NATIONAL ACADEMY OF SCIENCES

MOSES KUNITZ

1887—1978

A Biographical Memoir by

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The Rockefeller University

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December 19, 1887–April 20, 1978

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MOSES KUNITZ is best remembered for his isolation and crystallization of a half-dozen enzymes and precursors. This work had two important effects. First, the variety of the enzymes he isolated and the clarity of his evidence that the enzymes were proteins convinced those who had reserved judgment on the earlier reports of Sumner, Northrop, Caldwell, et al. Second, his reports of the crystallization of ribonuclease and desoxyribonuclease, which appeared just as the functions of nucleic acids were beginning to be explored, provided information on the high specificity of these enzymes. This information made them valuable tools for other researchers in their purification or the assignment of a biological function to either RNA or DNA, the two types of nucleic acid.

Moses Kunitz was born on December 19, 1887, in Slonim, Russia, where he was educated before emigrating to the United States. In 1909, he took up residence in New York City. Entering the Cooper Union School of Chemistry in 1910, he graduated with a bachelor of science degree in 1916. In the fall of that year, he entered the Electrical Engineering School of Cooper Union, where he studied until 1919 when he transferred to the Columbia University School of Mines Engineering and Chemistry. In 1922 he matricu-

lated as a graduate student in Columbia's Faculty of Pure Science, which awarded him a Ph.D. degree in biological chemistry in 1924.

Kunitz derived much of his formal education in graduate science from evening classes he attended while working full time as a technical assistant in Jacques Loeb's general physiology laboratory at the Rockefeller Institute for Medical Research. Loeb quickly recognized Kunitz's fine work habits and encouraged his educational development. When Kunitz received his doctorate, Loeb secured his appointment to the staff. He also collaborated with Kunitz in studies of protein-ion equilibria and related phenomena, and many of the original measurements on this subject appear in Kunitz's doctoral thesis and early publications.

Upon Loeb's death in 1924, John H. Northrop was appointed his successor. He invited Kunitz to continue with his fundamental studies of viscosity, swelling, and the effect of certain salts on the properties of proteins. Northrop joined Kunitz in many of these investigations—a happy, productive collaboration that was to last for more than thirty years.

Northrop and Kunitz moved to the Princeton branch of the Rockefeller Institute in 1926, and soon after the move, their interest shifted to the isolation of proteases. Northrop's choice of pepsin and Kunitz's choice of trypsin for these studies were due in part to the commercial availability of these substances. Although crystals of trypsin were obtained in 1931, the procedure was long and tedious, and the yield was low. In 1933 Kunitz devised a better approach. Preliminary experiments revealed that unlike the common structural tissue components, trypsinogen, the precursor of trypsin in beef pancreas, was soluble and stable in cold, quarter-normal, sulfuric acid. This information led him to develop a unique method of extracting trypsinogen and several other precursors and enzymes.

Variations of the fractions in Kunitz's assays soon revealed the presence of another protease precursor and enzyme. Because the new protease had strong milk-clotting action (a property not held by trypsin), Kunitz termed the precursor chymotrypsinogen and the enzyme chymotrypsin. In a relatively short time, Kunitz crystallized both chymotrypsinogen and trypsinogen and, soon after, the active enzymes themselves. Solubility studies that Northrop and he had developed showed these four proteins to be homogeneous. Kunitz also found conversion of trypsinogen to trypsin to be autocatalytic—that is, tryspin catalyzed the conversion. Trypsin also converted chymotrypsinogen to chymotrypsin, the kinetics in this case being first order.

Kunitz's extreme care in all of his experiments frequently led him to discoveries that the average worker might well have missed. Two instances illustrate this point. Kunitz investigated a slow change in the activity of a stored preparation of chymotrypsin that he believed to be stable. In the course of the work, he isolated two new autolysis products, still proteolytically active, which he named *beta* and *gamma* chymotrypsins, designating the original enzyme *alpha*. Again, when his trypsinogen preparation became active in acid solution—a result contrary to his earlier studies—he discovered that his stock HCl solution was contaminated with a mold that liberated a protease that had brought about the activation. He isolated the mold and then the mold protease. He then used the protease to convert trypsinogen to trypsin in an acid medium, obtaining a cleaner preparation of trypsin than was possible by any previous procedure.

The presence of substances inhibitory to trypsin in the original pancreatic extracts, and in certain soybean meal fractions, led Kunitz to the crystallization of a polypeptide inhibitor from the pancreas and a protein inhibitor from soybean. Isolation of the inhibitor from the pancreas answered a ques-

tion Kunitz had already posed: Why does trypsinogen remain inactive in pancreatic tissue when the pH is optimal for its activation?

Kunitz's interests, however, were far broader than merely the isolation of enzymes or inhibitors. In each instance, he studied the interaction of the inhibitor with the enzyme and isolated the complexes, studying their stoichiometry and other properties. One of his finest papers details the study of the kinetics and thermodynamics of the reversible denaturation of the soybean trypsin inhibitor.

From 1939 to 1940, Kunitz worked to isolate ribonuclease from beef pancreas. He found it to be one of the smallest proteins and extremely stable—even to boiling. This enzyme liberates only pyrimidine mononucleotides from ribonucleic acids. The isolation of desoxyribonuclease came ten years later, after Maclyn McCarty had obtained its partial purification. Very low (nanogram) levels of this enzyme destroyed the pneumococcal transforming activity of Avery, MacLeod, and McCarty's DNA preparations—a finding that led many investigators to believe that DNA carried hereditary determinants.

With the onset of World War II, when the laboratory's attention was focused on government projects, Kunitz was asked to isolate hexokinase, thought to be highly sensitive to the action of a poison gas. He isolated the hexokinase in crystalline form, but had to isolate three other crystalline proteins before the hexokinase crystallized.

The following anecdote reveals Kunitz's remarkable faculty for crystallizing proteins. Another laboratory had devoted considerable effort to the isolation of a plant protein of great interest to the Department of Defense, but the investigator had been unable to crystallize the protein. A package of the material was sent to Kunitz with the request that he attempt to crystallize it. The package arrived late one

afternoon. Kunitz dissolved some of the dry powder in water and placed small aliquots in a series of test tubes to which he added drops of dilute HCl, increasing the number of drops in each successive tube. A precipitate soon began to appear in the middle of the series, and Kunitz held a turbid tube to the light from the window, remarking, after a few moments, "It looks granular." He placed the tubes in the refrigerator, and the next morning several tubes had crystals of what proved to be the active protein.

It is unfortunate that more beginning investigators did not get the chance to work near Kunitz in the early years. Margaret McDonald and this author were certainly enriched by our long association with him. After his return to New York in 1952 and the conversion of the Rockefeller Institute to its present university status, Kunitz was named professor emeritus and continued to work daily in the laboratory. Many staff and students then had an opportunity to see how the master worked.

Kunitz's papers, models of scientific reporting, also illustrate his reliance on the results of broad experimentation rather than on preconceived notions. His procedures of exposing proteins to strong acid or high temperatures—unique at that time—were avoided by most investigators.

There is no more appropriate testimony to the esteem in which Kunitz was held by his peers than the comments of John Northrop, which were included in a review of Kunitz's work when he was awarded the Carl Neuberg Medal in 1957: "Dr. Kunitz possesses to a rare degree the abilities of a research worker of the first rank in his chosen field—imagination, ingenuity, persistence, great technical skill, mathematical facility, and a thorough theoretical knowledge. It is not surprising, therefore, that he has been able to solve almost every problem he has attempted. Some of them are of great importance. The isolation and crystallization of ribo-

nuclease, hexokinase, and deoxyribonuclease placed the protein nature of enzymes, in general, on a firm experimental foundation. In addition, the nucleases have been invaluable tools in the elucidation of the chemistry of the nucleic acids, those remarkable substances that appear to be the very 'stuff of life'."

Moses Kunitz was a modest, gentle, considerate person who loved his work and his family. His association with the Rockefeller Laboratories spanned a period of fifty-seven years. He died April 20, 1978, in Philadelphia, Pennsylvania. He is survived by a daughter, Roslyn Albert, and a son, Jacques Kunitz.

HONORS AND DISTINCTIONS

Moses Kunitz was associated with The Rockefeller University for almost sixty years (1913–1972). He was elected associate member in 1940, member in 1949, and professor emeritus in 1953. Kunitz was awarded the American Society of European Chemists and Pharmacists (New York City) Carl Neuberg Medal in 1957. He was elected to the National Academy of Sciences in 1967 and received an honorary degree from The Rockefeller University in 1973. He was a member of the American Association for the Advancement of Science, the Society for Experimental Biology, the American Society of Biological Chemists, and the Society of General Physiologists.

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