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A Biographical Memoir by
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ALFRED NISONOFF

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ALFRED NISONOFF, WHO DIED on March 12, 2001, was a major contributor to many basic aspects of immunology throughout his career. In addition to fundamental work that helped to define the nature of antibodies and the genes encoding them, he was an astute critic with penetrating analytical skills. His monograph *The Antibody Molecule* stands as the definitive reference work on the subject to 1975, the time of its publication.

Nisonoff's parents immigrated to the New York area from Hungary and Russia as teenagers. Al was born in Corona on January 26, 1923. When he was about two years old, his parents moved into a working-class, largely immigrant community in South River, New Jersey, to join other family members. They operated a kosher butcher shop and grocery store. Al's parents had little formal education and his initial exposure to books and reading was in school, where his exceptional intelligence was soon recognized. At age 6 he found himself in third grade, and by 15 he had graduated from high school. One of the few students in his school to go to college, Al received a state scholarship and enrolled at Rutgers, which was within hitchhiking distance and allowed him to live at home. He became interested in chem-

istry when a high-school friend gave him access to his home laboratory, and decided to major in this field. It also seemed to offer opportunities for practical future employment.

Upon graduation from Rutgers in 1942 at age 19, Al set off in a Model A Ford to take up a job with the U.S. Rubber Company in Detroit. It was the first time he had been more than 50 miles from home. He later recalled being told by an upper management person that he should be proud because U.S. Rubber did not ordinarily hire Jews. He was probably not surprised to hear this, because it was generally accepted at this time that many chemical companies would not hire Jews (see Dan A. Oren, *Joining the Club: A History of Jews and Yale,* p. 357, footnote 28. New Haven: Yale University Press, 1985).

Although Al was assigned to a fairly routine task, testing various latex compounds for their ability to adhere to the nylon cord required to strengthen airplane tires, he soon made a critical observation that changed the production of these tires so important for the war effort. One day while walking through the plant, he stopped to watch the construction of self-sealing gasoline tanks made from rubber and strengthened with nylon cord dipped in a water-based latex adhesive. Combining the keen power of observation and imagination that was to characterize his future research, Al adapted this process to the problem of adhering nylon cord to rubber tires—so U.S. Rubber was launched into making nylon-belted tires. Describing this practical discovery many years later, Al with typical self-deprecation said it was "primitive stuff . . . a mindless sort of thing." Another significant event of the time in Detroit was that Al met Sarah (Sally) Weiseman at a Jewish community center. They corresponded through the war years and were married immediately after his discharge from the Navy.

By 1943, with the war raging, Al became anxious to join

the armed forces. Even though he had an occupational deferment, he enlisted as a midshipman in the Navy. He served until the end of the war, missing the invasion of Okinawa only because his ship developed an engine problem. He had not given much thought to the future, but a college friend whom he met while passing through San Diego told him about the G.I. Bill, and he decided to pursue graduate work in chemistry. He was discharged from the Navy in July 1946 and entered graduate school at Johns Hopkins University in September, receiving his M.A. in 1948 and Ph.D. in 1951. His research, supervised by Frederick W. Barnes, Jr., was on the enzymatic mechanism of transamination.

Following graduate school and on the strength of his previous success at U. S. Rubber, Al joined a branch of the same company in Naugatuck, Connecticut. After two years, however, he decided to return to work related to biochemistry and took a position with David Pressman's group at the Roswell Park Memorial Institute in Buffalo, beginning work that set the direction for much of his research in the remainder of his career.

In the early 1940s David Pressman, then working in Linus Pauling's group, carried out an extensive series of experiments exploring the specificity of antibodies directed against haptenic determinants. These studies introduced the technique of quantitative hapten inhibition, an important extension of the experimental approach pioneered by Karl Landsteiner. Throughout his career Nisonoff applied quantitative approaches, often with anti-hapten antibodies, to a number of problems in immunology. With Pressman, Nisonoff explored the heterogeneity in the binding of antibodies with haptens and introduced means of estimating this heterogeneity quantitatively. In an important paper from this period he demonstrated that the two combining sites on a single antibody molecule have the same specificity. This

result, which confirmed earlier experiments by Landsteiner, Felix Haurowitz, and Herman Eisen using precipitin methods, was important in that it rendered unlikely that specific antibody sites would simply be generated by folding around an antigen template, as had been specified in the "instruction" theories of antibody formation, in most detail by Pauling.

Toward the end of his stay at Roswell Park, Nisonoff initiated experiments on the enzymatic cleavage of rabbit antibodies, which contributed importantly to the growing understanding of their structure. Rodney Porter had shown that two active univalent fragments, now known as Fab, could be produced from each antibody molecule by digestion with papain. Because papain is always used in the presence of a mercaptan, Nisonoff originally proposed that two steps, proteolysis and disulfide cleavage, were needed to generate the active univalent fragments. Accordingly, he repeated Porter's experiment with a different enzyme, pepsin, which does not require activation by a mercaptan. Although the initial premise was incorrect, as Nisonoff himself later pointed out, the experiment led to an even more interesting conclusion. Disulfide bond cleavage is not required to produce the active univalent antibody fragments after papain cleavage, but it is required to produce univalent fragments after limited digestion with pepsin. The explanation is that papain cleaves on the amino-terminal side of the single disulfide bridge connecting the two heavy chains in rabbit IgG, whereas pepsin cleaves on the carboxyl-terminal side of the same bond, generating a single bivalent fragment, $F(ab')_2$. Reduction of the inter-heavy chain bridge in the bivalent fragment yields univalent Fab′ fragments.

Nisonoff's studies provided critical insights into the nature of the fragments produced by digestion with papain and their disposition in the intact antibody molecule. His experiments with pepsin digestion implied that the two frag-

ments containing the active site (Fab or Fab′) are located on the same side of the molecule, away from the Fc fragment. The deliberations by Porter's group that led to the formulation of the polypeptide chain structure of IgG were summarized by Julian Fleischman in a "citation classic" review of the work. As Fleischman recalled, Porter initially favored the then popular cigar-shape model in which the two active fragments are disposed on either side of a central Fc. However, this model was not easily reconciled with Nisonoff's results with pepsin digestion and was therefore abandoned in favor of the now familiar four-chain model in which the two Fab fragments are on one side of the molecule. Nisonoff's work also clarified the nature of chromatographic fractions I and II obtained by Porter after papain digestion of rabbit antibodies. The similar yield initially found for fractions I and II was fortuitous, the result of charge heterogeneity in the antibody population and the choice of column conditions. In fact, the more negatively charged antibody molecules were found to contain two Fab fragments of type I and the more positively charged two Fab fragments of type II.

The $F(ab')_2$ fragment produced by pepsin retains the bivalence of the original antibody molecule and therefore the ability to precipitate or agglutinate antigen. However, it lacks the Fc fragment and will not bind to cells expressing Fc receptors, eliminating much undesired "non-specific" antibody binding. The next logical step, taken by Nisonoff just as he was moving from Roswell Park to a position as associate professor of microbiology at the University of Illinois, Urbana, was to show that the univalent Fab′ fragments generated by successive pepsin digestion and reduction could be recombined into the bivalent $F(ab')_2$ fragment by oxidation, allowing the creation of bivalent antibodies of mixed specificity. Such hybrid antibodies have had many practical

uses, for example, bringing a pharmacological agent into contact with a particular cell type.

Throughout his career Nisonoff retained an interest in the three-dimensional structure of antibodies. As early as the late 1950s this led to collaboration with Cecil Hall and Henry Slater at the Massachusetts Institute of Technology in an attempt to visualize the antibody molecule by electron microscopy. Although resolution was insufficient to discern the shape, the data provided a reasonable estimate of the size of the molecule. Methods of X-ray crystallography began to be applied to proteins in the 1960s. Recognizing the importance of applying these techniques to antibodies, Nisonoff soon succeeded in obtaining crystals of Fab fragments derived from human IgG myeloma proteins. Preliminary structural work was carried out in collaboration with Roberto Poljak's group; later more detailed studies carried out by Poljak and others led to a detailed understanding of the structure of the Fab fragment, including localization of the active site, the basic features of the Ig fold, and the orientation of V and C domains.

In 1966 Nisonoff moved from Urbana to the University of Illinois College of Medicine in Chicago, where in 1969 he assumed the chair of the Department of Biological Chemistry. In Chicago he continued a fruitful collaboration with Sheldon Dray, relating structural features of rabbit antibodies to genetic variations known as allotypy. In work using Nisonoff's characteristic quantitative approach, they showed that the population of IgG molecules in a rabbit heterozygous for allotype consists only of molecules displaying one or the other allotypic determinant, but not both. Coming on the heels of the proposal of the four-chain model for IgG, this finding suggested that the IgG molecule is symmetrical (i.e., with two identical heavy chains and two identical light chains).

Thinking that quantitative immunochemical methods would also be useful for investigating idiotypy (the unique antigenic specificity possessed by individual antibody molecules), Nisonoff embarked on a series of studies that laid the groundwork for the widespread use of idiotypes as genetic markers. Indeed, studies of idiotypy were to occupy him for the rest of his career. He drew on his experience with antibodies directed against haptenic determinants to address such questions as the relationship of the idiotypic site of an antibody molecule to the antigen-binding site. In other studies Nisonoff addressed such questions as the size of the repertoire of antibody-binding sites and the relationship of the idiotypic site of an antibody molecule to the antigen-binding site. For example, an important insight provided by Nisonoff, as well as others, was the recognition that some idiotypic specificities can be shared by different individuals, so-called public idiotypes, and that these are probably encoded by germline genes. The relationship between idiotypes and genes encoding antibody V regions provided means for tracking clonal lines of B-lymphocytes.

In the 1960s evidence began to accumulate indicating that a single germline gene could not encode both the variable and constant regions of an Ig heavy or light chain. One of the critical findings was provided by Nisonoff, who in collaboration with Hugh Fudenberg showed that IgG and IgM myeloma proteins obtained from the same patient had identical idiotypes, and therefore identical V regions, as was later confirmed by sequence analysis. Because the C regions of the gamma and mu chains must be encoded by distinct genes, the V and C segments had to be specified by separate genetic units.

He showed that antibodies directed against idiotypic determinants could compete with hapten in binding to antibody-combining regions. Nisonoff and J. Donald Capra in a

collaboration that extended over a number of years showed that idiotypes could be defined structurally and that idiotypic differences between different strains of mice reflect genetic variability. With Paul Gottlieb, Nisonoff provided evidence that both heavy and light chains in an immunoglobulin molecule are required for expression of the idiotype. Nisonoff's work and that of others on idiotypy is summarized in his presidential address for the American Association of Immunologists meeting of 1991.

A major occupation and preoccupation of the later Chicago years was the writing, together with John Hopper and Susan Spring, of the monograph *The Antibody Molecule* (1975), a monumental annotated work providing a scholarly and comprehensive review of what we now call the B-cell receptor. Choosing to review the field at this time was a reflection of Nisonoff's astute insight, for it provided a definitive summary of the protein phase of molecular immunology and set the stage for the genetic era that was soon to follow. A decade later Nisonoff wrote an introductory text of molecular immunology that showed not only his superb command of the subject but also his skill in presenting complex material. His extensive knowledge and clear, rigorous thinking made him an excellent teacher both in the laboratory and in the classroom.

In 1975 Nisonoff moved to the Rosenstiel Research Center at Brandeis University, where he helped to bring younger colleagues to a group whose focus was research in immunology. His own work continued to be centered on idiotypy. Interestingly, he was recruited to Brandeis by Harlyn Halvorson, then director of the Rosenstiel center, whose father, H. Orin Halvorson, had brought Nisonoff to the Microbiology Department at the University of Illinois, Urbana, at the outset of his academic career.

Nisonoff's well-deserved reputation for critical scientific

insight and sound judgment meant that he was much sought after to serve on review panels, advisory committees, and editorial boards. In what may be a record, he served three terms, once as chair, on the allergy and immunology study section of the National Institutes of Health. He was president of the American Association of Immunologists in 1990-91. Even after giving up his research program in 1996, Nisonoff continued as an advisor to NIH and played a major role in preparing a comprehensive report on current knowledge and future directions in immunology (*Report of the NIAID Task Force on Immunology,* National Institutes of Health, 1998). Retirement was not easy for him, as he missed the give and take of the lab, but he remained as sharp as ever and was always available for criticism and discussion with former colleagues.

One of Nisonoff's outstanding qualities was intellectual honesty. Always direct and outspoken, he disliked pretense in any form and was himself without any pretension. He saw right to the heart of any question and brooked no fuzziness of thought. The high standards he set for himself were a model for colleagues, as well as for the many students he mentored. He paid little attention to the external trappings of fame, his or anyone else's. He was interested in science for its own sake and was forever in pursuit of the rigorous experiment that would provide an unambiguous answer to a meaningful question.

Nisonoff's characteristic modesty is epitomized in the format of his curriculum vitae in which his honors are buried in a section captioned "Other Data." He received the Medal of the Pasteur Institute in 1971, was a foreign correspondent of the Belgian Academy of Medicine in 1977, a fellow of the American Academy of Arts and Sciences in 1982, and became a member of the National Academy of Science in 1984.

As a young person Nisonoff had little exposure to the visual arts or to music, but he came to love classical music passionately and was a regular at Boston Symphony concerts. He loved the works of many twentieth-century composers but was generally not enamored of the newly commissioned works that are often included in these concerts. He was moved to express this view in a letter sent to the Boston Symphony just a few months before his death. Why, he asked, were new compositions so rarely played again after their premiere? If they are worth hearing once, he reasoned, surely they are worth hearing twice. As far as is known, the question remained unanswered.

Nisonoff's sense of fun and good humor were legendary. In addition to music, he loved to play tennis and did so regularly. He was invariably good for a lively discussion of the current political scene. He had a strong lifelong sense of social justice and always took the side of the underdog. He quietly set about doing what he could to make his corner of the world a better place. After retirement from Brandeis he coached kids in math and recent immigrants in English, and he was planning to take a course on teaching English as a second language.

Nisonoff was as honest in his human relationships as he was in his science. To colleagues and students, as well as family and friends, he was loyal and committed. Although he was divorced from Sally in 1978, he continued to care for her on a daily basis. He had a close relationship with his children, Don and Linda, and was a devoted grandfather. He was the best friend of his younger sister, Lorraine. The last decade of his life was immensely enriched by his friendship with Patricia Carella, who shared his love of music and travel.

Al Nisonoff was one of a small number of investigators whose accomplishments span the classical and modern eras

in immunology. His work provided critical insights into the molecular nature of the antibody molecule and the genetic basis for antibody diversity. He approached important biological questions with the rigor of the chemist. His understanding of this complex field was as broad as it was deep. His publications stand as a model of clear thinking and vision.

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