Miami Winter Symposia
Volume 9

Molecular Approaches To Immunology

Edited by
E. E. Smith
D. W. Ribbons

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DEPARTMENT OF BIOCHEMISTRY
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PREFACE

This volume is the ninth of a continuing series published under the title: "Miami Winter Symposia." In January 1969, the Department of Biochemistry of the University of Miami and the University-affiliated Papanicolaou Cancer Research Institute joined in sponsoring and presenting two symposia on biochemical topics as an annual event, now in its seventh year. The two symposia were published as a single volume in 1970, the volumes were expanded to include the discussions that followed each presentation in 1971 and, in 1972, we initiated the publication of the proceedings of the two symposia as separate volumes in the series to allow greater flexibility in the choice of future topics for the joint symposia.

The major emphasis in the selection of the topics for our symposia has been to identify the frontier areas in which progress in biochemistry is leading toward an understanding of the molecular bases of biological phenomena. We follow a pattern in which a common theme is dealt with, in our symposium, on a fundamental basis, and then in the Papanicolaou Cancer Research Institute Symposium, as it relates to an understanding of malignant processes. This volume contains the proceedings of the Biochemistry Department's Symposium on "Molecular Approaches to Immunology" and will be published simultaneously with the proceedings of the Papanicolaou Cancer Research Institute's symposium (Volume 10) on "Critical Factors in Cancer Immunology." The word "Enzym" was first introduced by W. Kühne to describe pancreatic trypsin at a scientific meeting held 4 February, 1876. The proceedings of the meeting were published the following year (W. Kühne, 1877, *Verhandl. Naturhist Medic. Ver. Heidelberg,* 1, 194–198). It is, therefore, appropriate that, one hundred years later, the theme of the Miami Winter Symposia taking place during 12–16 January, 1976, should be enzymology; the topic for the first symposium is the role of proteases in biological regulatory mechanisms and this will be followed by a symposium on cancer enzymology.

Associated with the symposia is a featured lecture, the Feodor Lynen Lecture, named in honor of the Department of Biochemistry's distinguished Visiting Professor. Past speakers were George Wald, Arthur Kornberg, Harland G. Wood, Earl W. Sutherland, Jr., and Luis Leloir. This year the Lynen Lecture was delivered by Gerald M. Edelman. These lectures have provided insights of the history of discovery, and personal and scientific philosophies of our distinguished speakers. As such they appeal also to non-scientific members of the audience, and for ourselves and our colleagues in Miami, remain a source of inspiration. The Lynen Lecturer for 1976 will be Professor A. H. T. Theorell.

This volume opens with the Sixth Lynen Lecture and is followed by an introduction, also by Dr. Edelman, in which he provides, for the benefit of those not directly in the field, some indication of the basic assumptions, approaches and directions of modern immunological research. If others find this overview as helpful as did the editors of this volume, then it will have served its purpose admirably. To bring forward as much of the recent work as possible a session of short communications is included in these meetings. This year, these were presented in a joint poster session for the two symposia. This session proved so successful that we propose to continue with this arrangement in future years. Abstracts of the short communications have been assigned, whenever possible, according to their relevance to each symposium, but in a number of cases an arbitrary decision has been made. Thus, sixteen abstracts appear in this volume and the remainder are published in Volume 10 of the series.

Our arrangement with the publishers is to achieve rapid publication of these symposia and we thank the speakers for their prompt submission of manuscripts and the secretarial staff for their unstinting efforts which enabled us to bring this about. Our thanks also go to the participants whose interest and discussions provided the interactions that bring a symposium to life and to the many local helpers, faculty and administrative staff who have contributed to the success of the present symposium. Our special gratitude goes to the organizers and coordinators of the program: W. J. Whelan, K. Brew, Sandra Black and Olga F. Lopez, and to our two consultants Drs. Gerald M. Edelman and Matthew Scharff for their advice and help in organizing the scientific program.

The financial assistance of the University of Miami Departments of Medicine, Pathology and Radiology, the Howard Hughes Medical Institute, Dermatology Foundation of Miami, Abbott Laboratories, Boehringer Mannheim Corporation, Eli Lilly and Company, Hoffman-La Roche Incorporated, MC/B Manufacturing Chemists and the Upjohn Company, is gratefully acknowledged.

CONTENTS

Poster Session: Communications

THE SIXTH FEODOR LYNEN LECTURE

THE SHOCK OF MOLECULAR RECOGNITION

GERALD M. EDELMAN

The Rockefeller University

G: "If the world were not this way then it would be that way."
E: "Yes, but what way is it?"

"I see nobody on the road," said Alice. "I only wish I had such eyes," the King remarked in a fretful tone. "To be able to see Nobody! And at that distance too!"

Through the Looking Glass
L. Carroll

"Musicology is to music as ornithology is to the birds."

A Player

It is particularly gratifying to be the Lynen Lecturer this year, not only because of the honor it represents, but because it was my good fortune to have been here with Fritz Lynen on the occasion of the first of these meetings. My being here now prompts warm recollections of that occasion and of this fine tradition.

I hope you will forgive me if I do not indulge myself in recounting autobiographical details. Scientific reminiscence has always struck me as an almost impossible genre. It has none of the juicy quality of gossip and, in any case, the accidental elements of a scientist's life have no necessary relation to the order he hopes to bring out of his work. For

these reasons, I do not intend to recollect here at any length my own course of scientific development.

Instead, I hope to take this opportunity to comment in a personal mode upon some psychological aspects of discovery and the nature of scientific insight. The method I have chosen is to compare my present recollections of the state of immunological thinking at a given time with my own recorded scientific guesses at that time, and then to comment with hindsight on those guesses, correct and incorrect. This method has obvious limitations, but I hope it will at least stimulate some of your own recollections. I expect that the results of these efforts will be extremely limited and idiosyncratic, and therefore, I do not hope to draw many general conclusions or give lasting general advice based on my restricted experience. But I do hope to provoke some discussion by reaching some admittedly very personal conclusions.

The concern with creation and form has prompted comparison between art and science. I think it is worth comparing the creative procedures of scientists with those of artists, but it seems to me that although they are very much alike in the beginning, they are very different in the end. I hope that the examples I give will make this clear. But, independent of these acts of creation, there are, of course, great personal and aesthetic similarities between science and art in the act of perceiving or grasping an idea of order in either endeavor.

What I wish particularly to convey here is that in the process of creation, a shock occurs that is very much out of the ordinary when, for example, one recognizes the shape of a molecule or the order of events in a metabolic pathway. The shock is a very complex one, consisting of surprise, wonder mixed with some shame at the simplicity of the picture as compared to one's prior thoughts, and a relief, which I suppose comes from the removal of confusion. Then the shock disappears, the discovery becomes commonplace, is accepted as part of the furniture of the world, and one then goes on hopefully to new subjects. This whole process happens to individuals but it is shared and altered by groups. It seems to me to be the central experience in science.

THE PREVAILING PARADIGM: 1958

In his book on scientific revolutions, T.S. Kuhn (1) has promoted the very useful idea that a period in a particular

science is characterized by a paradigm, consisting of the communally shared view of that science, a view made up of both rational and unconscious elements. Following Kuhn, let me take you back to the time that I began to work in immunology and attempt to recollect the paradigm concerning antibody formation and the nature of antibodies.

Although Jerne had published his natural selection theory in 1955 (2), I think it is fair to say that most immunologists then believed in the instructive theory of antibody formation. Linus Pauling (3) was the most lucid and recent expositor of this theory and it was only in the late fifties that his views came under fire. As for antibody structure, what was known came mainly from hydrodynamic and electrophoretic analysis. In accord with the convention of the day, the antibody protein was assumed to be an ellipsoid of revolution. The main point that deserves mention, I suppose, is that this prevailing picture of the antibody was a neutral one, i.e. it was not yet crucial for a choice of a particular theory of antibody formation. And, if I remember correctly, no one seemed to feel that it would be crucial.

I did not share this view. Just after my first glimpse of the multichain structure of antibodies and the gel electrophoretic analysis of their chains, I had occasion to present my work at the Kaiser Foundation Symposium in San Francisco. It was my bad fortune to have to speak after Pauling, whose oratorical gifts matched his scientific brilliance. Because I felt that my data were at variance with his theory, I arranged through a mutual friend to have dinner with him. Although we spent a pleasant evening together, he didn't seem much interested in the chain structure of antibodies and the next day, he presented his original and classical theory with great flair and success. Then I followed with my factual contradictions, which were I am sure, not understood by any of the audience except Pauling, who sent me a terse note with no further comments. It said "Edelman - send reprints." I have not spoken with him about this but I suspect he was the only one in the room who began to see that the jig was up. In any case, at the press conference, he did not talk about antibodies but rather about his new theory of anesthesia and he did not publish anything in immunology after that.

LEEWAY - OR THE ADVANTAGES OF PUBLISHING IN THE WRONG JOURNAL

Independent of theoretical matters, there was a distinguished line of studies establishing the protein nature of antibodies (4), their divalence and binding properties (5) and the suggestion of classes (6). But no one except Pauling insisted very strongly that if you knew what an antibody really looked like, you would know a good deal about the important issues of immunology. Most of the emphasis was upon antigen structure, largely because of the enormous influence of Landsteiner's work and because instructive theories made the antigen crucial in antibody formation.

Not one word about shape, sequence or genetics - but this was hardly surprising. Insulin was just being analyzed by Sanger and ribonuclease by Hirs, Stein, and Moore. These molecules were 25 and 10 times smaller than immunoglobulin G. Nevertheless, we should note the early efforts of Pappenheimer, Petermann and Northrop on the cleavage of antibodies by proteases (7). This work had its natural fruition in the justly acclaimed work of Porter (8).

My own conviction was very strong that antibodies were the key to immunology. I distinctly remember the experiment that led eventually to the conclusion that antibodies consisted of multiple polypeptide chains. Perhaps prompted by Deutsch's work on macroglobulins (9) and Sanger's concern with disulfide interchange, I wanted to correlate the number of disulfide bonds with the shape and activity of antibodies. I proceeded by reducing the immunoglobulin, amperometrically titrating the free SH groups, alkylating them, and examining the products in the ultracentrifuge. At low pH, after cleavage of 6 or 8 S-S bonds, I noticed a peak with a sedimentation coefficient of about 2.3S. My colleagues put it off to conformational change, particularly in view of Porter's paper (10) concluding that rabbit Ig had one polypeptide chain. But it seemed too large a fall in the sedimentation coefficient to me and so I began a systematic study.

These studies led me to publish a paper (against advice, for no one believed the results - who would do ultracentrifugation in weird solvents such as urea-water mixtures?) - in the JOURNAL OF THE AMERICAN CHEMICAL SOCIETY (11). This one page note or letter was terse, dense, and to the point. Its beginning occupied one sentence - "Sir: Reaction of human γ-globulin with sulfhydryl compounds, sulfite, or performic acid resulted in marked diminution in the sedimentation co-

efficient and molecular weight." Its conclusion was equally as brief:"These findings suggest that human γ-globulin contains subunits linked at least in part by disulfide bonds. The possibility that linkages other than disulfide bonds are involved has not been excluded." No one believed it, it was received lackadaisically by an uninterested chemical audience, and in all I received seven reprint requests.

I am convinced now that being ignored in this way was a stroke of great good fortune. I was working alone, and had any one of the larger protein chemistry establishments taken the problem up, they would have moved it ahead much more swiftly than I could have hoped to do. My estimate is that I had three years of leisurely work given to me by the atmosphere and the paradigm. But I didn't feel this way at the time. I was working in the kind of oscillatory state of excitement and confused despair that comes from being against the paradigm.

CONVICTION AND ACCIDENTS OF NATURE

Although it seemed to me that the instructive theory was inadequate, I had not yet become fully convinced by the selective theory in 1960. I was more obsessed with the notion of fractionating the chains of immunoglobulins. This brought about an impasse in my thinking, not because progress had not been made, but because of the heterogeneity of this class of proteins. I had been able to fractionate chain components with different amino acid compositions by chromotography in 6M urea, but I know that they were likely to be microheterogeneous because electrophoresis in urea showed charge heterogeneity.

At this time, it occurred to me that perhaps myeloma proteins would be valuable to study. A mild controversy was then going on as to whether these proteins were "pathological" or were single normal immunoglobulins. Having various fractions in hand, I discussed this question with M.D. Poulik and suggested to him that we try the new procedure of starch gel electrophoresis in urea. We decided to compare a number of samples of multiple myeloma proteins and Waldenström macroglobulins.

My guess was that each of these would show a different and unique band in the faster moving fractions (now known to be the light chains), and I predicted that these bands would cover the range of diffuse protein staining exhibited

by the light chains of normal immunoglobulin. We had labored rather hard to accumulate our samples and with some hesitation, loaded the precious small amounts on one last gel to run overnight.

The next morning, I was confronted by a sad Poulik who told me he had dropped the gel upon staining it. I groaned, at which point he lifted out of a pan a gel that showed just the predicted features topped by his very wide grin. We also noticed a difference in the bands corresponding to heavy chains of myeloma proteins and macroglobulins (12).

At this point, in early 1961, I began to glimpse a general unifying picture of the immunoglobulins. I think it is instructive to examine this picture as revealed in the paper (12) reporting our results, for it has the beginnings of the shock of molecular recognition. I say beginnings because of its obviously general nature and lack of precision:

> "A unifying hypothesis may be formulated for the structure of proteins in the γ-globulin family based on the findings presented above as well as on findings of other investigators. 7S γ-globulin molecules appear to consist of several polypeptide chains linked by disulfide bonds. Bivalent antibodies may contain two chains that are similar or identical in structure. The 19S γ-globulins would be composed of 5 or 6 multichain units of the size of 7S γ-globulin. A provisional explanation for the wide molecular weight range of antigenically related globulins from Bence Jones proteins to macroglobulins is suggested by this model. Heterogeneity and differences in isoantigenicity may arise from various combinations of different chains as well as from differences in the sequence of amino acids within each type of chain.
>
> The finding that γ-globulin contains dissociable subunits has a possible bearing upon the pathogenesis of diseases of γ-globulin production. A primary defect in macroglobulinemia and multiple myeloma may be a failure of specificity and control in production and linkage of the various subunits to form larger molecules. Bence Jones pro-