NATIONAL ACADEMY OF SCIENCES

JULIUS AXELROD
1912–2004

A Biographical Memoir by

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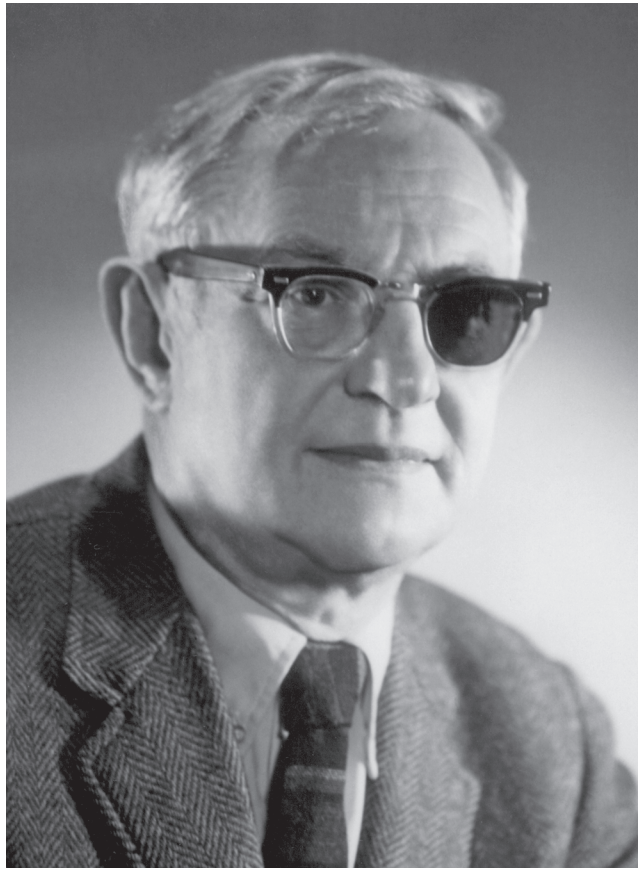
Biographical Memoirs, VOLUME 87

PUBLISHED 2005 BY
THE NATIONAL ACADEMIES PRESS
WASHINGTON, D.C.



Julius Axelrod

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May 30, 1912–29 December 29, 2004

BY SOLOMON H. SNYDER

ON THE MORNING OF Wednesday, December 29, 2004, I was visiting the offices of the *Proceedings of the National Academy of Sciences* in Washington, D.C., handling some editorial chores and staring repeatedly at my watch. Whenever in Washington for a morning meeting, I would try to get away early to visit either my father, who lives in nearby Rockville, Maryland, or my mentor Julie Axelrod, who lived only a few miles away from my father's home. I had decided that day to call Julie for a lunch date when my cell phone rang with a message from my secretary that Julie had died early that morning. He had evidently arisen from bed and collapsed of a heart attack. Till the day of his death, Julie had been alert, visiting his office at the National Institutes of Health several times a week to keep up with the literature and chat with colleagues. He had even flown to Wisconsin to visit his grandchildren just a week prior to his death. In keeping with Jewish tradition, Julie's funeral was held two days following his death. In keeping with Julie's own resistance to fuss and religious dogma, no rabbi was present nor were there any eulogies. Instead, a few longtime friends, including a high school classmate, provided warm reminiscences.

Simplicity and absence of pomp epitomized the life and scientific style of Julius Axelrod, arguably the greatest molecular pharmacologist of the modern era of drug research. His contributions were recognized in 1970 by the award of the Nobel Prize in physiology or medicine. Yet, Julie's scientific success story was most improbable for an individual who spent a major portion of his professional life as a technician without a Ph.D.

Julie's parents, Isadore and Molly, immigrated from Polish Galicia to the United States, where they met and resided in the lower East Side of Manhattan. Julie was born in a coldwater flat at 415 East Houston Street. His father, a basket maker, sold to grocers in lower Manhattan from a horse and wagon. Saturdays were a special treat for Julie, who could accompany his father and get a chance to drive. Though his family was not religious, Julie's parents were part of the East European Jewish immigrant culture so that Julie spoke Yiddish and attended religious school every day after emerging in late afternoon from a public school building that had been built during the Civil War era. His high school, Seward Park, subsequently became well known for its show business graduates, Tony Curtis, Walter Matthau, and Zero Mostel, but not for any intellectual tradition. Julie long maintained that his "real education" came from voracious reading of volumes from nearby Hamilton Fish Park Library.

Julie began college at New York University but ran out of money and transferred in 1930 to the City College of New York, which was tuition-free. Many poor New York citizens similarly attended CCNY, with seven Nobel laureates emerging in the middle to late twentieth century. Traveling an hour each way to college on the subway and maintaining a time-consuming job to support himself, Julie was not an outstanding student. Upon graduation, he applied to sev-

eral medical schools but was not accepted by any, in part because of the widespread Jewish quotas of those days.

When he graduated in 1933, the United States was in the midst of the Great Depression, and Julie was lucky to obtain a position in a laboratory at New York University paying $25 a month. In 1935, when the laboratory lost its funding, he obtained a position in the Laboratory of Industrial Hygiene, a nonprofit unit set up by the New York Health Department to evaluate vitamin supplements. Julie remained employed by that laboratory from 1935 to 1946. At the same time he enrolled in night courses at New York University, from which he obtained a master's degree in 1942.

RESEARCH WITH BERNARD BRODIE

In early 1946 Julie's laboratory was approached by the Institute for the Study of Analgesic and Sedative Drugs to help determine why the major nonaspirin analgesics, acetanilide and phenacetin, caused methemoglobinemia. In this condition toxic chemicals oxidize the ferrous iron of hemoglobin to the ferric form that cannot carry oxygen. It was recommended to Julie that he seek advice from Dr. Bernard Brodie, then at the Goldwater Memorial Hospital, a New York University division, who was an authority on drug metabolism. In their first meeting in February 1946, Julie and Brodie spoke for several hours about strategies to solve the problem, and Julie was invited to do some of the experiments in Brodie's laboratory. At that time no one knew anything about the metabolism of acetanilide, but an examination of its structure suggested that it might be converted to the dye aniline, already known to elicit methemoglobinemia. Julie developed a simple, sensitive, and specific assay for aniline in urine and plasma. Such elegant methodology, a hallmark of all of Julie's subsequent research,

was born at that time. A direct relationship between blood levels of aniline and methemoglobin was soon evident.

As negligible levels of acetanilide were detected in the urine, it seemed likely that the drug was metabolized to other substances. Within a few weeks Julie discovered that the major metabolic product of acetanilide is N-acetyl-p-aminophenol. He showed that this substance has analgesic activity and does not cause methemoglobinemia. Julie's first publication thus concluded, "the results are compatible with the assumption that acetanilide exerts its action mainly through N-acetyl-p-aminophenol. The latter compound administered orally was not attended by the formation of methemoglobinemia. It is possible therefore that it might have distinct advantage over acetanilide as an analgesic." Soon thereafter Julie and Brodie applied similar methodology to evaluate the metabolism of phenacetin, one of the active ingredients in what for years was the most popular headache remedy in the United States: APC (aspirin-phenacetin-caffeine). Phenacetin was also converted to N-acetyl-p-aminophenol as its active ingredient with p-phenetidine being responsible for methemoglobinemia.

These studies comprised Julie's first scientific publications, all in the *Journal of Pharmacology and Experimental Therapeutics.* They were soon recognized as among the most elegant and important drug metabolism studies of the modern era. N-acetyl-p-aminophenol, better known as acetaminophen, was subsequently marketed by the McNeil Drug Company as Tylenol. Neither Brodie nor Axelrod received any royalties, as it never occurred to either of the two to patent their discovery.

Julie remained with Brodie and participated in numerous drug metabolism studies. When James Shannon, director of the Goldwater Memorial Laboratories, was chosen in 1949 to head the recently established National Heart Institute in

Bethesda, Maryland, Brodie brought Julie with him to the NIH along with a number of talented Goldwater staff: Sidney Udenfriend, Thomas Kennedy, Robert Bowman, and Robert Berliner. In the early 1950s at the National Heart Institute, Julie and Brodie elucidated the metabolism of a number of drugs, including pioneering investigations of caffeine disposition. Then, working on his own, Julie began studying the metabolism of sympathomimetic amines, such as amphetamine and ephedrine. He discovered a variety of metabolic pathways, including hydroxylation, demethylation, deamination, and conjugation. These studies led to a major breakthrough, Julie's discovery of the liver's microsomal drug-metabolizing enzymes.

Julie was so impressed with the complete metabolism of amphetamines in animals that he decided to seek enzymes that might degrade the drug. Lacking expertise in this area, he obtained the assistance of Gordon Tompkins and in early 1953 was able to demonstrate that in liver homogenates, amphetamine could be degraded but only if cofactors such as NAD, NADP, and ATP were added. In assessing the role of various subcellular fractions, Julie noted that enzyme activity required a combination of the microsomal fraction (obtained when one centrifuges supernatant fractions of mitochondria at 100,000 times gravity) and cytoplasmic preparations. By selectively heating subcellular fractions, he soon discovered that the enzyme was located in a microsomal fraction and that the cytoplasm provided a critical cofactor. With the assistance of Bernard Horecker, Julie discovered the cofactor to be NADPH. Though nominally only a technician, Julie was able to publish these papers as sole author. Subsequently other workers in the Brodie group showed that the drug-metabolizing system, now known as the cytochrome-P450 mono-oxygenases, metabolizes a wide range of drugs.

CATECHOLAMINE METABOLISM

Julie was more than 40 years old when he discovered the drug-metabolizing enzyme system and still did not have a Ph.D. His friends urged him to go to graduate school, but the logistics of supporting a wife and two young sons seemed to preclude further educational endeavors. Finally, Julie spoke with Paul K. Smith, chair of pharmacology at George Washington University, who agreed that Julie's already published papers could constitute his thesis. Since he had a master's degree already, course requirements were relatively minor. Hence, Julie was able to devote only a single year to graduate school, taking courses and preparing for the comprehensive examinations. In 1955, at 42 years old, he received his Ph.D.

About this time Seymour Kety, the scientific director of the National Institute of Mental Health, set up the Laboratory of Clinical Science under the neurophysiologist-psychiatrist Edward Evarts with a mandate to set up multiple sections with a long-term target to understand schizophrenia. Evarts offered Julie the opportunity to head the Section on Pharmacology, which initially consisted only of Julie himself.

In 1956 Kety presented to the NIMH staff a highly publicized and provocative publication by the Canadian psychiatrists Abram Hoffer and Humphrey Osmond purportedly showing that epinephrine, the hormone of the adrenal medulla, was transformed in the blood of schizophrenics but not of normals to an oxidized, pink-colored substance called adrenochrome. Julie attempted to identify an enzyme that might transform epinephrine into adrenochrome but was unsuccessful. Then, in April 1957, he noted an abstract in *Federation Proceedings* (Federation of American Societies for Experimental Biology) by Marvin Armstrong and Armand McMillan reporting in the urine of patients with

pheochromocytomas, catecholamine-secreting tumors, a new metabolic product of norepinephrine or epinephrine, 3-methoxy-4-hydroxymandelic acid, also called vanillymandelic acid (VMA). In VMA a methyl group had been added to one of the two hydroxyl constituents of the catechol ring. Julie wondered whether he might be able to find an enzyme that carries out this process. Biological methylation had only recently been discovered by Giulio Cantoni, an NIMH scientist, to be mediated via a single universal methyl donor, S-adenosylmethionine (SAM). As SAM was precious, Julie in his initial experiments made do with adding to liver extracts the amino acid methionine and ATP, which should generate SAM, and observed a rapid disappearance of added epinephrine. When he finally obtained a sample of SAM, it worked like a dream.

A number of Julie's important advances in catecholamine metabolism depended heavily on identification of key metabolic products. The collaborative atmosphere of the NIH provided Julie with the assistance that facilitated many of its discoveries. The distinguished organic chemist Bernhard Witkop and his associate Siro Senoh provided the chemical synthetic efforts enabling Julie to show that his enzyme did indeed methylate epinephrine and norepinephrine to form, respectively metanephrine and normetanephrine. With Irwin Kopin he elucidated the relative roles of COMT and monoamine oxidase in human catecholamine disposition. Another member of Witkop's group who attained great distinction in his own right, John Daly, was also a key collaborator. Julie dubbed the enzyme catechol-O-methyltransferase (COMT), because it was clearly capable of adding methyl groups to all catechols, not just catecholamines.

The discovery of COMT revolutionized research into catecholamine metabolism. It was already known that catecholamines—such as dopamine, epinephrine, or norepi-

nephrine—were metabolized by monoamine oxidase. Julie rapidly established how the two metabolic pathways interface, resulting in VMA, the final common product identified by Armstrong and McMillan. VMA measurements in the urine became the standard method for diagnosing pheochromocytomas, epinephrine-secreting adrenal tumors that cause hypertension.

With the availability of SAM radiolabeled in the methyl group, Julie proceeded to identify a variety of important methylating enzymes, such as the enzyme that methylates and inactivates histamine and the enzyme that converts norepinephrine to epinephrine in the adrenal gland and elsewhere. One of the most fascinating of these discoveries is worth recounting, because it illustrates Julie's approach to the discovery process. Eager to seek new methylating enzymes, Julie incubated offbeat tissues with radiolabeled SAM, extracted the products into an organic solvent that he could readily evaporate to dryness, and then conducted paper chromatography to isolate and identify the radiolabeled methylated product. In the case of the pituitary gland, he found an enzymatic activity whose product was volatile, because whenever he would evaporate the organic solvent to a small volume for chromatography, the radiolabel vanished. His collaborator John Daly added a derivatizing agent to stabilize the product and discovered that Julie had found an enzyme that methylates water to form methanol. Subsequently other investigators showed that the enzyme, renamed protein carboxylmethyl transferase, methylates proteins on the carboxyl groups of glutamyl and aspartyl residues, an important regulatory step. The methylated carboxyl is a labile ester that undergoes hydrolysis with water giving rise to methanol.

CATECHOLAMINE UPTAKE

In 1970 Julie shared the Nobel Prize in physiology or medicine with Ulf von Euler and Sir Bernard Katz "for their discoveries concerning the humoral transmitters in the nerve terminals and the mechanism for their storage, release and inactivation." Julie's portion of the prize clearly was for his discovery that the synaptic actions of norepinephrine are terminated by reuptake into the nerve ending that had released it. This mechanism for neurotransmitter inactivation is now regarded as the most frequent means for terminating synaptic transmission. At the time that Julie began this work it was thought that neurotransmitters were inactivated by metabolic alterations as was well known for the first characterized neurotransmitter acetylcholine, which is inactivated by the enzyme acetylcholinesterase. Enzymatic degradation of norepinephrine by COMT or monoamine oxidase was assumed to serve as the inactivating mechanism, but inhibiting these enzymes did not terminate the effects of injected epinephrine or norepinephrine.

Julie was not initially investigating those questions. Rather, Seymour Kety was continuing his efforts to examine the validity of the Hoffer-Osmond hypothesis that schizophrenics convert epinephrine to adrenochrome. He obtained custom-prepared tritium-labeled preparations of epinephrine and norepinephrine and provided samples to Julie to do with what he wished. Julie decided to investigate catecholamine disposition simply by injecting these substances into animals. With Hans Weil-Malherbe he administered [^{3}H]epinephrine to cats and found it concentrating and persisting in sympathetically enriched tissues such as the heart, spleen, salivary, and adrenal glands. With Gordon Whitby, he obtained similar findings in rats with [^{3}H]norepinephrine. Particularly crucial experiments were

carried out with a visiting scientist, George Hertting, who lesioned the sympathetic nerves by unilaterally removing the superior cervical ganglion of a cat and observed a loss of norepinephrine accumulation into the salivary gland and eye muscles of the lesioned side. Based on these data, Julie postulated that uptake into the sympathetic nerves accounted for inactivation of norepinephrine.

Julie soon explored actions of drugs and discovered that cocaine, amphetamine, and other sympathomimetic amines blocked this uptake process. Inhibition of norepinephrine uptake could explain the ability of drugs to potentiate the effects of sympathetic nerve stimulation, providing compelling evidence that reuptake is the physiologic mode for neurotransmitter inactivation. Julie also found that the major tricyclic antidepressants inhibited the uptake of norepinephrine into the heart. The blood brain barrier precluded studies of the brain in initial experiments. When Jacques Glowinski and Leslie Iversen joined Julie as postdoctoral fellows, they used a technique developed by Glowinski for introducing [^{3}H]norepinephrine directly into the brain via injections into the lateral ventricle, dissecting various brain regions and monitoring accumulated norepinephrine. Glowinski showed that the ability of a variety of antidepressants to inhibit norepinephrine accumulation in the brain paralleled their antidepressant efficacy. It was proposed that this means of potentiating the effects of norepinephrine is responsible for antidepressant actions. We now know that inhibition of the uptake of serotonin as well as norepinephrine participates in antidepressant efficacy. To this day, inhibition of amine uptake remains the accepted mechanism of action of tricyclic antidepressants and has driven the development of several generations of novel antidepressants during the succeeding 40 years.

The above description highlights only a few of Julie's

scientific contributions. In the interest of brevity I haven't mentioned his research on the pineal gland, which opened up an entire new field. When the pineal-gland hormone melatonin was discovered by Aaron Lerner to be 5-methoxy-N-acetylserotonin, Julie identified the methylating enzyme that generates melatonin. With Richard Wurtman he then showed that melatonin is the active principle of the pineal gland, which mediates organismic influences of light, presaging a vast body of work establishing melatonin as a regulator of sleep and circadian rhythms.

JULIE'S STYLE

Julie's extraordinary creativity as a researcher was virtually unparalleled. Thus, understanding Julie's "magic" might provide insight into what makes for innovative discovery in science. All his students echo a few common themes. One was Julie's skill at getting to the heart of complex problems. He had less formal training in complex areas than most scientists, and his eyes would glaze over when he was presented with convoluted intellectual schemes involving multiple equations. He eschewed statistics and often said, "If the difference between the two groups is so small that you need a statistical test to prove its significance, then it might not be a very important difference." In speaking to the scientific historian Robert Kanigel, Julie commented, "I don't like to do complex experiments. I'm not a complicated person. . . . Picasso makes a single line, but it takes a lot of time and thought."

Similarly, Julie did not like complicated theoretical schemes in which one predicts the results of an elaborate series of experimental studies. Rather, he felt that the data should tell you where to go next. "Just follow your nose." He was irritated by scientists who spend weeks or months in the library developing a project, remarking to Kanigel, "You

don't learn anything by thinking about what to do . . . just by going into the lab and doing it."

In the biomedical sciences researchers work through their students so that mentoring is key to discovery. Julie was an ideal mentor, teaching by gradations of positive reinforcement. Even if a student had a result that was only modestly interesting, Julie would provide encouragement and brainstorm with the student trying to find gold in the dross. I remember many occasions when I felt my results were not even worth showing to him, but Julie was able to find promising hints in what seemed to be drab data.

Julie also conveyed an infectious exhilaration in the discovery process. Research for Julie was genuine fun, and almost all his students came away from time in his lab with the same attitude. I remember vividly a lecture he was giving soon after receiving the Nobel Prize. He opened the talk by saying, "It seems that all these speaking invitations are a conspiracy to get me out of the lab. I find it hard to imagine that I am paid a good salary for doing things that are so much fun that I'd work in the lab for no pay." When a critical experiment was completed, he was often so excited that standing in front of the liquid scintillation counter, which records radioactivity levels, he almost jumped up and down using body English to accelerate or decelerate the accumulation of radioactive counts, depending on what result he was seeking.

Despite his skills as a mentor Julie always saw himself as a bench scientist. Because of his own many years as a technician, Julie felt uncomfortable if he was not himself carrying out an experiment. Each day he arrived in the laboratory at 8:15 a.m. and by 8:30 was incubating a set of test tubes. He generally completed his experiment by noon and then joined one of the "boys" for lunch. He religiously spent an hour each day after lunch in the library keeping up with

current literature and then devoted the remainder of the afternoon to working on manuscripts together with students. His style of manuscript preparation differed markedly from most senior scientists. Instead of the student preparing a draft that the mentor would then review, Julie and the student would sit together and review the data and its significance, whereupon Julie would write out the manuscript by hand, incorporating the student's input. Julie was a superb scientific writer, presenting his story in simple declarative sentences and providing no more discussion than was warranted by the data. He hated elaborate pretense and so was irritated when scientists wrote that the rats were "sacrificed." He admonished, "There aren't any altars in our laboratory; we just kill the rats."

Julie was a quiet, self-effacing, mild-mannered individual who rarely was angered. Though he and his wife did not socialize much with students, he was always empathic, inquiring about events in our lives and immensely helpful in our career decisions.

Julie married Sally Taub in 1938. She had grown up in the same lower East Side environment as Julie and worked for many years as a second grade school teacher. She died in 1992 from complications of diabetes. Their elder son, Paul, is a professor of anthropology at Rippon College in Wisconsin, where he lives with his wife, Michelle, and their children, Sonya and Sander. Their younger son, Fred, is a forestry consultant in Wisconsin; he and his wife, Johanna, had two children, Julia a student at Bethel College in St. Paul, Minnesota, and Nathan, who died in 2004. Julie was immensely devoted to his children and grandchildren and frequently traveled to Wisconsin to visit them.

Julie's humility is best conveyed by his own statement: "I soon learned that it did not require a great brain to do original research. One must be highly motivated, exercise

good judgment, have intelligence, imagination, determination and a little luck." Then Julie got to the heart of the matter, commenting, "One of the most important qualities in doing research, I found, was to ask the right questions at the right time. I learned that it takes the same effort to work on an important problem as on a pedestrian or trivial one. When opportunities came, I made the right choices."

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