MOLECULAR BIOPHYSICS AND BIOCHEMISTRY*

by William C. Summers
(Professor of Therapeutic Radiology, Molecular Biophysics and Biochemistry, and History of Medicine, and Lecturer in History)

MOLECULAR BIOPHYSICS

During World War II many university physicists undertook new research programs aimed at wartime goals. Notable among these goals were the Manhattan Project to investigate and exploit nuclear energy for military purposes and the project, based at the MIT Radiation Laboratory, to develop radar as a military surveillance tool. Yale nuclear physicist Ernest Pollard, a student of Chadwick and Rutherford, was recruited for the Radiation Lab (known informally as the "Rad Lab") by Ernest Lawrence. Prior to the war, Pollard had been carrying on a modest program of teaching and research in nuclear physics at Yale in the Department of Physics, then chaired by William Watson. In Pollard's view, wartime physics research fundamentally changed the style and form of physics in America. Nuclear physics had become big science, requiring expensive equipment and teams of scientists, not an activity for a university professor with a small research group and major teaching obligations. In addition, having spent the war years working on microwave research, Pollard and other members of the Rad Lab had lost out on the excitement and new advances, many of them still classified, in nuclear physics coming out of the Manhattan Project.

When Pollard and his small group of students, including Franklin Hutchinson, returned to Yale after the war, he considered two new directions for his research, cosmology and biology. In Pollard's view, he was not temperamentally suited to be a cosmologist, and he thought it might be hard to start up in that field at Yale at that time. Thus, he settled on "biophysics" as his new research and intellectual program. Still, for a while after returning to Yale, Pollard carried on in nuclear physics as he was beginning to delve into biophysics. He noted: "I was most comfortable describing the effects of physical agents: temperature, pressure, ultraviolet light, electric fields, and ionizing radiation on almost anything biological" (Pollard, p. 29). In the course of this work, he read the recent work of Douglas E. Lea of Cambridge University on the use of ionizing radiation to investigate cells and viruses. The approach that Lea employed was known as "target theory" and had evolved out of the work of J.J. Thomson and Ernest Rutherford on studies on the internal structure of the atom.

The "target theory" is the name given to a model for the way radiation interacts with cells. The two basic features of this model are that the radiation is considered to be random projectiles, and the components of the cell are considered as the targets to be bombarded by these projectiles. From the nature of the dose-response relationship for a specific type of radiation, and inactivation of a specific biological function, the number and size of the subcellular target for that particular function can be calculated. This approach was used to estimate the size and shape of enzymes, viruses, and even genes. It has also been applied to study the subcellular apparatus that synthesizes proteins and DNA, as well as such global physiologic processes as respiration and ion transport. From the mid-1930s to the mid-1950s, the target theory and its applications were a major preoccupation of the field that became known as biophysics.

Pollard and his colleague Fred Forro adapted the Yale cyclotron to this work. The cyclotron had the distinct advantage over previous target theory work in that it delivered a measurable beam of radiation for which accurate doses could be calculated, and also it allowed for the variation in particle energy and

hence spatial distribution of ionization events that occurred within the biological specimens. Their first experiments employed accelerated deuterons (heavy hydrogen ions) to kill bacteria. Pollard's initial group working on bombarding cells with high energy particles included Richard P. Setlow, a spectroscopist already at Yale, Franklin Hutchinson and Harold Morowitz, both recent Ph.D.s in physics from Yale, and Alexander Mauro, a beginning graduate student. Their first laboratory for biophysical work at Yale was in the attic of the Sloane Physics Laboratory.

Setlow and Donald Fluke built a large water prism to provide high intensity light of different wavelengths to investigate the spectral dependence of ultraviolet light effects on biological targets. This homemade monochromator became famous, and years after it became obsolete and was mothballed, visitors to Yale would still ask to see it. This work on ultraviolet radiation biology has continued at Yale and led to the discovery of the mechanisms of repair of DNA damage and the identification of genes that control sensitivity to DNA damage, genetic recombination, and ultraviolet light-induced skin cancer.

A central question in target theory since its formulation in 1924 by J.A. Crowther was "What is the nature of the critical radiosensitive target?" While target theory allowed measurement of the size and shape of the entity in the cell that was inactivated ("hit") in the lethal action of the radiation, it did not give any chemical information about that entity. Debates on this question followed the fashion of the times: at one point some key enzyme was the favorite candidate; at another time, chromosomes were in vogue; some scientists favored membranes as the critical target. By the mid-1950s, however, many scientists thought the best candidate was DNA because the efficiency of the lethal effects of different wavelengths of light exactly matched the absorption of light by the DNA molecule. At Yale, Walter Guild exploited the ability to study the genetic function of pure DNA in the test tube (so-called bacterial transformation) to examine the effects of ultraviolet light directly on DNA itself. His work contributed to the ultimate acceptance of DNA as the critical radiosensitive target.

Pollard and his associate Marjory Reaume extended their studies to bacterial viruses (bacteriophage) and were surprised to find that some viruses and some enzymes could survive in the dried state in the high vacuum in the cyclotron, so they were able to initiate a research program applying target theory to study the structure and function of purified biological molecules. At this time (mid-1950s) scientists had three general ways to study the structure of molecules, especially the large molecules such as proteins and nucleic acids of interest to biologists: chemical analysis of the components of the molecules (this approach had just yielded the amino acid sequence of a very small protein, insulin); solution hydrodynamics (sedimentation and diffusion rates), which provided the molecular weight and a general notion of the shape of the molecule, i.e., globular, oblong, fibrous; and x-ray diffraction analysis (not yet successful with any molecule as large as a protein), which was capable of providing molecular structures at atomic resolution. The biophysical approach pioneered by Pollard and his associates was an additional tool in this attack on the structures of interesting biological molecules and viruses. While it was not able to provide the detailed information of x-ray diffraction, this method was much easier and did not require the high purity and homogeneity needed to apply the hydrodynamic methods. Another new approach to low resolution molecular structure was the use of small angle x-ray scattering, and Donald Caspar set up this method at Yale and employed it to study the structures of viruses.

Between 1948 and the mid-1950s, the biophysics research at Yale concentrated on the studies of the target analysis of viruses, enzymes, and bacteria. The target theory was refined by the inclusion of effects due to the presence of water (diffusion of ionized molecules), temperature, and the nature of the ionizing radiation. Molecular sizes and shapes determined by the target theory approach were in remarkably good agreement with those determined by hydrodynamic method in cases where

comparable measurements had been made. Several interesting, yet puzzling, results were obtained that in subsequent research were to be more fully explained: Fred Forro found that a virus had two components, an "essential" part, needed for its infectivity, and a "non-essential" part, the inactivation of which did not interfere with infectivity. This result presaged the findings of Alfred Hershey and Martha Chase that showed that only part of the virus, the DNA genome, is injected into the cell when a virus infects its host. In a similar vein, Pollard investigated the intracellular machinery that synthesizes proteins, and he found that the target, the inactivation of which stopped protein synthesis, was very long and very thin. This was a puzzle at the time (late 1950s) because protein synthesis was believed to occur in small, found particles called microsomes. Only later was it found that the complete protein synthetic apparatus was, indeed, a long, thin assembly of many ribosomes attached to the messenger RNA (the polyribosome or polysome, for short).

In 1955 the biophysics group received substantial support from the Hartford Foundation, and for a while most of the group actually worked in laboratory space provided by the foundation at the Hartford estate in Valhalla, New York. For several reasons this distant arrangement was not ideal, and in 1958 it was terminated and the biophysics group returned to the campus and in 1959 moved into the newly constructed sixth floor of the Josiah Willard Gibbs Laboratory.

In 1960 Ernest Pollard was attracted to Pennsylvania State University, and Franklin Hutchinson succeeded him as chair of the Biophysics Department from 1961 to 1963; Frederic M. Richards was recruited from the Department of Biochemistry to assume the chair in 1963, a post he held until 1969.

Major changes in the intellectual direction of the Biophysics Department followed Pollard's departure, partly because of new trends in basic biological research, and partly because of the influence of Kingman Brewster, Yale's new provost. Brewster arrived in New Haven from Harvard in 1961 and had been alerted by several of his scientific colleagues in Boston that biophysics at Yale presented an opportunity to build up research on topics loosely called molecular biology. In 1962, the Biophysics Department changed its name to the Department of Molecular Biology and Biophysics to better represent these intentions and aspirations. In 1964, in response to new initiatives in the Department of Biology, the Department of Molecular Biology and Biophysics changed names again and became the Department of Molecular Biophysics.

In the mid-1960s, new faculty were brought to Yale, and existing faculty in other departments who had interests relevant to molecular biophysics were recruited. Alan Garen, Peter Lengyel, Donald Marvin, Irwin Rubenstein, Dieter Söll, Harold Wyckoff, Robert Wilhelm, and Peter B. Moore were hired to strengthen research in the work combining genetic, biochemical, and physical approaches. From the Department of Chemistry, Donald Crothers and Oktay Sinanoglŭ were recruited as joint appointees. Major research programs now focused on structural studies of proteins and nucleic acids, and on how these structures could be used to understand biological functions. In particular, there was a shift away from organic chemistry as the fundamental approach and toward the use of the methods and principles of physical chemistry.

Richards and Wyckoff collaborated to investigate the structure of pancreatic ribonuclease at the atomic resolution by means of x-ray diffraction analysis of crystalline ribonuclease. This structural approach to the study of enzymes and enzyme mechanisms established a paradigm for subsequent development of macromolecular biochemistry at Yale. They successfully extended this approach to the study of bacterial alkaline phosphatase, an enzyme with interesting regulatory properties, and on which extensive genetic analysis had been carried out, by Alan Garen in particular. This structural approach to

the understanding of macromolecular functions has developed into a dominant research theme in biological research at Yale since this early work in the 1960s.

MOLECULAR BIOPHYSICS AND BIOCHEMISTRY

With the merger of the faculties of the Department of Molecular Biophysics and the Department of Biochemistry in 1969 with Frederic Richards as chair, Yale invested significant new resources in this endeavor by providing newly renovated space in the School of Medicine and new faculty appointments. The faculty residing in the School of Medicine, formerly from the Department of Biochemistry, were augmented by recruiting of several faculty in other departments of the School of Medicine to accept joint appointments in the new amalgamated department. This strategy provided for a rapid infusion of both new enthusiasm as well as an expansion of the research interests represented in Molecular Biophysics and Biochemistry (MB&B). Charles M. Radding and Sherman M. Weissman, both members of the Department of Internal Medicine, joined MB&B as did W. Dean Rupp and William C. Summers, both from the Department of Therapeutic Radiology. These four scientists had ongoing research programs in the molecular biology of genes and viruses, and brought strengths in those areas to the newly renovated department. On the main campus, joint appointees were recruited from Biology (Gerald Wyatt, Joseph Gall, and Norman Giles) and Chemistry (Jui Hsin Wang was added to existing joint faculty members Sinanoglŭ and Crothers). Active recruiting soon brought in Thomas A. Steitz, Joan A. Steitz, David M. Ward, John E. Cronan, Donald M. Engelman, and Lubert Stryer.

Since its founding in 1969, research in MB&B has evolved along three major lines, molecular genetics, structural biology, and biochemistry of proteins and nucleic acids. The interdisciplinary nature of these research programs has been nurtured by the inclusion in MB&B of faculty from related departments. Close ties with Chemistry; Molecular, Cellular, and Developmental Biology; Genetics; and some of the clinical departments in the School of Medicine have strengthened both the research and educational work in MB&B.

Undergraduate teaching became an important focus of MB&B, and the major attracted a growing population of committed and talented students. Teaching of biochemistry in the School of Medicine was given new priority and both introductory and advanced disease-oriented courses were developed as offerings to the first-year medical students with diverse backgrounds in the biochemical sciences.

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