowledge of intramolecular and intermolecular forces and which was not at variance with the facts.

His approach adopted the following basic assumptions:

1. That antibody has a complementary steric relationship to specific patterns on the antigen surface.
2. That such complementary patterns must be laid down against the antigen molecule itself.
3. That to account for this the simplest postulate is that all antibody molecules contain the same polypeptide chains as normal globulin and differ from normal globulin only in the configuration of the chain, that is, in the way the chain is coiled in the molecule. The coiling is pictured as taking place against the surface of the antigen.

That theory is still the working hypothesis of biochemists and immunologists who are concerned primarily with the use of antibodies as specific chemical reagents. It is not adequate for those who are concerned with more biological topics such as the nature of homograft immunity, the existence of blood group chimeras or the maintenance of "immunological memory" for many years. Alternative hypotheses are necessary, but it seems likely that for the foreseeable future they

will have to be approached in the same way that Pauling did. They can at best be no more than the simplest logical structure that can be suggested from current knowledge of the fields concerned and which is not at variance with the facts.

Until a year or two ago, the only current alternative to the Pauling theory, which was itself a development of the ideas of Haurowitz, Mudd, and Alexander, was that put forward by Burnet and Fenner (3) in 1949 and restated in slightly altered form by Burnet (1) in 1956. The approach here was based on four postulates:

1. That some means of recognizing self from not self material must be provided.
2. That the capacity for antibody production is transferable to descendant cells.
3. That the antigen has a positive effect in modifying the cell to produce a new protein pattern and to lay down an appropriately inheritable mechanism to do this.
4. That a useful analogy to this can be found in the process by which adaptive enzymes are induced in bacteria.

This theory has been extensively discussed in relation to the phenomenon of immunological tolerance and, it may be claimed, was in part responsible for the development of an experimental as well as an observational approach to such phenomena.

The next serious attempt to provide a theory of antibody production is due to Jerne (4). This seems to have originated from his finding that normal horse serum contained small amounts of a globulin which had the curious effect of stabilizing tryptophane activation of the tryptophane dependent strain of phage T4. In the earliest stage of specific immunization of a horse with this phage the agent increased 100 to 1000 fold, and although it had no inactivating effect on the phage Jerne felt that it must be regarded as an antibody. In none of the previous theories had any effort been made to cover the existence of normal antibodies and their relation to classical antibody. Another reason for dissatisfaction with Burnet and Fenner's theory was the change in attitude that had taken place toward the phenomenon of adaptive enzyme formation in bacteria. It is now generally agreed that the pattern of an adaptive enzyme is genetically determined, the function of the inducer being merely to call into being conditions needed for its increased production and liberation. Jerne's "natural selection" theory of antibody formation took as its starting point two new postulates:

1. A specific antibody conforms to pre-existent patterns which are represented often in undetectably small amounts in the populations of γ-globulin molecules present in normal serum.

2. The action of antigen is not to enforce changes of globulin pattern but to select out for differential proliferation those pre-existent patterns which have an appropriate steric relationship to the antigen.

It will be noted that each new formulation retains most of the preceding one but adds and modifies to allow the inclusion of a wider range of immunological phenomena. To most biochemists, however, the mechanism devised by Jerne to account for the proliferation of the appropriate type of γ-globulin was unacceptable. He gave to antigen "the sole role of carrying such specific globulin molecules from the circulation into cells in which these molecules can induce the production of more of their kind."

It seems quite out of line with current views on protein synthesis to suggest that, when a globulin molecule of appropriate pattern united to an antigen molecule is taken up by a mesenchymal cell, the antigen is discarded and the globulin molecule now provides a model for the continuing production of more globulin molecules to its specific pattern. This would demand at least as elaborate and unlikely a collection of *ad hoc* hypotheses as is required to elaborate the genocopy mechanism of the earlier hypothesis by Burnet (1).

Despite these unattractive features, Jerne's hypothesis has many virtues. Its great contribution is to draw attention to the theoretical possibility that the differentiation of self and not self could be achieved in another fashion than by the recognition of self patterns postulated by Burnet and Fenner. We were aware of the difficulties of the self-marker idea but at the time we could see no conceivable alternative to allow the differentiation of self from not self. The clonal selection theory which I am going to discuss is an attempt to modify Jerne's theory in such a fashion as to retain its essential features but to replace the unsatisfactory idea of replication of globulin molecules taken into the antibody-producing cells. Following the same practice as for what can be called theories 1, 2, and 3, we can tabulate the basis of the fourth approach as:

1. The pattern of a protein is genetically determined. When a cell and its descendants produce functionally specific protein the pattern of that protein depends on the presence in the genetic mechanism of appropriately coded "information."

2. Somatic mutation of a cell can modify the pattern of the specific protein it produces.

3. Mesenchymal cells can carry on their surface the same type of specific pattern that characterizes an antibody globulin molecule. Contact of this with antigen can stimulate a variety of cellular reactions.

These requirements are fulfilled by taking over from Jerne the idea that there is a wide variety of pre-existent γ-globulin patterns, but ascribing each pattern to a clone of mesenchymal cells of which it is a genetically determined character. For the sake of completeness the differences between clones are ascribed to changes in the nuclear (chromosomal) mechanism, but it is immaterial whether they involve that or some lower level genetic mechanism. The requirement is simply that different clones produce specifically different globulins and that the difference is inheritable through successive cell generations.

The appearance of a large number of distinguishable clones of mesenchymal globulin-producing cells is regarded as part of the general process of differentiation during embryonic and postnatal development. In the absence of any accepted interpretation of the genetics of differentiation a process of randomization of patttern has been assumed, but again this is unessential and will naturally be modified in any fashion that is required by increasing knowledge of the process of differentiation.

Subject to these preliminary qualifications the clonal selection theory assumes that in the course of embryonic development a large number of clones of lymphoid cells develop which amongst them carry potential antibody globulin patterns sufficient to react to some extent with all possible antigenic determinants. In embryonic life, little or no globulin is secreted, but an active process is postulated by which all clones that carry active sites corresponding to antigenic determinants present in the accessible parts of the body are eliminated. The assumption is that if a cell carries on its surface an active site *a*, corresponding to a determinant group A, present, say, in one of the plasma proteins, interaction will result in cell changes which will either destroy the cell completely or inhibit any further proliferation. In this way, clone *a* will be eliminated along with all others capable of specific interaction with body constituents. Such a process of eliminating self-specific clones will be completed before birth or hatching, and somewhere around the critical point a sharp change in the reactivity of the potential globulin producers is postulated. Instead of inhibition or destruction, contact after the critical point is assumed to stimulate the cell to functional activity. As the simplest and most

likely of the possible alternatives, we assume that the lymphoid series of mesenchymal cells are the potential antibody producers, and we will refer to the cells of the clones being discussed as lymphocytes.

We may consider a lymphocyte of clone x which carries on its surface several active sites of x specificity as a result of the differentiation process which has been described. When this encounters antigenic determinant X, there are two main possible types of reaction. (i) The cell itself can be stimulated to liberate globulin of specific type, either by the liberation of preformed antibody or as a result of synthesis. (ii) The cell can be modified so that when it passes to an appropriate tissue location it takes on a more active primitive form and acts as a stem cell from which a clone of descendant cells is rapidly produced. There is much to suggest that if such a cell lodges in the medulla of a lymph node it will give rise to plasma cells or in a lymph follicle to lymphocytes.

Eventually it may become necessary to add that, when it lodges in some situations in the bone marrow, eosinophils are produced.

No biological process can be simple, and no theory of antibody production can hope to be expressible in a single sentence. All that can be asked is that it should form the basis on which with the help of self-consistent *ad hoc* hypotheses all the relevant phenomena can be brought naturally together. My objective today is to support the thesis that the clonal selection theory is capable of providing such a basis and that it brings a wider group of phenomena into relevance to the immune process than any of the earlier views. I am not concerned overmuch whether the theory is true—if that expression, in fact, has any real meaning. I am very much concerned, however, to point out that it does suggest and call for a wide variety of new experiments, some of which are already being undertaken in my laboratory.

The experiments suggested are not necessarily experiments to differentiate between the four hypotheses. It is particularly interesting that in my laboratory we have firm supporters of both #2 (Burnet-Fenner) and #4 (clonal-selection) hypotheses. After many discussions we have been unable to devise a crucial experiment to differentiate between what seem to be completely different approaches. We find that the crucial experiment either demands a technique not yet available or would be dependent on a fortuitous and improbable observation. If we could cultivate lymphocytes and plasma cells *in vitro* producing clones from single cells and if such clones retained their functional activity, we should be in a position to make a direct attack on the clonal selection hypothesis. Puck's methods may eventually be elaborated along these lines but at the moment the prospects seem

dubious. We are experimenting with a micro drop technique which may make it possible to detect antibody liberation from a single cell, but here we are forced to use heavily immunized animals and if, as preliminary results suggest, only one in ten or one in twenty cells produce antibody, we should, after all, expect such a result from any of the four theories of antibody production. If we could obtain a population of cells all producing one antibody and then show that they could also be made to produce any other antibody we choose

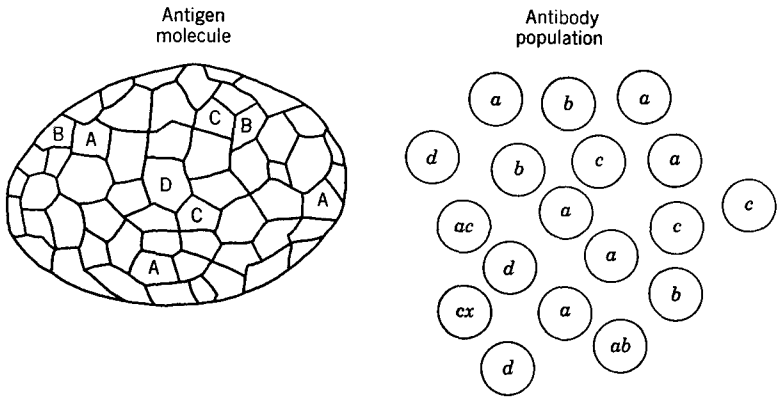

Figure 1. Schematic representation of an antigenic (protein) molecule and the corresponding population of antibody-globulin molecules.

Note that the great majority of potential antigenic determinants on any protein will correspond to similar groupings in the animal producing the antibody and hence will be immunologically inert. [From Burnet (2).]

to call for, the clonal selection hypothesis is disproved. The only way we have been able to conceive such an experiment demands that we discover a tumor or a leukemia in which the malignant cell comes from a clone that is an active antibody producer and which retains that character despite its malignant change. The major difficulty if such a malignant strain existed would be to find which of perhaps 10,000 antigenic determinants its globulin was complementary to.

Clearly every effort must be made to develop techniques which in one way or another allow the isolation of a pure clone of antibody-producing cells with retention of the antibody-producing capacity. Until such techniques are available, however, any choice between the four theories will have to be on the basis of their convenience as a framework for the particular experiment or discussion at hand. It is highly probable that where an antiserum is prepared as a specific reagent for a practical purpose it will always be convenient to use

the fiction, if it is a fiction, that an antigen provokes the animal to produce a corresponding antibody, the process being effectively visualized as an antigenic pattern acting like a die to impress a complementary pattern on antibody. The only elaboration necessary for such purposes (and this is almost irrelevant to the problem of how antibody is produced) is in regard to the nature of the component molecules in an antiserum against a single type of antigenic molecule. Most immunologists will probably accept the view that any antiserum contains more than one type of antibody-globulin molecule and is in fact a population of molecules, each of which carries an average of two reactive groups directed against one antigenic determinant.

It is now generally accepted that an individual antigenic determinant, defined as the molecular configuration with which the specific reactive group on an antibody molecule combines, is rather small perhaps averaging about 1000 Å^2 in area. In general this will correspond to three or four amino acid or monosaccharide residues. Every antigenic macromolecule will carry many such potential antigenic determinants, most of which since they correspond to configurations present in components of every animal body will not be antigenic.

When, however, we look at what may be called the biologically more sophisticated aspects of immunology, this simple practical approach becomes more or less irrelevant. To such questions as the nature of antenatal or postirradiation tolerance of foreign tissues, the persistence of measles or yellow fever immunity for 50 years in the absence of the antigen, and the existence of some types of immunity in persons who cannot produce antibody, there is no simple answer possible. Whatever theory of antibody production is supported, a number of *ad hoc* qualifications will have to be produced to provide a useful-seeming generalization which will cover the facts. We feel that until crucial experiments can be devised, the choice between theories of antibody production will only be made on the basis of the ease with which such *ad hoc* qualifications can be made to seem natural and in accord with general biological experience.

Let us take the question of tolerance first, on the grounds that the experimental and observational facts are fully established and that the requirement for some means of differentiating self from not self is therefore the first criterion that any elaboration of the Haurowitz-Pauling theory must satisfy. It is the rule that no "accessible" component of an animal's body is antigenic to itself though many will be antigenic to another species and sometimes to another individual of the same species. This is a situation with which theory 1 is in no way concerned. It could be said by supporters of this theory that

body cells are specially adept at breaking down homologous components so that these do not exist long enough to be able to serve as templates. But this still requires some machinery which can "recognize" whether a given antigenic determinant is of self pattern or of not-self pattern.

Broadly there are only two ways in which this can be done. They can be exemplified by two alternative ways that might be used to sort out English speakers from a polyglot crowd. A sorter speaking English, but knowing no other language, could recognize all other English speakers in the crowd. Equally a person knowing all the other languages represented, but quite ignorant of English, could recognize English speakers in a negative but probably adequate fashion as those people he could *not* understand. The crux of the second method is of course that the sorter understands *all* but the one language.

In the immunological situation, it may be found either that there is developed some machinery by which body components can be recognized as such—something Fenner and I called "self markers"—or alternatively that there is some type of organization which, after being rendered nonreactive to all self patterns, can "recognize as foreign" all potentially antigenic patterns not present amongst the self components.

Neither alternative is particularly easy to satisfy, and one cannot see that anything would be gained by postulating a simultaneous existence of both types. Although the last possibility must be kept in mind, we shall keep the discussion to the merits and probabilities of the two primary alternatives.

Positive recognition of self components could be pictured in at least two ways. The first is that used by Burnet and Fenner (3). It was postulated that a small number of "self markers" would be sufficient to provide the "information" needed to switch intracellular processes to a rapid disaggregation of the organic material down to a nonantigenic level. If none of the self markers were present, the cell taking in the foreign material was switched to the alternative process of antibody production.

A second approach might be along the lines that antigenic potentiality of body components only arises when denaturation of protein occurs. On denaturation, a number of antigenic determinants are exposed to the plasma or other body fluids. We might assume that the body fluids contain a sufficient number of types of naturally patterned globulin molecules to ensure that any denatured cell or component will be coated with globulin which will then act as an "opsonin" in the slightly variant sense of facilitating its rapid and complete dis-

integration without antibody production when it enters the appropriate cell. A foreign pattern, on the other hand, fails to be so coated and when taken into the macrophage provokes the process leading to antibody production.

In the first hypothesis, the "recognition" is regarded as intracellular and in the second, extracellular, but otherwise there is little difference between the two. If we concentrate on the problem of why the worn out erythrocyte at the end of its span is nonantigenic, it seems axiomatic that any answer along the line of positive recognition of self components must involve the phagocytic cells responsible for the scavenging process. Since the phagocytic cells are definitely not concerned with the actual production of antibody, this leaves an awkward gap between the macrophage which takes up the antigen and the plasma cell which produces it. The gap can be bridged by assuming (*a*) that a macrophage can become a plasma cell or (*b*) that a protein synthetic organelle can be transferred to a primitive reticulum cell from which a brood of plasma cells develops.

On either of these views, the machinery of recognition is developed during embryonic life, when by some means or other the organism develops a capacity to produce appropriately patterned recognition units, either intra- or extracellular, to correspond to all potential antigenic determinants that are present either by right or fortuitously. It is characteristic of this type of hypothesis that the development of recognition units as of antibody is an active process by which new patterns are positively impressed upon cells not genetically organized to produce them.

For the sake of convenience, we may refer to these hypotheses as:

A1. Self marker—intracellular recognition.
A2. Self marker—extracellular recognition.

The alternative approach is the negative one in which alien patterns are recognized and self pattern is known only by the fact that it neither reacts with nor provokes immunological reaction in body cells or tissues. The second part of the statement is in itself a strong preliminary argument for a theory of this type. It is aesthetically and logically more satisfying to have a lack of response to X ascribed simply to the fact that X is inert rather than to postulate that X provokes some active process which prevents the body from making its natural reaction to X. The difficulty lies in the first part, which demands in effect that the body possesses "information" which will allow the recognition as alien of at least some hundreds, and possibly many thousands, of antigenic determinant patterns.

As with A1 and A2 the recognition mechanism could be either cellular or extracellular. The extracellular possibility is Jerne's theory as already described. We believe that it is inadmissible in Jerne's original form by which a randomly (i.e., not genetically determined) patterned globulin molecule which happens to fit the alien pattern is taken, along with the antigen, into a cell which thereupon develops the capacity to produce patterned globulin modeled precisely on that taken in. It is not difficult to conceive that globulin molecules may be produced which by accident, say of secondary coiling, have a wide variety of possible adsorptive capacities, but it is extraordinarily difficult to fit knowledge and theory of protein synthesis to any concept by which one such pattern can subsequently be reproduced by the cell into which it is taken.

Let us modify this hypothesis along the lines of A2 so that we have in the plasma a wide range of globulin patterns. By a mechanism operating presumably in embryonic life, all which could react with body components have been eliminated. Alien material X enters the blood stream and is immediately coated with any globulin molecules that are capable of specific adsorption. The next step in the argument is that coated X, but not uncoated X, will, on entry into an appropriate cell (macrophage), provoke that cell or its plasmacytoid descendants to produce corresponding antibody. This, of course, is precisely the opposite postulate from that used in A2, but, since there is in fact no evidence whatever that coating or lack of coating with globulin influences the fate of a substance or particle taken into a macrophage, neither possibility can be excluded.

In this variant of Jerne's hypothesis, which we can label B1, there is no reason why the natural globulins should not have a completely random structure subject only to the condition that in some way those reactive with body components are effectively removed from the body fluids or are prevented from being produced. Their function is merely to decide that the antigenic determinant they react with is to be regarded as foreign and to be reacted against immunologically.

I have no doubt that this hypothesis could be worked up to provide a self consistent theory in which the actual process of antibody production would be elaborated along lines essentially similar to Burnet and Fenner's theory (A1). A major difficulty may be to find an acceptable answer to the necessity for eliminating all globulin reactive with normal or partly denatured body components. We can hardly postulate a production of randomly patterned globulins and then claim that in some way a large and specifically determined segment of the whole spectrum is not produced. To account for its elimina-