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Introduction

By Paul Kallós, Helsingborg

The allergic state, the state of specifically altered reactivity of an organism to an antigen, attracts more and more interest from research workers and clinicians. The reviews, collected in this volume, bear witness to this. Methods and views change and become increasingly sophisticated, the basic problems concerning the very nature of antigens and antibodies and their interaction seem, however, to remain as yet unsolved. The complexity of these problems mirrors the complexity of Nature and is obviously the best stimulus for continuous search for new facts and explanations and the best source of new ideas.

The search for the chemical basis of the antigenicity of synthetic protein-like compounds has progressed very rapidly during the last few years. Contributions by P. H. Maurer and his team to this field are of greatest importance and his review of "Use of synthetic polymers of amino acids to study the basis of antigenicity" in the present volume not only summarizes our present knowledge but also clearly shows the remaining unsolved problems. The "synthetic approach" to the molecular basis of antigenicity is very rewarding. Many structural properties which are essential for antigenicity, have been unraveled and could be related to the structure of natural antigens. In spite of these results, it seems as yet uncertain which features "a molecule must possess in order to be antigenic (induce antibody formation)".

As Maurer proved antigenicity "appears to be related primarily to the genetic background of the host". In a recent paper by Levine et al. (21) this is emphasized further. The authors treated randombred Hartley-strain guinea-pigs with 4 different hapten-poly-L-lysine conjugates and could show that a few of them became allergic (positive Arthus-phenomenon and delayed skin reactions; circulating antibodies) to all conjugates, whereas the majority did not react to any of them. The authors conclude that the ability to be-

come allergic to a hapten-poly-L-lysine conjugate "is dependent on the guinea-pig's ability to metabolize the conjugate in the precise ways necessary to induce the immune response".

The properties of the species, the strain, the individual and last but not least those of the reacting cells, determine the response of an organism to the antigenic stimulus.

The most important response is the formation of antibodies. These are according to E. C. FRANKLIN's review in the present volume "the prime, if not the sole, mediators of immune reactions in mammals as well as a number of lower vertebrate species" and belong to "a group of structurally and functionally closely related proteins". According to FRANKLIN "because of their functional similarity, yet obvious structural heterogeneity, they have been collectively called the group of γ-globulins or immune-globulins".

In the Introduction to volume 6 of Progress in Allergy KALLÓS AND WAKSMAN reviewed the current theories of the cellular basis of antibody synthesis.

Experimental work in BURNET's laboratory (9, 10, 22) led BURNET to the conclusion (10) that his "clonal selection theory" of antibody production is 'inadmissible' in its original version. BURNET (10) now states that the supposition of a primary randomization of patterns (cellular or subcellular) corresponding to all foreign antigens is not tenable. Instead, there are only "a relatively small number of basic patterns". BURNET believes that "we may be concerned with two sets of patterns, both functional and specifically differentiated. The first set covers those patterns which, if they arose in the body would have, or would be associated with, harmful effects. The second set provides a basic array of patterns which can react with adequate number of common microbial antigens". BURNET (10) restates now the clonal selection "hypothesis" in the following terms: "The phenomena of immunity and of immunologic reactivity generally are based on the population dynamics of clones of lymphoid cells which are adapted to react with antigenic determinants and, as one result of such reaction, to produce antibody. The nature of the immunologically specific patterns concerned is determined genetically, and any modification of those patterns is brought about only by genetic processes—mutation, reproduction, selective proliferation, and survival with the possibility, still to be proved or disproved, of transfer of genetic information between cells of distinct clones." This modified hypothesis fits quite well in-

to the experimental facts, known hitherto. In fact, the protagonists of the "instructive hypothesis" of antibody formation seem to feel, as HAUROWITZ (18) expresses, that "at the present exciting period of biology where genetic codes are translated into the language of chemistry, the gap between the two viewpoints gets narrower from year to year. We may soon end with a unified theory of antibody formation according to which the antigen has two functions, *viz.*, to elicit by a nonspecific stimulus of the genetic apparatus the production of cells which are able to form antibodies, and, subsequently, to interfere with the formation of gammaglobulins in such a manner that the newly formed gammaglobulins are spatially adjusted to the determinant groups of the antigen molecules".

Immunoglobulins are produced by plasma cells. As mentioned in the Introduction to volume 6, it is quite possible that the antigen is first taken up by macrophages and metabolized there. The antigen so modified would then pass over to lymphoid precursors of plasma cells, which may originate from small lymphocytes (GOWANS et al., 16; cfr. also the discussion in 32) and after their transformation produce antibody. FISHMAN AND ADLER (14) inserted Millipore-diffusion-chambers charged with non-immune rat lymph-node cells and a cell free homogenate of rat-macrophages, which had been incubated with an antigen (bacteriophage T 2), intraperitoneally into X-irradiated rats. An intensive antibody production resulted. The specific stimulatory activity resides, according to FISHMAN AND ADLER (14) in a ribonucleic acid fraction of the macrophages, which could be purified and was of low molecular weight, non dialyzable and phosphorylated. It is quite possible that this fraction contained "antigenic fragments derived from bacteriophage, complexed with ribonucleic acid from the macrophages" CAMPBELL et al. (11) isolated from the liver of with an antigen pretreated rabbits "soluble ribonucleic acid or nucleotides" complexed with a small fragment of the antigen. This complex is, according to CAMPBELL, "extremely immunogenic". Microgram amounts of this material induce intense antibody production in guinea-pigs. As BURNET (10) points out, it is not impossible that "an antibody producing cell may derive the necessary information from another cell not in its own line of descent". The not entirely explained facts concerning "The competition of antigens", thoroughly reviewed in this volume by F. L. ADLER, fit well into this concept.

Phylogenetically, as PAPERMASTER et al. (30) have so excellently demonstrated in their investigations on the California hagfish, the absence of a primary lymphoid organ, such as the thymus, and of an organized lymphoid system, is tantamount to the total absence of plasma globulins, the capacity of antibody production and delayed reactivity. Allogenic grafts survive in these primitive cyclostome fishes and an eventual rejection does not show the vigorous cellular infiltration, which is characteristically seen in higher organisms.

Teleost fishes, such as bullheads, have an organized lymphoid system and plasma globulins. They easily form antibodies, develop delayed reactivity to different protein- or microbial antigens and reject homografts in the same manner as higher vertebrates. Newer developments in this field have recently been discussed by GOOD et al. (32). As MILLER AND DUKOR (28) point out ontogenetic studies are consistent with these findings.

MILLER (25, 26, 27, 28) has observed that the thymus is "the major lymphoid organ in perinatal life" and supposed that "thymectomy of the neonate might be associated with some detectable effect on the maturation of immunological faculty". As it is well known this idea opened an entirely new and most fruitful field of research and as SIMONSEN (35) remarks " in a most spectacular way has restored that half-forgotten organ to what seems to be its legitimate role in immunology".

From the research work of MILLER et al., MARTINEZ et al. (23), ARCHER et al. (1), ARNASON et al. (2, 3, 4), JANKOVIĆ et al. (19), WAKSMAN et al. (38) on mice, rats, hamsters and rabbits and from that of ASPINALL et al. (5), GRAETZER et al. (17) and SZENBERG AND WARNER (36) on chickens, a more complete picture of the immunological role of the thymus and that of the second primary lymphoid organ in birds, the bursa of Fabricius, emerges. This work and the pertinent literature, have recently been completely reviewed in a stimulating monograph by MILLER AND DUKOR (28).

It is only intended to give a very brief outline of these investigations here. In a coming volume of Progress in Allergy a complete review will be published.

In mammals, thymectomy performed at birth is associated with a severe depletion of small lymphocytes, atrophy of lymph nodes and spleen and a nearly total absence of the ability to form antibodies to protein- or microbial-antigens, to develop delayed re-

activity and to reject allogeneic and heterospecific skin grafts. There is no total lack of lymphoid cells and the globulin- (especially the γ-globulin) content of the blood is not impaired quantitatively *. Neonatally thymectomized animals, especially mice, seem to develop normally during the first months. After 2–4 months, however, many of the animals die from a syndrome which is, according to MILLER characterized by progressive wasting and diarrhoea. This wasting disease shows some similarities to the syndrome of runt disease, caused by graft-*versus*-host reaction. Splenomegaly, a prominent feature of runt disease (SIMONSEN, 34) is, however, absent in wasting disease. The reactivity of the lymphoid system of neonatally thymectomized animals and hereby even their ability to form antibodies, to develop delayed reactivity and to reject foreign skin grafts, can be restored by a neonatal or embryonic thymus graft.

MILLER showed in experiments with marker chromosomes in thymectomized and thymus grafted mice that nearly all dividing cells "in both thymus graft and lymphoid tissues were of host origin".

OSOBA AND MILLER (29) could show that the capacity of neonatally thymectomized mice to reject allogeneic skin grafts could be fully restored by implantation of Millipore diffusion chambers containing neonatal or embryonic thymus tissue. These very carefully conducted experiments strongly suggest that the enclosed tissue, namely its epithelial-reticular framework, produces a humoral factor, which enables the neonatally thymectomized mice to reject homografts. OSOBA AND MILLER (29) point out that "the lymphocyte population of neonatally thymectomized mice bearing thymus tissue enclosed in diffusion chambers was in most cases not significantly different from that of control thymectomized mice. It seems, therefore, that the capacity of mice to reject homografts is not dependent on the quantity of lymphocytes present in the animal".

As mentioned above fowls have two primary lymphoid organs, the thymus and the bursa of Fabricius. Neonatal thymectomy inhibits the development of the capacity of fowls to reject skin homografts. Antibody production and delayed reactivity are not or only slightly impaired. So called natural hemagglutinins are present. Neonatal surgical bursectomy or prenatal chemical (steroidal) burs-

* SHERMAN AND DAMESHEK (34a) found recently that in neonatally thymectomized hamsters the wasting syndrome is associated with severe hypogammaglobulinemia.

ectomy inhibits the capacity of chickens to produce antibodies (including natural hemagglutinins) and to develop delayed reactivity (5, 17, 28, 36, cfr. also 18a).

The leukocytes and spleen cells of thymectomized or bursectomized chickens retain their ability to form specific foci on the chorioallantoic membrane of a chick embryo of different strain ("Simonsen-phenomenon").

MILLER could further show that thymectomy after at least 3 weeks of age does not impair homograft rejection in mice. Thymectomized adult mice seem to be healthy, the only change attributable to the thymectomy is pronounced lymphopenia and reduction of the weight of lymph nodes and spleen. After a single dose sublethal x-irradiation thymectomized adult mice, however, cannot—in contrast to sham operated controls—regenerate their lymphoid system and are unable to homograft rejection and antibody production. Mice thymectomized in adult age and "given a potentially lethal dose of total body irradiation could be protected against death from acute radiation effects by an injection of syngeneic marrow" (26). The lymphoid system and the immunological functions (homograft rejection, antibody production) of these chimaeras were not restored. In contrast "syngeneic chimaeras with the thymus intact had normal lymphocyte populations and normal immune response" (26). Therefore, according to MILLER "the thymus seems to be essential for re-establishing immunological function under any circumstances in which the body's immunological potential has been largely destroyed or depleted".

The attractive hypothesis, widely held a few years ago, that all immunologically competen lymphocytes originate in the thymus and are distributed from there at birth, is unlikely. According to MILLER "whatever the truth may be, cells with immunological potential must be presumed to persist in the neonatally thymectomized mouse although they cannot express that potential in the absence of a thymus". The above quoted results in thymectomized syngeneic chimaeras suggest that "these cells may reside in the bone marrow".

Animals thymectomized at birth produce plasma globulins, even γ-globulin. Rats show increased amounts β_2M-macroglobulin (ARNASON et al., 3). MILLER AND DUKOR (28) give several examples that neonatally thymectomized mice treated with different anti-

gens simultaneously, could produce some antibody at least against one component of the antigen-mixture. It is very likely that blood globulins without antibody function and antibodies are produced by two different lymphocyte populations—the first of them thymus independent. In fowls we must assume that at least three different lymphocyte populations exist: the thymus dependent system responsible for homograft rejection, the thymus independent and bursa dependent system which produces antibodies and develops delayed reactivity and finally the system effective in the Simonsen phenomenon. The fact that neonatally thymectomized animals can produce some antibody can perhaps be explained—provided that the thymectomy is really total—by the fact that the thymus could already before the operation exert its influence on parts of the peripheral lymphatic system, thus giving them immunological competence. MILLER AND DUKOR assume that investigations on primitive marsupials, such as the opossum, the young of which are at birth (12 days after fertilization) very immature with no definitively formed organ systems, will help to clarify this matter. Lymphopoiesis in the thymus starts 5 days and on the periphery 10–20 days after birth in opossum embryos. The baby opossums are accessible in the pouch of the mother. In a recent paper KALMUTZ (20) has already published very interesting data about antibody production in opossum embryos. Important results can obviously be achieved in tissue cultures and I refer to the papers of AUERBACH (6, 7). The production of different globulins in spontaneously occurring or induced plasmacytomas in mice elucidate some features of globulin synthesis. I refer to the recently published proceedings of a conference on the "Neoplastic plasma cell" (MERWIN et al., 24). It is to be hoped that continued efforts in this field will finally clarify the complicated problems of globulin- and antibody synthesis and thereby the mechanism of the development of the allergic state.

It would be tempting to discuss the impact of these recent results on the concept of the clonal selection theory, immunological tolerance and paralysis and the genesis of the so called auto-immune diseases. However, I feel, that I must restrain myself and refer to MILLER AND DUKOR (28), BIELSCHOWSKY et al. (8), BURNET (9, 10), MACKAY AND BURNET (22), ARNASON et al. (2) and DAMESHEK (13), and the Transactions of the Fifth Symposium of the Collegium Internationale Allergologicum (37) and the Proceedings of the Third International Symposium on Immunopathology (32).

E. P. Benditt and B. Lagunoff contribute to the present volume with a review on "The mast cell: its structure and function". This critical analysis clearly shows that in spite of much work and great efforts "the answer to the riddle (of mast cells) will take more searching". The role of tissue and blood basophils in controlled experimental situations is as yet uncertain and gives place to different explanations. This fact should be considered by clinicians and should exclude broad generalizations. The *in vitro* degranulation of blood basophils from human or rabbit blood under the influence of serum of allergic patients and the allergen has been recently proposed as a rapid, simple and reliable test which "detects circulating antibody in any allergy" (33, 34). "Any allergy" means not only allergic diseases of the immediate type in which the Prausnitz-Küstner reaction is more or less frequently positive, but also drug allergies of different type, "atopic" and contact dermatitis, tuberculosis, syphilis, fungal allergies and a number of conditions, the allergic nature of which is very doubtful, such as ulcerative colitis and diarrhoea, migraine etc. Unfortunately this method is not as simple and far from as reliable as its proponents seem firmly to believe. Much caution and criticism is necessary before the "widespread use" of this method can be recommended and before it can be accepted that positive results "disclose the allergic nature of diseases now of obscure origin".

Another complex problem of great clinical importance "Drug fever", is thoroughly discussed in the present volume by L. E. Cluff and J. E. Johnson III. The ultimate mechanism of this quite frequent and disturbing clinical entity is not known. The possible role of allergy is carefully discussed by the authors and their contribution clearly indicates the directions which future research should take. I may perhaps add some unpublished observations. In 1951 M. Diamant and I administered in double blind trial an antihistamine and placebo to large groups of healthy individuals. On the eighth day of administration about 7% of the individuals in one group (which as it has been later disclosed received the antihistamine) reacted with fever (38–40° C). After discontinuation of the drug the fever subsided as suddenly as it appeared without any complication. No other symptoms, such as urticaria etc., occurred in these individuals. The other observation is, that drug fever occurs in some individuals not only after prophylactic or therapeutic administration of drugs but also as an occupational disease. I observed a number of such cases in different chemical factories, first

and foremost in pharmaceutical plants, processing antibiotics or enzyme preparations. Recently, in a pharmaceutical plant a miniature epidemic of drug fever occurred amongst the workers engaged in work with powdered streptokinase. I also observed drug fever, with or without "true" allergic manifestations, such as urticaria, rhinitis or asthma, in nurses handling chlorpromazine solutions for injection rather carelessly. In all these cases inhalation of and/or contact with the drug caused the fever.

Leprosy is a great and embarrassing epidemiological and social problem, affecting hundreds of thousands of people in many countries. In spite of this sad fact, as R. J. W. Rees states in the present volume, "Yet the leprosy infection and the leprosy bacillus are still near Cinderellas in the field of immunology". It is to be hoped that this review will arouse the interest of immunologists the world over in the important and intriguing problems, which this unfortunately so widespread disease poses. As Rees points out the tuberculin reaction is far more thoroughly investigated and elucidated. This is well documented in a recent review by Arnason and Waksman (4).

In the field of treatment of allergic diseases there has been no spectacular progress during the last few years. An entirely new and hopeful approach seems to me to be the use of different antimetabolites in experimental and clinical allergy. At a recent conference at the U. S. National Institute of Health, H. C. Goodman and his co-workers (15) reported their own experimental and clinical work and reviewed the pertinent literature. Further work in this field is awaited with greatest interest.

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In his review on "Competition of antigens" in the present volume F. L. Adler states that "it is tempting to summarize present knowledge of the mechanism of antigenic competition in one word: *unknown*". I am afraid that this statement also applies to many other biological processes discussed in this volume. Fortunately, this seems not to discourage workers to carefully and meticulously search for more facts and better understanding. Co-ordinated efforts will perhaps soon enable us to believe that "unknown" is only a temporary state which can be changed in the not all too distant future.

As I said at the beginning of this Introduction, methods and views in our field, as in every other area of biology, have become

increasingly complicated. I feel that many of us agree with the view of W. B. Wood Jr. (39) expressed recently in his presidential address, read to the Association of American Physicians: "We would gladly sell our soul to Mephistoteles on the stipulation that we would have no more trouble reading the Journal of Molecular Biology than the Archives of Internal Medicine". I am glad to say that the contributors to this volume have tried their hardest to save our souls from the devil at least this time. I would like to express the sincerest thanks of the editors to all of them. The thanks of the editors are also due to the Publisher, Mr. Thomas Karger, for his splendid co-operation.

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Drug Fever

The Mast Cell: Its Structure and Function

The Significance of the Lepromin Reaction in Man

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Use of Synthetic Polymers of Amino Acids to Study the Basis of Antigenicity*

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I. Introduction

During the past decade, a tremendous effort has been made to learn about the molecular basis for the biological activity of many kinds of protein molecules, i. e., enzymes, hormones, antigens and antibodies. The intriguing problem of correlating the structure and function of a protein has vexed investigators, including the immunologist. In addition, the immunologist has been concerned not only with the serological specificity of the antigen-antibody interaction, which was shown many years ago by Landsteiner to have a definite chemical basis (1), but also with the perplexing question dealing with the structural and chemical requirements which a protein must fulfill in order for it to be antigenic. The two central problems which, at present, are being studied by several groups are: a) what structures must be present in a protein or polypeptide molecule to render it antigenic (immunogenicity) and b) what is the chemical nature of the sites responsible for the antigenic specificity. Until recently, answers to the second question about the nature of the antigenic determinants, or those areas of a protein which impart immunological specificity were approached by two methods: a) chemical alteration of the antigen and a study of the subsequent changes in immunological reactivity and specificity and b) degradation of the antigen and an examination of the digests for fragments which retain some serological activity.

With the development of 1) methods for controlling the polymerization of N-carboxy-α amino acid anhydrides onto proteins to

* Aided by grants from the USPHS and Department of Defense. Research career investigator of the National Institutes of Health.

produce *polypeptidyl proteins* and 2) methods for the production of synthetic polymers of amino acids *(poly-α amino acids)* from the amino acid anhydrides (2), it has been possible to employ proteinlike polymers in attempts not only to answer the question of the nature of antigenic determinants, but also to learn some of the factors influencing immunogenicity.

Several review articles have summarized recent investigations of the chemical modification of proteins (3, 4, 5) and the degradation of protein antigens (6, 7, 8). This review will deal with the rapidly developing knowledge of polypeptidyl proteins and synthetic polymers and the contributions which they have made so far to problems in immunology and immunochemistry.*

A. Polypeptidyl Proteins

An interesting approach to investigation of the nature of determinants is to attach amino acids to proteins and study the properties of the new derivatives. LANDSTEINER AND VAN DER SCHEER (9) were the first investigators to couple peptides to proteins through *azo* linkages. These proteins had the amino acids glycine, leucine, glutamic acid or tyrosine coupled to them. It was observed that azo proteins from amino acids produced highly specific antisera. Cross reactions were observed only among the related amino acids glycine and alanine, valine and leucine or aspartic acid and glutamic acid. Another technique of attaching amino acids to proteins first described by BECKER AND STAHMANN involves the polymerization of N-carboxy-α amino acid anhydrides in aqueous media using the NH_2 group of the lysyl residues of the protein as the initiator (10). The synthesis of polypeptidyl proteins proceeds under mild conditions, so that most proteins remain "native", in contrast to the drastic reaction conditions used by LANDSTEINER. Peptides of different sizes can be built on the free amino groups. This technique has been employed to modify antigens, enzymes and hormones. (See reviews in 2, 11,and recent Symposium on Polyamino acids, Polypeptides and Proteins, 12).

The first report dealing with the modification of an antigen was that of MAKINODAN et al. (13). Polyglycyl bovine serum albumin (BSA) was prepared and shown to have a 70% cross reaction with rabbit anti BSA. This confirmed the non-importance of the

* The survey of published articles was concluded November, 1962.