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A Biographical Memoir by

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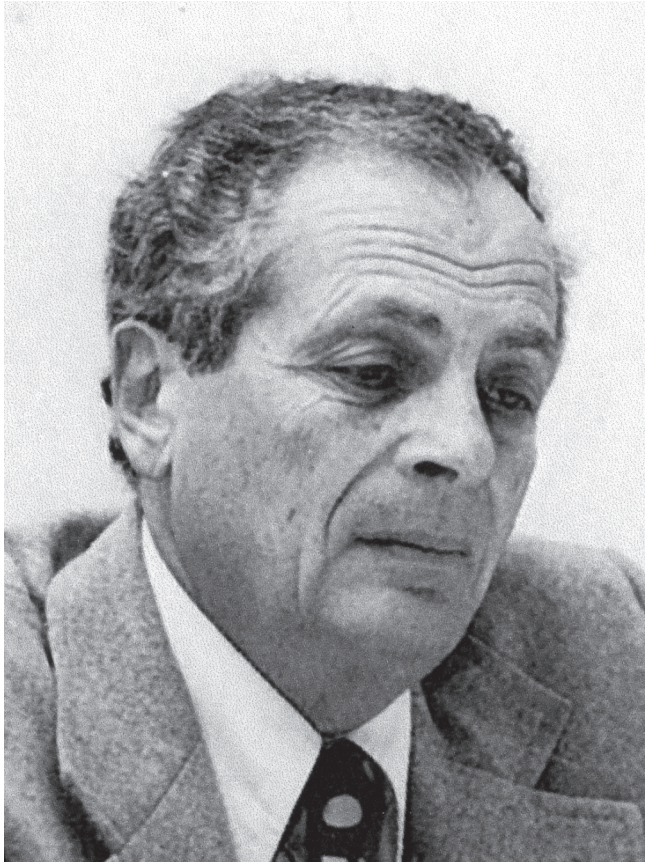

Sidney Udenfriend

Taken from the Annual Report of the Roche Institute of Molecular Biology

SIDNEY UDENFRIEND

April 5, 1918–December 29, 1999

BY HERBERT WEISSBACH AND BERNHARD WITKOP

SIDNEY UDENFRIEND'S PARENTS emigrated to the United States from an Austro-Polish region in central Europe in 1913. They had three children; the oldest was Sidney, who was born in Brooklyn, New York, on April 5, 1918. After attending public schools in Brooklyn Udenfriend entered the City College of New York (CCNY) in 1935. At that time CCNY was the dream for so many of the immigrant parents who wanted their children to obtain a college education. Supported by public funds, with no tuition, CCNY provided that opportunity for those students who could pass the rigid requirements for entrance. The Chemistry Department was well recognized in the field of physiological chemistry (or biochemistry) thanks in large part to Benjamin Harrow, who wrote a widely used textbook.

Harrow had a great influence on Udenfriend, and after graduation in 1939 Udenfriend was set on a career in biochemistry and determined to go to graduate school. In 1940 he was accepted at New York University Graduate School in the Department of Biology working with Kenneth Blanchard. At nights he had a position with the New York City Department of Health directing other graduate students in carrying out Wasserman tests on draftees for the Army. In 1942

he received his M.S. degree and with the country at war, he took a position as a biochemist in the New York University malaria program at Goldwater Memorial Hospital in New York City. James Shannon directed this program, and Udenfriend was placed in a group headed by Bernard B. ("Steve") Brodie. His research involved developing new analytical methods for drugs and studying drug metabolism (1943). The malaria program was considered vital defense research and Udenfriend was deferred from the draft, and obtained valuable research experience in this exciting environment until the end of the war. During this period at Goldwater he married Shirley Reidel. They remained together for 56 years, until his death in 1999, and they had two children, daughter Aliza and son Elliot.

In the fall of 1945 he returned to New York University to complete his graduate studies, initially working with Severo Ochoa in the Department of Biochemistry of the Medical School. Ochoa left the department after one year, and Udenfriend changed mentors and continued his thesis work with Albert Keston. Together they developed the isotope-derivative method for the assay of amino acids and for determining amino terminal residues in proteins (1949). He received his Ph.D. in biochemistry from New York University in 1948 and accepted a position as instructor in Carl Cori's Biochemistry Department at Washington University in St. Louis. Udenfriend could not imagine, as he and his wife Shirley left for St. Louis, that several of the scientists with whom he had interacted at Goldwater Memorial and New York University, including Shannon, Brodie, Julius Axelrod, John Burns, and Ochoa, would cross his path again in the years to come.

The Department of Biochemistry at Washington University, headed by Nobel laureate Carl Cori, was one of the most prestigious biochemistry departments in the country.

Udenfriend applied his isotope-derivative methodology to enzymes that were under investigation in the Cori laboratory. One of his close colleagues during that period was Sid Velick, and their studies resulted in several papers on amino acid analysis and protein N-terminal analysis (1951,1-2).

On April 7, 1949, the *New York Times* informed the public on the appointment of James Augustin Shannon as associate director in charge of research at the National Heart Institute created in June 1948 by an Act of Congress signed by President Truman. This was the beginning of the meteoric rise of the National Institutes of Health (NIH), of which Shannon became director in 1955, from a routine government laboratory to the world's center of biomedical science

Udenfriend received the letter of invitation to join Shannon's expanding research team in 1950 while in St. Louis. His answer when he asked Cori for advice was: "If you join a little known government laboratory, this will be the end of your scientific career!" At that time Udenfriend also had an application pending for an assistant professorship at Columbia University, with little chance of success. So he did not hesitate to ignore Cori's advice and accepted the position of biochemist (at the GS-13 level) in the Laboratory of Chemical Pharmacology under his old boss Brodie in the National Heart Institute, which started in Building 3 on the NIH campus in Bethesda, Maryland. By the early 1950s NIH had attracted a large group of scientists from Goldwater Memorial Hospital, in addition to Shannon and Brodie. At that time NIH was still a fledgling research center, but the scientific talent present in Building 3 in the early 1950s was extraordinary.[1] In Axelrod's words: "Never had such a small group of promising scientists reached such Olympic heights."

In a letter dated June 5, 1950, Shannon informed one of us (B.W.) of the complementarity of current projects at

Harvard University with those of Udenfriend, and so it happened that Udenfriend became a colleague and friend until his passing. In 1953 one of us (H.W.) was recruited by Udenfriend from his alma mater, CCNY, and became his first Ph.D. student, thanks to a graduate program that Udenfriend helped establish between the Brodie laboratory at NIH and the Departments of Biochemistry and Pharmacology at George Washington University. To a young graduate student the quality of science and the excitement and talent that surrounded him in Udenfriend's laboratory and all of Building 3 left a lasting impression never to be equaled.

In this convivial atmosphere at NIH Shannon initiated a weekly interdisciplinary seminar supplemented by more relaxed gatherings of the "Applied Statistics Club," a euphemism for the poker games with high stakes, where Irish Mist was served under the motto "The Irish never missed!"

Just as Shannon never forgot his famous mentor Homer Smith, so Udenfriend acknowledged throughout his scientific lifetime that he stood on the broad shoulders of Shannon. On the occasion of a festive banquet of the Committee for the Weizmann Institute in New York, "godfathers" Udenfriend, Axelrod, and Witkop decided to move the authorities to name the pillared central administrative building, referred to as Building 1, the James Augustine Shannon building. After high-level and congressional deliberative delays a solemn celebration—in the presence of a smiling Shannon—preceded the official christening on January 18, 1983. This was the first and unfortunately the last time that an NIH building was named after a scientist and not a member of Congress.

Of the more whimsical talks on this occasion Hans Stetten compared the Shannon building at NIH to the CNS with numerous afferent and efferent channels, which Shannon successfully controlled in spotting action potentials amidst

much background noise. Like Ben Franklin he looked for helpful temperature-dependent currents to move the large NIH vessel through stormy seas and to avoid unfavorable counter currents the same way as Franklin had advised transatlantic shipping in 1786 (*Transactions of the American Philosophical Society* 2(1786):294-329). Measuring the "temperature" on the "climate of expectancy" in institutes and laboratories and at the same time respecting their integrity and independence was Shannon's style, and therefore, "The style is the man." Hans Stetten later became the first chair of the Scientific Advisory Board to Udenfriend at the Roche Institute of Molecular Biology.

The transition of pharmacology, based on physiological evaluation, to a science based on quantitative analysis using exact colorimetric, fluorescence, or radioactive-isotope methods gave Brodie's laboratory the title " chemical pharmacology" and goes back in part to investigations by Udenfriend with Keston and Velick. Udenfriend always believed, regardless of the project, that the time best spent was working out a rapid and sensitive assay. Here we also have the beginning of research that used isotopically labeled substrates to quantitatively determine enzyme activity, which led to the discovery of the famous "NIH shift," as discussed below.

During the 1950s hydroxylation was a common theme in Udenfriend's research, and it was during that period that he became especially interested in aromatic hydroxylation. His first studies on the enzymatic conversion of phenylalanine to tyrosine were done with Jack Cooper (1952), and this research soon broadened to include studies on tryptophan hydroxylation and the biosynthesis of both norepinephrine and serotonin, and later proline hydroxylation and collagen synthesis. He was intrigued by the discovery of serotonin, which was isolated, identified, and crystallized in 1948 by Maurice Rapport in the laboratory of Irvine Page,

who was then the director of the Research Division of the Cleveland Clinic. These collaborative studies developed into both personal and productive relations between the two groups. The first step in the serotonin biosynthetic pathway studied in detail was the conversion of 5-hydroxytryptophan (5HTP) to serotonin (1953,1; 1954,1) At first it was thought that this enzyme was distinct from the decarboxylase that used dihydroxy-phenylalanine (DOPA) as substrate, but upon purification the enzyme, called aromatic amino acid decarboxylase, was shown to be able to decarboxylate not only 5HTP and DOPA but also tryptophan, tyrosine, and phenylalanine, although to a lesser extent.

By 1953 it became clear that serotonin biosynthesis involved two steps, hydroxylation to 5HTP and decarboxylation to serotonin. By then it was also apparent that serotonin was not only a neurotransmitter but had a role as a vasoconstrictor and potentially other roles because of its high concentration in both platelets and intestinal mucosa. This surge in the central and peripheral importance of serotonin led to extensive basic and clinical investigations in which Udenfriend and his colleagues or disciples, such as Herb Weissbach, Walter Lovenberg, Elwood Titus, and the clinical group headed by Albert Sjoerdsma, were involved. Carcinoid syndrome is just one example of the productive collaboration between the Udenfriend and Sjoerdsma group. These tumors produce large amounts of serotonin that cause the gastrointestinal symptoms and blushing seen in these patients. Weissbach had already developed an assay for 5-hydroxyindole acetic acid (5HIAA), the primary urinary metabolite of serotonin. Thus a simple diagnostic test for the malignant carcinoid syndrome was developed based on the determination of 5HIAA in urine (1955,1). An interesting sidelight to these studies was the observation that Weissbach was routinely running high levels of 5HIAA in

his urine while others in the lab had normal levels. There, of course, was concern that he might have a carcinoid tumor until the high 5HIAA levels were traced to his daily ingestion of bananas that contain high levels of serotonin and other amines. This work was extended to other fruits and vegetables, which brought Udenfriend into contact with nonscientists like the president of the United Fruit Company, which led to an award to Udenfriend and Sjoerdsma sponsored by United Fruit.

The need to localize and assay serotonin was one of the reasons that Robert Bowman, the chief of the Laboratory of Technical Development, helped Sid to design a spectrofluorometer (SPF) with quartz optics that not only extended fluorescence assay into the ultraviolet region but also permitted one to change both the activation and fluorescent wavelengths to achieve increased sensitivity and much higher specificity (1955,2). The initial instrument, put together by Bowman using some parts from an Army and Navy store in Bethesda, took up half a laboratory and because there was no shield to prevent room light from activating the SPF photomultiplier, the room had to be kept dark during the measurements. Using this instrument the sensitivity of the serotonin assay increased by orders of magnitude and it was now possible to assay endogenous serotonin in virtually any tissue (1955,3). This dramatically changed the research efforts and opened up a new dimension in biogenic amine research. The development by the Aminco Company of a small well-designed SPF (called the Amino-Bowman SPF) also made it possible for the scientific community to have access to this new instrument. Numerous assays were developed for all sorts of compounds using the SPF as described in the book Udenfriend first published on fluorescence assay in biology and medicine in 1962, with a second edition in 1969. How this story evolved in 1955 is also described by

Udenfriend in a nostalgic retrospection 40 years later published in *Protein Science* (4[1995]:542-51). In a surprising about-face the mentor-disciple role with Sidney Velick was reversed when the two Sids collaborated on the use of the SPF on novel and previously inaccessible problems such as enzyme-coenzyme complexes or antigen-antibody interactions.

Several Nobel Prize winners have relied on the SPF as an indispensable tool. In collaborative studies Axelrod identified labile metabolites of lysergic acid diethylamide (LSD), mescaline, and norepinephrine. In Axelrod's words,

> The SPF made it possible to measure noradrenaline and serotonin . . . practically. This changed the direction of the whole field of neurobiology. Quantitive studies established the relationship of the level of these transmitters to certain mental illnesses and aided in the development of mental tranquilizer and energizer drugs. Continued studies in this area will yield additional information on the basis for mental illness.

The adage "Transmission is as important as discovery" could be applied to the time that Udenfriend spent as a graduate student with Ochoa in the Department of Biochemistry at NYU Medical School in 1946. Udenfriend became aware that hydroxyproline was uniquely present in collagen from his earlier days at NYU, since Joseph Bunim, a professor at the NYU Medical School, had impressed on him how collagen was intimately involved in the health and disease of connective tissue, in arthritis and other disorders. Bunim and Stetten soon joined NIH at the Institute of Experimental Biology, which was not accepted by Congress as a serious "disease" and so became the National Institute of Arthritis and Metabolic Diseases (NIAMD).

That the hydroxylation of proline does not occur in the free form, but at some step in the formation of collagen was the discovery of Marjorie ("Marnie") Stetten and in-

spired Udenfriend, already involved in hydroxylation reactions, to pinpoint the exact step at which proline was hydroxylated. Udenfriend had the good fortune of having Beverly Peterkofsky join the laboratory at that time. Peterkofsky, a graduate student with Ochoa at NYU, moved to NIH when her husband Alan Peterkofsky accepted a position in the NIAMD. As Udenfriend said of Beverly Peterkofsky, "It was one of the best things that ever happened to me." She finished her graduate studies in Udenfriend's lab, where she obtained a cell-free system from chick embryos (1961) that incorporated *cis*-and *trans*-4-H^3-L-proline into peptide-bound hydroxyproline in a front-side displacement with complete retention of configuration at C-4 (1964). This reaction was comparable to other enzymes, which directly use molecular oxygen in the formation of hydroxylated products. Years later, in 1975, at a Collagen Symposium at the Roche Institute of Molecular Biology, Udenfriend fondly remembered these early events in the collagen saga that was completed by Darwin Prockop, another of Udenfriend's students. A major discovery was the finding that alpha-ketoglutarate was the cofactor of proline hydroxylase and of a totally new class of enzymes. This enabled Prockop to study in detail the nature of the hydroxylase and of the transformation of "protocollagen" into collagen. Carl Piez at NIH then carried out a kinetic study of collagen biosynthesis, and Prockop showed a role for hydroxyproline in stabilizing the triple helix of collagen that Lubert Stryer in his famous textbook *Bio-Chemistry* likened to a Bach fugue. Prockop continued his studies on collagen after leaving the Udenfriend lab and moving to Philadelphia, where he showed that mutations in the genes for collagen, caused osteogenesis imperfecta, or brittle bone disease in children, or dwarfism (chondrodisplasias), not to mention the role of collagen in more common syndromes,

such as osteoporosis and osteoarthritis. These insights round out the clinical observation by Udenfriend and Sjoerdsma of the increased excretion of hydroxyproline in Marfan's syndrome, which goes back to 1958 and even further when we consider Egypt's eighteenth dynasty with Amenopsis and Tutankhamen being possible victims of this disorder.

As early as 1953 Udenfriend and Samuel Bessman published on the hydroxylation of phenylalanine in patients with the genetic disease phenylpyruvic oligophrenia, called phenylketonuria or PKU (1953,2). This work preceded the exhaustive research on phenylalanine hydroxylase by NIH colleague and friend Seymour Kaufman that extended over 20 years. A model system for aromatic hydroxylation published in 1954 with Brodie and Axelrod was intended to throw some light on the mechanism of this oxidation (1954,2). Witkop informed Udenfriend that his system consisting of oxygen, ferrous ion, and ascorbic acid in the presence of ethylenediaminetetracetic acid (EDTA) is a modification of a system that Heinrich Wieland described for the oxidation of formic acid by ferrous ion, dihydroxymaleic acid, an agent forming metal complexes and oxygen, as mentioned in his Silliman memorial lectures (Yale University Press, 1932, p. 86). "Progress is tradition preserved." Curiously enough this rediscovery went into the literature as "Udenfriend's reagent" (Michael B. Smith, *Organic Synthesis*, McGraw-Hill, 2002, p. 296). Here we deal with the name game (Alex Nickon, *Modern Coined Terms and Their Origins*, Pergamon Press, 1987) to which we will return subsequently. There is a similarity of this Wieland-Udenfriend system with the requirements of proline hydroxylase for alpha-ketoglutarate, ferrous ion, ascorbate and oxygen as found in 1966 (1966,1). The years 1966 and 1967 were the time when insight into the mechanism of hydroxylation was obtained because of the availability of a tritiated substrate,

p-H^3-phenylalanine, for phenylalanine hydroxylase, the important enzyme missing in PKU. When the results of the experiment on the fate of the tritium came in, Udenfriend was perplexed, for there was much tyrosine formed but no loss of tritium (1966,2). Questioning the location of the tritium in the substrate, Sid angrily scolded the chemist, "Can't you put the label in the right place?" So John Daly, who felt hurt in his professional competence, ruefully extended the study to p-H^2-phenylalanine, in which NMR clearly showed the correct p-position of the deuterium. Using this as substrate for the enzymatic hydroxylation some deuterium was lost but most was found in the *meta*-position. This was the birth of the happy child christened the "NIH shift," with several proud parents involved in paternity (1967). There even was a twin: Not only did tritium and deuterium slide over to the neighboring *meta*-position but so did halogen (1968). To strengthen the case for an arene-oxide intermediate Emanuel Vogel in Cologne, the pioneer in the arene oxide field, was asked for a sample of the novel, more suitable naphthalene 1,2-oxide, which indeed could be observed when naphthalene is converted to 2-naphthol by the action of liver microsomal hydroxylase[2] (1970).

The hydroxylation of the antipyretic and antirheumatic acetanilide to the more active p-hydroxy-metabolite acetamidine, later sold as Tylenol, was an Axelrod discovery in Brodie's laboratory. Axelrod often mentioned that he missed becoming a millionaire many times over by not patenting this process. When this same transformation was reinvestigated in 1967 with 4-tritioacetanilide, Tylenol was formed in vivo in rats or in vitro in rabbits with rabbit or rat microsomes (1967,2). The migration and retention of tritium ranged between 38 percent and 56 percent. Nonenzymatic hydroxylations of aromatic substrates lead to NIH shifts only with peroxytrifluoroacetic acid, a much stronger oxidant than

the Udenfriend reagent. Of course, tryptophan-5-hydroxylase, the first step in serotonin biosynthesis, was the next enzyme to be tested. The reaction had been demonstrated only in whole cells of *Chromobacterium violaceum*. Using as substrate 5-tritiohydroxytryptophan, 4-tritio-5-hydroxytryptophan was formed with little release of tritium into the medium[3] (1966,3).

Udenfriend always had close contact with Seymour Kety, because they both tried to find the biochemical basis for mental disorders, a fact that led to the first International Symposium on Catecholamines at NIH in October 1958. Udenfriend and Witkop presented there the observation on the conversion of dopamine to 6-hydroxydopamine, which selectively destroys catecholamine-containing nerve terminals and was at one time thought to be a possible endogenous agent involved in mental diseases.

Sid had an unfailing eye for budding talent, and it is not possible to document the large number of successful scientists who passed through his lab or the impact he had on so many others. One such example is the case of Paul Greengard, who came to Udenfriend's laboratory in the mid-1950s to learn assays and some of the procedures being routinely done in the amine field before beginning a position at Ciba-Geigy. Greengard studied the uptake of tyrosine in the rat brain, a beginning that he gratefully remembered when he received the Nobel Prize in 2000 for extending this initial interest in the brain to highly refined receptor studies.

The fact that Marshall Nirenberg, who received the Nobel Prize in 1968, remained at NIH after his initial experiments in the early 1960s that cracked the genetic code was in large part due to Udenfriend's efforts, and the coincidence that Nirenberg's wife, Perola, was Udenfriend's assistant. Weissbach remembers clearly when Udenfriend called a lab

meeting to tell us that there was a chance that Nirenberg would leave NIH unless he had more space to continue his experiments: "We all agreed to cooperate, and soon thereafter Nirenberg's group moved into the space we made available. I benefited greatly from the proximity of the Nirenberg group and within a short period of time was actively engaged in experiments to elucidate how the genetic information was used in the translation process."

Marshall Nirenberg sums up his memories as follows.

My wife, Perola, worked as a technician for Sid Udenfriend for about ten years, from 1958 to 1968. Perola had enormous admiration for Sid and he valued her work greatly. Theirs was probably the best working relationship I have ever seen.

One of the reasons why it was so successful is that Sid would outline a problem to Perola and suggest a possible mode of attack and Perola then would set up the assays and see if it would work. After a month or so when she had some data she would go back to Sid and show him the data or discuss problems she had encountered. So Perola had all of the fun of solving most of the problems she encountered on her own, and Sid could do exploratory research while investing very little of his own time. It was an ideal arrangement for both of them.

One day Perola asked Sid for a few days leave so that she could go with me to visit an academic institution that had offered me a position, and she told him that I probably would accept the position. By the time we returned to Bethesda Sid had worked out a plan to keep me at NIH by offering me some space and support within his laboratory, which would enable me and my colleagues to continue our work. And so I moved to Sid's lab. Years later Sid often enjoyed telling me that the reason that he had offered me the position in his lab was to keep Perola from leaving NIH, and I would counter by saying that he was just plain lucky to have gotten me to go to his lab. In fact, this arrangement proved mutually beneficial because Sid and Perola continued to work with one another, and my colleagues and I were able to finish deciphering the genetic code. Our presence in Sid's lab made it easy for Herb Weissbach to begin working on protein synthesis, since we were experts in the field.

Sid Udenfriend always was bubbling over with enthusiasm and ideas for the projects that he was involved in. He had a superb mind and would have been successful in almost any field of endeavor. He always tried to help me in a fatherly way by giving me the benefit of his own experience.

Sid called me a few weeks before his death to find out how Perola and I were, and tell me about his plans for the future. He especially wanted me to convey his best wishes to Perola. I think that Sid was an outstanding human being as well as an outstanding scientist.

Udenfriend's career was flourishing at NIH and by the early 1960s he was continually being approached about positions in both academia and industry. With only a few exceptions he expressed little interest in leaving the wonderful, stimulating environment in Bethesda. It would take a unique challenge to pull him away from this research Mecca, and in 1967 such an opportunity appeared, due in large part to old friendships. John Burns, a former colleague of Udenfriend's from the Goldwater Memorial period, had moved his laboratory to NIH in 1957. Burns remained there for only a short period and then became vice-president of research at Burroughs Wellcome in 1960. In January 1967 he moved to Hoffmann La Roche as vice-president of research and met Udenfriend at a cocktail party in Bethesda shortly after assuming his post at Roche. Burns was anxious to make innovative changes in Roche research, and Udenfriend suggested that Roche establish a basic science institute as part of the company's research effort. Unlike existing programs at most pharmaceutical companies this institute would not be product driven but function much like the intramural NIH, with the scientists having direct funding, a reasonable time commitment, and freedom to pursue a research project of their own choosing. The benefits to the company would come from the cutting-edge research that would place the company in a unique position to move rapidly into new areas of biology and develop

novel therapeutics. It was becoming clear even by 1967 that the discoveries in molecular biology and molecular genetics that Udenfriend was so aware of because of his association with the Nirenberg laboratory, would be the driving force for the development of new drugs in the decades to come.

From this brief casual discussion at a social gathering arose the concept of the Roche Institute of Molecular Biology (RIMB). Within the short span of four months, thanks to the efforts of Udenfriend, Herb Weissbach (whom Udenfriend had asked to join him), and Burns, the RIMB came into being. By April Burns presented a summary proposal and detailed budget to the Roche Executive Committee. Approval from Nutley and Basel came quickly, thanks in part to Alfred Pletscher, who was head of research in Roche Basel. Pletscher had spent time in the mid-1950s in Udenfriend's laboratory working directly with Weissbach and was supportive of the concept. Indeed, due to the efforts of Pletscher, within two years the Basel Institute of Immunology, the sister institute to the RIMB, was established in Basel near the Roche facilities.

The period between May and July 1967 was a critical time in the history of the Roche Institute. Udenfriend, Weissbach, and others were unsure whether a move to industry, despite the attractiveness of what was being planned, was too big a career risk to take. At that time basic scientists were extremely wary of industry. It was clear that Udenfriend would not make the move without a solid contingent of committed scientists. With Shannon the man and his talent came first and then the mission. In this way he assembled the stellar cast that led NIH to such scientific success in the same way as Udenfriend, after he moved to Roche, had the satisfaction of assembling a similar group. Whether Roche would keep its promise to establish and maintain a basic

research institute for a reasonable period (e.g., a 10-year commitment) was the major question. Finally a meeting was scheduled in June of 1967 with V. D. Mattia, then president of Roche in Nutley. A group of scientists from NIH that Udenfriend wanted to recruit, all with great concern, met with Mattia. By the end of the meeting it was clear that the tide had turned. Although a time period was never put in writing, the scientists came away convinced that the Roche commitment was long-term and most of the scientists at that meeting eventually joined the RIMB. Shortly thereafter the freedom the scientists desired would be clearly stated in a charter signed by Mattia on July 14, 1967. That was the day the Roche Institute of Molecular Biology came into being. The RIMB lasted 28 years and during its existence the commitment that Mattia made to the NIH scientists in 1967 was never broken. Although Mattia passed away before construction of the institute was finished in 1971, succeeding presidents, such as Robert Clark and Irwin Lerner, respected the provisions in the RIMB charter.

Once the charter was in place events moved quickly. Within months Udenfriend obtained commitments from a number of young NIH scientists, including Herb and Arthur Weissbach, Nathan Brot, Sydney Spector, Sidney Pestka, Ronald Kaback, and Aaron Shatkin. Richard Snyder was hired to handle the administrative affairs, and temporary office space was rented in Bethesda. A distinguished Board of Scientific Advisors was established and by the summer of 1968 temporary space was available in Nutley and Udenfriend and scientists in his department set up the first laboratories. In 1971 Udenfriend was elected to the National Academy of Sciences, which gave prestige to both the RIMB and the company. By 1971 construction of the Roche Institute was completed, and the RIMB scientists who had been housed in temporary laboratories throughout Roche and in labora-

tories across the nation and abroad were able to move into the new building. For the first time the institute staff was together under one roof.

Udenfriend was ideally suited to be director of a basic research center serving the pharmaceutical industry. Although the scientists in the institute had free rein, Udenfriend had the unique ability of seeing a practical application to many of the programs. In this way the company always had a direct line to what was happening in the institute and the opportunity to have the technology transferred without interfering with the research philosophy the institute was built on.

Two of the initial members of the RIMB who Udenfriend had brought from NIH made important discoveries early on that were of interest to the parent company. Sydney Spector developed an assay for drugs of abuse, which became a major product of Roche Diagnostics, and Sidney Pestka, whose work on interferon brought Roche into the field of biotechnology, were clear examples of how the concept of a basic research institute within a pharmaceutical company could be successful. Under Udenfriend's leadership the environment at the Roche Institute was conducive to doing good science and the careers of many of the scientists flourished there. Based on the work done at the RIMB, three of the members, Aaron Shatkin, Herb Weissbach, and Ronald Kaback, were elected to the National Academy of Sciences and at one point the RIMB had seven members of the Academy among a staff of less than 30 scientists.

In addition, the RIMB had a training mission. From the initial discussions in 1967 it was clear that the long-term success of the RIMB as a basic research center would depend on being able to attract postdoctoral fellows and graduate students. Udenfriend was determined that this would be the case. The charter clearly stated training as a mission

of the RIMB. At that time, in the late 1960s, universities were reluctant to accept industry scientists (as the institute scientists were viewed) as adjunct faculty and there was a period of great concern for Udenfriend that institute scientists would not have university affiliations and thus not be able to have graduate students. Udenfriend, thanks to old friendships with faculty members at City College, such as Abe Mazur and Mike Fishman, obtained the first appointment from his alma mater, City College. Within a short period a strong relationship was built between the RIMB and Columbia University, thanks to the efforts of Udenfriend and Sol Spiegelman who was the new chair of the Department of Human Genetics at Columbia. Eventually RIMB scientists had appointments at most of the large universities in the New York-New Jersey region. Postdoctoral fellows were anxious to come, and there was no aspect of the RIMB that Udenfriend was more proud of than the fact that through the 28 years the RIMB was in existence more than 1,000 postdoctoral fellows and close to 50 graduate students received their training at the RIMB.

Udenfriend's own research never faltered during the period he was director from 1968 to 1983. He continued his studies on the hydroxylation of proline, tyrosine, and dopamine (1971,1-2; 1972). In addition to a basic interest in the mechanism of these reactions, Udenfriend always considered the in vivo ramifications and attempted to understand how proline hydroxylase was involved in collagen synthesis and how tyrosine hydroxylase and dopamine beta-hydroxylase were involved in the regulation of norepinephrine synthesis. During this period his love and knack of developing assays led to the use of fluorescamine as a sensitive reagent for the assay of amino acids, peptides, and proteins (1973). The development of the fluorescamine assay made it possible to detect small amounts of peptides

and proteins during purification and was especially valuable in Udenfriend's studies on the enkephalins, opioid peptides, as well as the separation and isolation of various species of natural α-interferon by HPLC.

The studies on interferon deserve special attention, for this was the first example of how research in molecular biology proved valuable to the company. The interferon project was initiated at RIMB by Pestka, who felt that this naturally occurring protein might have both antiviral and antitumor activity. In order to clone the gene for this chemokine it was necessary to purify it first from white blood cells and obtain a partial amino acid sequence. The lack of large amounts of cells and the realization that there may be a family of interferons made the task much more difficult. Without the analytical procedures that were available in Udenfriend's laboratory it is doubtful that the isolation of the first natural interferon species would have been achieved so quickly. Once the purification was achieved the sequencing and cloning of an interferon gene was accomplished in Pestka's laboratory. By the early 1980s, in collaboration with scientists at Genentech, α-interferon became the first Roche drug produced by recombinant DNA technology. It served as a prototype for other biotechnology products (e.g., interleukin-2), and it is well accepted that the RIMB was the prime factor in making Roche one of the first, if not the first, large pharmaceutical companies to move into biotechnology. The influence that the RIMB had on the course of Roche research was living proof of Udenfriend's vision of the role of the institute when it was first conceived in 1967.

In 1983 at the age of 65, Udenfriend stepped down as director of the RIMB, and the reins were passed to Herb Weissbach. Udenfriend, of course, was not ready to retire and continued to direct a productive laboratory. His pri-

mary research during the late 1980s and early 1990s centered on alkaline phosphatase and its attachment to the cell membrane by a phosphatidylinositol containing a glycolipid anchor. Udenfriend's studies helped to elucidate the biogenesis of this unique linkage, cleavage, and processing of the anchored proteins.

Like the other scientists at the RIMB, many of them younger additions to the staff, he was looking forward to productive years at the Roche Institute. However, Hoffmann-La Roche, although one of the major large pharmaceutical companies in the world, was facing financial constraints that were initially apparent after the expiration of the Valium patent in the early 1980s. By 1994 major long-term decisions were being made about the future direction of the company research, and to the surprise of the RIMB staff Weissbach, who was director at the time, was informed that the RIMB would be phased out. Weissbach had the unpleasant task of terminating the institute in a manner that was least destructive to the institute staff. For both Weissbach and Udenfriend this was the most difficult period in their long careers. What they had started together almost 30 years ago was coming to an end. Weissbach worked with Roche management to insure that all of the members of the institute would leave with their equipment, as well as some support if they were moving to a university position. It took almost two years for everyone in the institute to be placed.

At times Udenfriend found it difficult to deal with the dismantling of the institute, which had meant so much to him, although he and Weissbach kept in touch during the long negotiations. By December 1995, about a year after the initial announcement of the closing of the RIMB, most of the institute staff had left. Weissbach had decided he would not leave until everyone was placed, and still maintained a functioning laboratory. He would soon leave the

institute building, which was being closed, and move to another location within Roche. Dreadful as the closing of RIMB was for Udenfriend, in December of 1995 he would face a major unexpected challenge that would obscure all other concerns.

Early in that month Udenfriend and his wife, Shirley, had stopped at a pharmacy in Cedar Grove, New Jersey, to pick up a prescription. He had parked facing a brick wall and put the car in reverse as he was preparing to return home. What happened next is still not clear. It appears that when the car was put into drive, it accelerated rapidly and crashed into the brick wall some 30 feet in front of the car. Both Udenfriend and Shirley suffered multiple fractures, and Udenfriend was in a coma for several days after the accident. Although both would survive the accident, in that one split second their lives were irreversibly changed. After months of rehabilitation they both were finally able to return to their home. Weissbach had set aside an office for Udenfriend in his new space at Roche, and Udenfriend would come in about once a week, more to chat with Weissbach than to do science. By the fall of 1996 everyone in the institute had been placed, and Weissbach was planning on closing down his laboratory in December and relocate to a position at Florida Atlantic University. The equipment was being moved on a dreary, damp Saturday in December of 1996, and Weissbach, there alone, was unaware that Udenfriend had made it a point to come in that day, since this was the last day of the RIMB. Weissbach did not have to ask Udenfriend why he had bothered to come. Udenfriend's first words were, " We started this institute together and I wanted to be here when it ended." By noon the two left the building in the freight elevator, through the loading dock. They realized that for the first time in more than 40 years their career paths would diverge.

The reasons for the demise of the Roche Institute of Molecular Biology are still not entirely clear. The end of this world-renowned research center that housed so many outstanding investigators touches at the root of the reasons for research support that perhaps was expressed nowhere better than by Arthur Kornberg (Nobel Prize, 1959).

> The difficulty with research support in our society, I have come to realize, is the failure to understand the nature and importance of basic research. This failure can be seen among members of the lay public, political leaders, physicians, and even scientists themselves. Most people are not prepared for the time-scale of basic research and the need for a critical mass of collective effort. Fragments of knowledge [unwelcome] and unexploited are lost, as were Gregor Mendel's basic genetic discoveries. The vast majority of legislators and some scientific directors cannot accept the seeming irrelevance of basic research. Were there a record of research grants in the Stone Age, it would likely show that major grants were awarded for proposals to build better stone axes and that critics of the time ridiculed a tiny grant to someone fooling around with bronze and iron. People do not realize that when it comes to arguing their case for more funding, scientists who do the basic research are the least articulate, least organized, and least temperamentally equipped to justify what they are doing. In society where selling is so important, where the medium is the message, these handicaps can spell extinction.

Udenfriend was an outstanding researcher and teacher but perhaps his greatest contribution to science was in establishing the Roche Institute of Molecular Biology, and during his tenure as director, in creating one of the outstanding industry-supported biological research institutes in the world. The success of the Roche Institute is not measured only by the papers published or the accomplishments of the individual scientists or the impact on the company. What will be its greatest legacy is the large number of individuals trained at the institute, scattered throughout the world, who remain to this day a living reminder of the Roche Institute. Udenfriend's dream had come true.

When Udenfriend left Roche in December 1996, he already had accepted a position at Drew University as director of the Charles A. Dana Research Institute for Scientists Emeriti (RISE). This institute was specifically established to encourage interaction between some of the top retired scientists from industry in New Jersey and undergraduate students at the university. Udenfriend remained in that post through 1999, and under his leadership the institute expanded its membership and broadened its sphere of activities. Udenfriend obtained great satisfaction from working closely with undergraduate students and his caring for both science and people were apparent to all who knew him at Drew University.

In 1999 Udenfriend made the difficult decision to step down as director of the RISE. He and Shirley had decided to move to Atlanta, where their daughter lived, since it was becoming clear that because of age and the aftereffects of the accident, they both needed help to carry on many of their daily activities. The move south was made in 1999, but soon after they were settled Udenfriend was showing symptoms of coronary artery blockage. In the early winter of 1999 he entered the hospital for a bypass operation, which appeared to be successful; however, during recovery he apparently suffered a massive stroke and remained in a coma for several days until his death on December 29, 1999. The funeral was held on December 31, and because of the time factor and location, only about 20 people, mostly his close relatives, attended the graveside service. Weissbach was able to fly up from Florida and was the only scientific colleague from the past to be present.

Weissbach planned on having a memorial event in Udenfriend's honor for the many scientists whose lives Udenfriend touched. Working with Ashley Carter, the new director of the RISE and Barbara Petrack a RISE member,

a half-day symposium was held on May 25, 2000, on the Drew University campus. A scientific lecture was presented by Greengard, and the list of scientific colleagues who made short remarks, in addition to the organizers, included Witkop, Burns, Nirenberg, Axelrod, Spector, Arthur Weissbach, Sjoerdsma, Ron Kuntzman, and Fishman.

Udenfriend leaves a scientific legacy that includes close to 500 publications and major contributions to the fields of analytical biochemistry, fluorescence, hydroxylation reactions, serotonin and norepinephrine biosynthesis and metabolism, collagen biochemistry, encephalins, amino acid transport, and protein anchoring to membranes. Although research and not formal teaching was the focus of his career he trained dozens of postdoctoral fellows; through his university appointments at George Washington University, City College, and Columbia University, among others, he trained a large number of graduate students. His role in establishing the Roche Institute was a major accomplishment, but what will be missed most is the enthusiasm and love of science that were an integral part of his being.

Sid Udenfriend is gone but not forgotten.

NOTES

1. Included in this list are Nobel laureates Chris Anfinsen, Julius Axelrod, and Arthur Kornberg. Several scientists from that early permanent staff in Building 3 later were members of the National Academy of Sciences: Bruce Ames, Robert Berliner, Donald Fredrickson, Leon Heppel, Bernard Horecker, Earl and Theresa Stadtman, Herbert Weissbach, Bernhard Witkop, and James Wyngaarden. Fredrickson and Wyngaarden eventually became directors of NIH. Other outstanding postdoctoral fellows and visiting scientists who worked in Building 3 at that time included Paul Stumpf, Horace Barker, Gerard Hurwitz, Paul Marks, and Arthur Weissbach. The authors realize that this is a partial list and apologize to the many talented scientists who worked in Building 3 but have not been mentioned.

2. The First Symposium on Arene Oxides in Biochemistry and Metabolism (*Science* 178[1972]:779-81) was held at Roche in April 1972 with Udenfriend presiding and pointing out that as early as 1947, E. Boyland, who was present, had postulated arene oxides as reactive intermediates in the metabolism of polycylic aromatic substrates, an immense area of research for the carcinogenic effects of tobacco smoke and benzopyrene keeping investigators, such as Harry Gelboin (NIH), Allan Conney, (Roche), Don Jerina, (NIH), Charles Heidelberger (University of Wisconsin), and many others busy for years.

3. A tryptophan research meeting on a regular international basis was eventually organized in 1971, mainly as a result in the growing interest in the role of serotonin in depression and moods and the wider consequences for neurochemistry, psychiatry, cardiovascular studies, and more recently immunobiology and neuroimmunobiology. The acronym for these biannual symposia is ISTRY, or International Study Group for Tryptophan Research.

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