

## Molecular Biology and Pauling's Immunochemistry: A Neglected Dimension \*

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**ABSTRACT** - This paper argues that there is a substantial overlap between the history of immunology and the history of molecular biology, an overlap manifested in the researches on antibodies during the 1930s and 1940s. This common ground is a product of intellectual developments, as well as institutional trends. Viewed from an intellectual vantage point of the 1930s and 1940s, molecular biology was essentially the study of the biological specificities of the so-called 'giant protein molecules'. Within the conceptual framework of early molecular biology, which was rooted in the protein view of life, the concepts of protein template, autocatalysis, and heterocatalysis were central in explaining the protein syntheses of genes, viruses, enzymes, hormones, and antibodies. Immunochemistry and serological genetics were at the heart of that research agenda. This paper also shows that the immunochemistry program of Linus Pauling, which focused on molecular mechanisms of antibody structure and function, and the projects in serological genetics at Caitech's biology division were supported by the Rockefeller Foundation under the aegis of its molecular biology program. Based on the close examination of intellectual and institutional factors, the histories of molecular biology and immunology in the pre-DNA era are seen as closely linked.

### *Introductory Remarks*

Before discussing the relation between molecular biology and immunochemistry it would be helpful to provide a cursory, or working definition of the terms 'molecular biology' and 'immunochemistry'. The term 'molecular biology', as I employ it, encompasses intellectual as well as institutional connotations. Molecular biology refers to the intellectual program that developed in the 1930s, a scientific agenda which focused on physico-chemical investigations of physiologically active molecules with emphasis on problems related to biological specificities. Within that agenda, physiological studies of the gene, its structure and function, occupied a central place. Molecular biology also refers to the Rockefeller Foundation's program of physico-chemical biology, a term coined in 1938 by

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Warren Weaver, the Foundation's director of the Natural Sciences Division.<sup>1</sup>

Immunochemistry refers to the subspecialty which emerged early in this century as a result of the influence of physical chemistry on immunology. The earlier work, with its physiological focus on the role of immune cells which defend the body against foreign antigens had given rise to cellular (and humoral) immunology. Advocates of the physico-chemical approach to immunology, on the other hand, stressed the specificity of chemical reactions by which antibodies neutralized the effects of antigens, reactions governed by weak intermolecular forces. This approach gave rise to immunochemistry (or molecular immunology), which focused almost exclusively on antibodies as the determinants of immunity.<sup>2</sup>

By the 1930s, the structure and mode of action of antibodies had become an active area of study for researchers from several fields. If we were to visualize the convergence of disciplinary paths in terms of Venn diagrams, research on antibodies would be the area of intersection of four circles representing microbiology, biochemistry, immunology, and molecular biology; the history of antibody research would thus fall within the domain of the histories of these disciplines.

These neat operational definitions are formulated with the benefit of hindsight, however. My interest in the history of immunology, which arose out of my work on the early history of molecular biology, was wholly unexpected. The standard histories of molecular biology, such as F. Portugal and J.S. Cohen, *A Century of DNA*, R.C. Olby, *The Path to the Double Helix*, J. Fruton, *Molecules and Life*, and H.F. Judson, *The Eighth Day of Creation*, do not include discussions on immunology. With the exception of the chapters on O.T. Avery's studies of the 'transforming principle'<sup>3</sup> in pneumococci, there has been no attempt to explain the relationship between antibody research and those researches in microbiology, biochemistry, and genetics which converged as molecular biology in the 1930s and 1940s.<sup>3</sup>

<sup>1</sup> R.B. Fosclick, *The Story of the Rockefeller Foundation*, New York, Harper and Brothers, Publishers, 1952, pp. 156-180, R.E. Kohler, 'The Management of Science: The Experience of Warren Weaver and the Rockefeller Foundation Programme in Molecular Biology', *Minerva*, 14 (1976), 249-293, E.J. Yoxen, 'Giving Life and New Meaning: The Rise of the Molecular Biology Establishment', *Scientific Establishments and Hierarchies; Sociology of the Sciences, IV*, edited by N. Ellas, H. Martins, and R. Whitley, Dordrecht, D. Reidel, 1982, 123-143, and P. Abir-Am, 'The Discourse of Physical Power and Biological Knowledge in the 1930s: A Reappraisal of the Rockefeller Foundation's "Policy" in Molecular Biology', *Social Studies of Science*, 12 (1982), 341-382.

<sup>2</sup> See for example P. H.M. Mazumdar, 'The Antigen-Antibody Reaction and the Physics and Chemistry of Life', *Bulletin of the History of Medicine*, 48 (1974), 1-21, idem, 'Karl Landsteiner and the Problem of Species' (Ph.D. dissertation, The Johns Hopkins University, 1976), and K. Landsteiner, *The Specificity of Serological Reactions*, 2nd edition, Cambridge, Harvard University Press, 1945.

<sup>3</sup> F. Portugal and J.S. Cohen, *A Century of DNA*, Cambridge, The MIT Press, 1977, R.C. Olby, *The Path to the Double Helix*, London, The Macmillan Press, 1974, H.F. Judson, *The Eighth Day of*

Thus, upon undertaking the project on the rise of molecular biology at the California Institute of Technology (Caltech) - the study of the large scale joint-venture between the biology and chemistry divisions supported by the Rockefeller Foundation - I was not at all prepared to deal with major research programs in immunology. Upon finding out that between the late 1930s and early 1950s (under the aegis of the molecular biology program) Linus Pauling's chemistry division had a substantial program in immunochemistry; and that projects in serological genetics occupied a central place in T.H. Morgan's biology division, I began to explore this aspect of the history of molecular biology.<sup>4</sup> My search for clues in several histories of immunology ended up with finding a reciprocal lacuna.<sup>5</sup> While these studies were helpful in explaining fundamental intellectual issues in physico-chemical immunology, they did not address the relationship between gene and antibody; between molecular genetics and immunochemistry. A recent study by Anne Marie Moulin, 'La Creation de Systeme Immunitaire, 1880-1980', which devotes a chapter to the connection between immunology and molecular biology, is a timely contribution; but more needs to be done.<sup>6</sup>

One reason that immunology has been excluded from accounts of molecular biology is rooted in the whig approach to history, and anachronistic uses of the term of molecular biology. If one perceives the history of molecular biology to be essentially the path to the double helix from which 'wrong turns' are either excluded or become scholarly apologia, then immunology indeed would seem largely an irrelevant detour. If the history of molecular biology is reduced to the story of DNA, then immunology - specifically chemical studies of antibodies which deal exclusively with proteins - would occupy at best a marginal place in such accounts.

However, if we were to remain historically authentic; erase from our minds present-day meanings of biological specificity and current explanations of protein synthesis based on the 'central dogma', and instead examine the molecular biology agenda as it was perceived and practiced in the 1930s and 1940s, another dimension is revealed. Within that older conceptual framework serological genetics and Pauling's program of immunochemistry assume a central role in the history of molecular biology.

*Creation*, New York, Simon and Schuster, 1979, and J. Fruton, *Molecules and Life*, New York, John Wiley and Sons, 1972.

<sup>4</sup> L.E. Kay, 'Cooperative Individualism and the Rise of Molecular Biology at the California Institute of Technology, 1928-1953' (Ph.D. dissertation, The Johns Hopkins University, 1986),

<sup>5</sup> A.M. Sirverstein, 'History of Immunology', in *Fundamentals of Immunology*, edited by W.H. Paul, New York, Raven Press, 1984, pp. 9-50, idem, 'History of Immunology: Development of the Concept of Immunologic Specificity', *Cellular Immunology*, 78 (1983), 174-190, idem, 'History of Theories of Antibody Formation', *Cellular Immunology*, 91 (1985), 263-283.

<sup>6</sup> A.M. Moulin, 'La Creation de Systeme Immunitaire, 1880-1980' (Ph.D. dissertation, University of Leon, 1986).

### *Biological Specificity and the Protein View of Life*

Early molecular biology was rooted in the 'protein view of life'<sup>3</sup>, the premise that proteins were the primary determinants of biological specificities. According to the protein view of life, all vital processes: reproduction, growth, and regulation were determined and governed by biological specificities inherent in the chemical structures and modes of action of proteins. Understanding the molecular mechanisms of the so-called 'giant protein molecules' - genes, viruses, enzymes, hormones, and antibodies - held out the promise of solving the riddle of life on the most fundamental level.

One of the best accounts on the primacy of the 'giant protein molecules' within the molecular biology agenda was given in 1939 by Warren Weaver.

All that association of phenomena which we term life is manifested only by matter made up to a very large extent of proteins, and is never exhibited in the absence of these substances. [Weaver's quote from T.R. Parsons, *Fundamentals of Biochemistry*, 1935, p. 1] ... (Proteins) enter into nearly every vital process. They are the principal component of the chromosomes which govern our heredity; they are the basic building stuff for the protoplasm of each cell of every living thing. Our immunity to many diseases depends upon the mysterious ability of serum globulin, a protein in the blood stream, to form specific antibodies when foreign proteins are introduced. Several of the hormones., including insulin, are protein in nature... The invasion of certain huge protein molecules, otherwise known as viruses, gives us common cold, influenza, encephalitis, certain forms of pneumonia, and many other diseases. Enzymes, those strange chemical controllers of so many of the detailed processes of the body, those perfect executives which stimulate and organize all sorts of activities without using up any of their own substance or energy - these enzymes are now believed to be protein in nature. Indeed many diverse scientists, each with his own special enthusiasm, would be willing to agree that these proteins deserve their names of 'first substance'.<sup>7</sup>

Based on these observations. Weaver concluded that the physico-chemical investigations of proteins lay at the heart of the molecular biology program. As a result of these views, studies of fundamental vital phenomena and questions of biological specificity were essentially recast as investigations of the 'giant protein molecules'<sup>3</sup>; the explanations were based mainly on concepts and theories from physical chemistry. Autocatalysis, or self-generating reaction, explained the self-duplication of protein molecules and was viewed as the mode of action by which certain proteolytic enzymes,

<sup>7</sup> Rockefeller Archive Center (hereafter RAG), RG2, General Correspondence 1939, 100, Box 170, file 1235; Warren Weaver, August 28, 1939.

genes, and viruses reproduced. Heterocatalysis, or the reaction governing the formation of non-identical protein molecules, explained the syntheses of most enzymes, hormones, and antibodies.<sup>8</sup>

The concept of a protein template was central to all these biological processes, supplying an analogy of a blueprint for autocatalytic and heterocatalytic constructions of proteins; all protein molecules were supposedly copied off pre-existing protein prototypes. The protein template concept, in turn, was predicated on two physico-chemical mechanisms: complementarity and intermolecular interactions. New protein molecules were synthesized off the protein template as a result of a perfect molecular fit between the old and new configurations, a molecular complementarity analogous to a dye and coin. The action of weak molecular forces, such as ionic interactions and van der Waals forces, gave rise to various chemical bonds, especially the flexible hydrogen bonds which determined the spatial configurations of proteins.

These physical explanations of molecular interactions in relation to biological specificity were articulated in 1940 by Linus Pauling and Max Delbrück in their joint-article, 'The Nature of Intermolecular Forces Operative in Biological Processes'. An outline of the protein-based approach to biological problems, this theoretical paper, in a sense, may be viewed as a manifesto of molecular biology and the conceptual framework for Pauling's 'instructive theory' of antibody formation.<sup>9</sup>

In this paper, the authors explained that fundamental biological processes involved the three-dimensional folding of highly complex protein molecules in the living cell. These configurations, the authors argued, were only partially determined by covalent bonds, The major role was played by weak physical interactions which gave maximum stability to a system of two molecules with complementary structures in juxtaposition, though not necessarily identical structures. The phenomenon of antibody formation was a case of non-identical complementarity, where an antigen acted as a template for the syntheses of complementary but structurally dissimilar proteins, through the formation of hydrogen bonds. By that time the seminal 1936 paper by Pauling and A.E. Mirsky on the role of hydrogen bonds as determinants of

<sup>8</sup> See For example II. C. Olby, 'The Protein Version of the Central Dogma', *Genetics*, 79 (1975), 3-27, A.W. Ravin, 'The Gene as a Catalyst; The Gene as Organism', *Studies in History of Biology*, 1 (1977), 1-45, and L.E. Kay, 'W.M. Stanley's Crystallization of the Tobacco Mosaic Virus,' 1930-1940', *Isis*, 77 (1986), 450-472.

<sup>9</sup> L. Pauling and M. Delbrück, 'The Nature of the Intermolecular Forces Operative in Biological Processes', *Science*, 92 (1940), 77-79.

three-dimensional configurations of proteins had been widely acclaimed, and the influence of physical chemistry on biology fully established.<sup>10</sup>

### *Immunochemistry and Serological Genetics*

According to Pauling's account, his initial involvement in immunology dated back to May 1936, when he gave a seminar on hemoglobin at the Rockefeller Institute. After listening to Pauling's seminar, so the story goes, Landsteiner invited him to speculate on possible explanations of the molecular mechanisms of antibody formation. Landsteiner's revolutionary studies on antibodies were then about two decades old. It was in 1917 that Landsteiner and his collaborators at the Rockefeller Institute first prepared artificially conjugated antigens by coupling simple inorganic compounds (haptens) to protein carriers and injecting them into animals. Under normal physiological conditions the organism could have never encountered these synthetic molecules. Yet the animals' antisera had been found to contain antibodies to these synthetic antigens. Landsteiner concluded from these results that antibodies to these non-physiological substances could not have preexisted in the cell surface, as Paul Ehrlich had proposed in his side-chain theory. Instead, Landsteiner reasoned, there had to be a chemical mechanism by which antibodies were synthesized *de novo*, in direct response to an injected antigen. It was the molecular mechanisms of this *de novo* synthesis of antibodies that Landsteiner sought to explain when he approached Pauling in 1936.<sup>11</sup>

Among his various projects during the next couple of years, Pauling also studied the literature in immunology, and had begun formulating theories of antibody formation based on Landsteiner's findings. In the October 1940 issue of the *Journal of the American Chemical Society*, Pauling published his classic article 'A Theory of the Structure and Process of Formation of Antibodies'. In this remarkably influential paper Pauling offered a detailed and creative explanation of antibody synthesis, a theoretical account based largely on the experimental works of several immunologists and biochemists, including Landsteiner, M. Heidelberger, Felix Haurowitz, and Jerome Alexander.<sup>12</sup>

<sup>10</sup> A.E. Mirsky and L. Pauling, 'The Structure of Native, Denatured, and Coagulated Proteins', *Proceedings of the National Academy of Sciences*, 22 (1936), 439-447.

<sup>11</sup> L. Pauling, 'Fifty Years of Progress in Structural Chemistry and Molecular Biology', *Daedalus*, 99 (1970), 909-910.

<sup>12</sup> L. Pauling, 'A Theory of the Structure and Process of Formation of Antibodies', *Journal of the American Chemical Society*, 62 (1940), 2643-2657, (RAG), RG 1.1, 205 D, Box 7, file 92; Pauling to Weaver, March 18, 1941.

Pauling simply assumed that all antibody molecules contained the same polypeptide chains as normal globulins. Based on the 1936 work of Pauling and Mirsky on hydrogen bonds and protein folding, Pauling concluded that antibodies differed from normal globulins merely in the configuration of the chain, in the way the two end-parts of the globulin polypeptide chains were coiled. In his elegant mechanism of antibody synthesis Pauling proposed six graphic steps that showed how the two ends of the globulin would assume many spatial configurations with nearly the same stability. Under the influence of an antigen molecule acting as a template, the globulins would assume configurations complementary to the surface regions of the antigens, thus forming two active ends. The central portion of the chain would fold up, freeing the oppositely directed ends to attach to two antigen molecules; the antibodies were therefore bivalent. Pauling admitted that there was no direct evidence to support some of his theoretical assumptions but he felt that these assumptions constituted the simplest and most reasonable mechanism which could account for diverse experimental observations.<sup>13</sup>

One interesting and important consequence of Pauling's proposed mechanism of antibody synthesis was his prediction that one could synthesize artificial antibodies around any given antigen. This promise carried revolutionary implications for basic research in the life sciences, as well as for the commercial products of research. While the repeated failure to obtain antibodies *in vitro* was a major factor in the later demise of Pauling's 'instructive theory'<sup>3</sup>, during the 1940s the potential practical returns intensified the Rockefeller Foundation's support for immunochemistry.<sup>14</sup>

The support by the Foundation for immunology at Caltech as part of its long-term commitment to molecular biology also extended to the biology division, where Pauling's 'instructive theory' inspired a flurry of activities in serological genetics. With Morgan's enthusiastic encouragement, A.H. Sturtevant and Sterling Emerson were raising rabbit anti-sera to various *Drosophila* genes in order to obtain antibodies directed against gene products, with the aim of producing mutations.<sup>15</sup>

In his 1940 paper, 'Can Specific Mutations be Induced by Serological Methods?'<sup>5</sup> Sturtevant gathered findings from the 1930s, among them works by J.B.S. Haldane and M. Irwin. These studies showed that there was a one-to-one correspondence between the presence of single genes

<sup>3</sup> L. Pauling (footnote 12), p. 2643.

<sup>4</sup> L. Pauling (footnote 12), p. 2656, and RAG, RG 1.1, 205 D<sub>5</sub> Box 7, file 92; Pauling to Weaver, January 2, 1941.

<sup>5</sup> RAG, RG 1.1, 205 D, Box 7, file 91; Grant for Serological Genetics, June 14, 1940.

and specific antigens; avian erythrocytes, in which specific antigens had been shown to be direct gene products, were a case in point. Sturtevant also drew on Landsteiner's findings that an antigen could directly induce the synthesis of antibodies, and on the molecular mechanisms proposed in Pauling's theory, which explained how antigens reacted serologically with antibodies based on specific chemical configuration. 'These considerations<sup>3</sup>, argued Sturtevant, 'led to the supposition that if a particular gene is responsible for the formation of a given antigen, then there is a possibility that the antibodies induced by this antigen may react with the gene. If these possibilities exist, there is a series of consequences that are of interest to the geneticist'. The induction of mutations by antibodies was a far-reaching consequence.<sup>16</sup>

Based on the judgement of prominent scientists, among them Morgan, Sturtevant and Pauling, the Foundation officers came to regard these projects as extremely promising. In appropriating a grant for work on serological genetics to Morgan's group, the officers predicted that 'it seems likely that in this field lies the best hope of attacking the general problem of gene action'.<sup>17</sup> During the next decade, based on G.W. Beadle's recommendation, the program in serological genetics expanded to include research on antibody-induced mutations in *Neurospora*.

In a 1949 article in *Scientific American* describing the growth and institutionalization of molecular biology at Caltech, Beadle was quoted as saying:

We are seeking to uncover the principles that govern fundamental processes of life... Science is still far from completely analyzing these biological agents [genes, antibodies, viruses, hormones, biological pigments, and related structures] but the investigations tend to show that the molecular form known as protein is the key structure... The genes, we believe, exercise an overruling control on all these activities. They do this, we think, by serving as the master patterns for the many proteins which function in the processes of life. Thus, there is probably a gene which serves as the template for the body's manufacture of insulin, another which provides the mold for pepsin, and so for albumin, fibrinogen, the polypeptide chain that forms antibodies, and all the rest.<sup>18</sup>

Clearly, by the late 1940s research on antibodies had become a salient feature in the protein-based molecular biology program.

<sup>16</sup> A.H. Sturtevant, 'Can Specific Mutations be Induced by Serological Methods?', *Proceedings of the National Academy of Sciences*, 30 (1944), 176-177. According to Sturtevant, the paper was written in 1940 but submitted for publication unaltered in 1944.

<sup>17</sup> Grant for Serological Genetics, 1940 (footnote 15), p. 1,

<sup>18</sup> G.W. Gray, 'Pauling and Beadle', *Scientific American*, 180, No. 5 (1949), 16-21; quotes on pp. 19-20.



*Final Reflections*

In describing the researches in serological genetics and immunochemistry at Caltech, I have attempted to show that these studies were central to the molecular biology program. As we have seen from an institutional point of view, these projects were supported by the Rockefeller Foundation under the aegis of its molecular biology program. From an intellectual vantage point of the 1930s and 1940s, molecular biology was essentially the study of the biological specificities of so-called 'giant protein molecules'<sup>3</sup>. As this paper has shown, within the conceptual framework of early molecular biology, which was based on the protein view of life, the protein template, autocatalysis, and heterocatalysis were the central explanations for protein syntheses of genes, viruses, enzymes, hormones, and antibodies. Immunochemistry and serological genetics were at the heart of that research agenda.

This close examination of antibody research in relation to gene action clearly shows that the omission of the history of immunology from the history of molecular biology is not only unjustified, but that it leaves out a very important dimension in the complex history of both fields.