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Stefan Emeis

AXELROD, JULIUS

(*b.* New York, New York, 30 May 1912, *d.* Bethesda, Maryland, 29 December 2004), *pharmacology, neurochemistry neurotransmitters, metabolism.*

Axelrod made fundamental contributions to understanding the mechanisms of chemical neurotransmission and drug metabolism. His discoveries contributed directly to the design of drugs to treat psychiatric disorders, to control physiological systems, and to relieve pain. He won the Nobel Prize for Physiology or Medicine in 1970 for his work on the reuptake and deactivation of neurotransmitters such as norepinephrine and serotonin.

Early Education and Training. Julius ("Julie") Axelrod was born and raised in a poor Jewish neighborhood on the Lower East Side of Manhattan. His parents, Isadore and Molly, immigrated to the United States from Poland. His father was a basket maker, and his mother worked in the home. Neither was functionally literate in English.

Upon graduation from Seward Park High School, Axelrod studied at New York University (NYU). He exhausted his family's savings after one year and transferred to the tuition-free College of the City of New York. His grades in chemistry were poor, especially in qualitative analysis, the area in which he would later build his reputation. He graduated in 1933, at the height of the Great Depression, with a BS in biology. In the same year, he lost sight in his left eye in a laboratory accident. As a result, he was deferred from the World War II draft and was free to pursue his dream of becoming a doctor.

Axelrod's applications to medical school were rejected, however, and he was hired as a laboratory technician at the Harriman Research Institute at NYU. He worked for K. G. Falk, preparing buffer solutions and assisting with research on enzymes in malignant tumors. When funding for the laboratory ran out in 1935, Axelrod turned down work with the U.S. Postal Service for a less lucrative position working for George B. Wallace, a retired pharmacology professor, in the Laboratory of Industrial Hygiene at the New York City Department of Health. Axelrod's job was to assay the levels of vitamins in fortified food products. He had to learn the available techniques for measuring vitamins and to modify them for application to food products. This work gave him early experience as an experimentalist. The lab also exposed him to the *Journal of Biological Chemistry*.

While working with Wallace, Axelrod married Sally Taub, who had a degree in chemistry from Hunter College. With the birth of their sons Paul (1946) and Alfred (1949), Taub quit her job in an insurance agency to work in the home. She later earned a teaching certificate from the University of Maryland and became an elementary school teacher. Until 1949, Axelrod and his family lived within blocks of his parents and his two sisters (Gertrude and Pearl) in Brooklyn, New York.

During this period Axelrod studied chemistry in postgraduate night courses at NYU. He wrote a thesis, "The Ester-Hydrolyzing Actions of the Tissues of Polyneuritic, Normal, 'Cured,' and Thiamin-Fed Rats." Rats deprived of thiamin (vitamin B1) develop polyneuritis, a general swelling of nerve tissues, which is one of the symptoms of beriberi. As part of an investigation of the mechanism producing this symptom, Axelrod ground the organs of rats, exposed them to different kinds of esters (an organic compound), and measured the ability of these tissues to hydrolyze the esters. For this research, he received his Master's of Science degree in chemistry in 1942. He continued to work with Wallace until he was thirty-four years old.

Bernard Brodie and Non-Aspirin Pain-Relievers. In 1946, Wallace gave Axelrod a funded project to determine why protracted use of non-aspirin pain relievers (such as acetanilide, an ingredient in the then-popular pain reliever Bromoseltzer) produce an abundance of methemoglobin, the oxidized form of hemoglobin, which fails to bind oxygen. Bernard B. Brodie, a pharmacologist, invited Axelrod to perform these studies at Goldwater Memorial Hospital (Welfare Island, later Roosevelt Island, New York). Brodie was part of a research group, headed by James A. Shannon, that was charged in part with running clinical trials on anti-malarial vaccines during the Japanese embargo on quinine during World War II. Shannon recruited a number of gifted scientists for this purpose, including Robert Berliner, Robert Bowman, Thomas Kennedy, and Sidney Udenfriend, all of whom made significant contributions to biochemistry. As Axelrod later wrote, "It was in this atmosphere that, in a period of a few years, I became a researcher" (1988, p. 4).

Brodie taught Axelrod that the body metabolizes drugs and that metabolites can have both beneficial and harmful consequences. It was known at the time that the acetanilide in Bromoseltzer can be metabolized into

N-acetyl-p-aminophenol and analine, and it was known that analine can produce methemoglobin. It was not known whether acetanilide metabolizes into analine in the human body. Axelrod developed a sensitive assay for analine levels in blood and urine. He and Brodie showed that analine levels rise after doses of acetanilide and that analine and methemoglobin levels are correlated. This finding led them to suggest that N-acetyl-p-aminophenol, later commonly known as acetaminophen, could replace acetanilide as a pain reliever without this potentially deadly side effect (Brodie and Axelrod, 1948). Amid worries in the 1970s that aspirin causes gastric ulcers, acetaminophen (marketed as Tylenol) became, and remained in the early 2000s, one of the best-selling pain relievers in history.

Metabolism of the Sympathomimetic Amines. In 1949, Shannon was appointed director of intramural research at the National Heart Institute (NHI), part of the National Institutes of Health (NIH) in Bethesda, Maryland. Axelrod, Brodie, and many of the other Goldwater scientists soon followed. Initially, Axelrod worked with Brodie in the Laboratory of Chemical Pharmacology, investigating analgesics and the effects of ascorbic acid (vitamin C) on drug metabolism. Again, Axelrod found himself in a stimulating environment: "Among the scientists working in Building 3 in the 1950's, more than half became members of the National Academy of Science, five became Nobel laureates, and three were appointed directors of NIH" (2003, p. 3).

By 1952, Axelrod was doing independent research in Brodie's lab. His first project was to describe the metabolism of caffeine. This work soon broadened to include the metabolism of a class of drugs that George Barker and Henry Hallett Dale named the "sympathomimetic" amines. These drugs, which include amphetamine, ephedrine, and methamphetamine, produce effects similar to the activation of the sympathetic nervous system. The sympathetic nervous system can be activated to produce a set of coordinated responses that enable the body to deal with stresses and threats. For example, activation of the sympathetic nervous system increases blood pressure and heart rate, accelerates breathing, constricts the arteries, and dilates the pupils. Axelrod found that the sympathomimetic amines are metabolized by a variety of pathways, including conjugation (by joining with other molecules), deamination (the removal of an amino group, NH_2), and hydroxylation (the addition of a radical hydroxyl group, OH). He also showed that the precise metabolic pathways vary across species (1953; 1954a).

Axelrod's work on the sympathomimetic amines led him to discover and localize a new class of liver enzymes. To study amine metabolism *in vitro*, Axelrod exposed livers from different species to sympathomimetic amines, measured the rate at which the drugs are metabolized, determined the metabolic end products, and inferred the pathways by which those end products are produced. His study of amphetamine metabolism was the centerpiece of this project (1954b; 1955a). When Axelrod applied amphetamine to sliced or homogenized rabbit liver, the drug was rapidly metabolized. He found that he could speed up the metabolism by adding triphosphopyridine nucleotide (TPN, later known as NADP, for nicotinamide adenine dinucleotide phosphate), which was already known to be a coenzyme in many biochemical pathways. Axelrod concluded that the metabolic pathway involves TPN as a coenzyme.

To determine which parts of liver cells metabolize the amphetamine, he centrifuged the cells, separating them into different cell fractions containing different cellular components. He then applied amphetamine and TPN to the different cell fractions. None of them metabolized the drug by itself. When he added amphetamine and TPN to

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Julius Axelrod. *Julius Axelrod in the laboratory.*

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a mixture of the cytosolic fraction (the intracellular fluid) and the fraction containing microsomes, the drug rapidly disappeared, leaving ammonia and phenylacetone as end products. This reaction led him to conclude that in rabbit liver, amphetamine is deaminated by an oxidative enzyme that uses TPN as a coenzyme.

Axelrod did not know whether the necessary enzyme was in the cytosol or in the microsomes. To decide, he heated the cell fractions to denature the heat-labile enzymes. When he heated the cytosolic fraction to 55°C and then added unheated microsomes, amphetamine, and TPN, the amphetamine was metabolized. When he heated the microsomal fraction to 55°C and added unheated cytosol, amphetamine, and TPN, the amphetamine was not metabolized. The crucial enzyme, he concluded, was in the microsomes, but the cytosol must contain a necessary coenzyme; amphetamine is not metabolized by the microsomal fraction and TPN alone.

To identify the coenzyme, Axelrod exposed the microsomal fraction to amphetamine, TPN, and to three different substrates (glucose 6-phosphate, isocitric acid, and phosphogluconate). The single quality that these substrates have in common is that they all reduce TPN to TPNH (or reduced TPN, later known as NADPH). The amphetamine disappeared. Perhaps, then, the coenzyme in the cytosol is necessary to reduce TPN to TPNH. When he added TPNH to the microsomal fraction alone in the presence of oxygen, the amphetamine was again metabolized, convincing Axelrod that he had discovered an enzyme in rabbit liver that deaminates amphetamine in the presence of oxygen and TPNH. The structure of amphetamine, and the necessity of TPNH for its metabolism, suggested that this was a new kind of enzyme. Similar enzymes were subsequently discovered for the metabolism of ephedrine and other drugs as well. These findings amounted to the discovery of a new class of liver enzymes, later known as cytochrome P450 enzymes. These enzymes, which are found in all lineages of life, metabolize most organic chemicals (such as bilirubin) and many drugs and pollutants. They are also involved in the synthesis, activation, and inactivation of a number of regulatory molecules.

Axelrod's Conversion to Neurochemistry. The work on sympathomimetic amines gave rise to an authorship dispute between Axelrod and Brodie, and Axelrod left Brodie's lab. Despite his already substantial contributions to science and his post as a senior chemist at NIH, Axelrod recognized that his chances for advancement were limited without a PhD. He took a leave of absence to do graduate work at George Washington University. His advisor, pharmacologist George Mandel, arranged for him to submit some of his work on the sympathomimetic amines as a dissertation, "The Fate of Sympathomimetic Phenylisopropylamines." In 1955, at the age of forty-two, Axelrod earned his PhD.

While Axelrod was working on his dissertation, Seymour Kety, scientific director of the joint intramural program of the National Institute of Mental Health (NIMH; established 1949), and Edward Evarts, director of the Laboratory of Clinical Sciences, invited Axelrod to set up a section in their laboratory. Kety became head of that laboratory in 1956. The explicit mission of the NIMH was to integrate basic and clinical research on psychiatric disorders. Although Kety allowed Axelrod considerable intellectual freedom, Axelrod felt obliged to do work relevant to mental health. His first project was to characterize the distribution and metabolism of lysergic acid diethylamide (LSD), an experimental psychiatric drug that would become widely used as a recreational drug (Axelrod et al., 1956). Axelrod did not take this drug himself.

Methyl Transferase Enzymes. Early in 1957, Axelrod attended a seminar in which Kety recounted evidence that epinephrine, a neurotransmitter and hormone (also known as adrenalin) is rapidly converted to adrenochrome when it is exposed to air. Kety also reported that ingestion of adrenochrome produces hallucinations like those experienced by people with schizophrenia, which suggested that schizophrenia might result from abnormal metabolism of epinephrine. Axelrod decided to study the metabolism of epinephrine and norepinephrine (also known as noradrenalin), using the same tools he had used to study other sympathomimetic amines.

At the time, it was widely believed that epinephrine and other catecholamines are metabolized in the body by the enzyme monoamine oxidase (MAO). This hypothesis conflicted with the fact that pharmacological inhibitors of MAO have no effect on the rapid recovery of the sympathetic nervous system from the effects of an injection of epinephrine. Axelrod surmised that a different enzyme was implicated. In March 1957, the biochemist Marvin Armstrong and Armand McMillan reported that patients with norepinephrine-producing tumors excrete large quantities of 3-methoxy-4-hydroxymandelic acid (VMA), a product that could be produced by the O-methylation (the addition of a methyl group, CH_3) and deamination of epinephrine and norepinephrine. Guessing that the donor of the methyl group in the methylation reaction might be S-adenosylmethionine, Axelrod added S-adenosylmethionine and epinephrine to a rat liver homogenate. He observed that the epinephrine was rapidly metabolized and that the product was an O-methylated form of adrenaline (which he called metanephrine), indicating the presence of an O-methylating enzyme. Axelrod purified this enzyme and then demonstrated that it could O-methylate

catechols but could not O-methylate monophenols. The enzyme, which he named catechol-O-methyltransferase (COMT), was soon found to be ubiquitous in the brain. (Axelrod and Tomchick, 1958). The discovery of this enzyme furthered the understanding of catecholamine metabolism, led to the development of drugs to inhibit COMT in the treatment of Parkinson's disease with L-dopa, and led to the development of new biological markers in biopharmacological studies. Axelrod later wrote, "As a result of these findings, I then considered myself a neurochemist" (1988, p. 13).

Regulation of Neurotransmitters at Synapses. Between 1958 and 1961, Axelrod carried out the experiments on the inactivation of neurotransmitters that earned him the Nobel Prize. His work focused on the inactivation of epinephrine and norepinephrine at synapses in the sympathetic nervous system. At the time it was known that acetylcholine, the neurotransmitter at the neuromuscular junction, is inactivated when it is rapidly metabolized by the enzyme acetylcholinesterase. Many suspected that all neurotransmitters are deactivated through enzymatic transformation.

Two preliminary findings set the stage for Axelrod's discoveries. First, Axelrod showed that inhibitors of COMT did not prolong the effects of norepinephrine on blood pressure, suggesting that something else must be responsible for its inactivation at sympathetic synapses. Second, Kety commissioned the New England Nuclear Corporation to manufacture tritiated epinephrine (epinephrine labeled with tritium, or [^{3}H]epinephrine) and norepinephrine ([^{3}H]norepinephrine). He used these to show that schizophrenics do not metabolize catecholamines differently from controls.

Axelrod borrowed the tritiated epinephrine and norepinephrine from Kety and developed sensitive methods for measuring their concentrations in tissues and blood. When he injected cats with large doses epinephrine or norepinephrine, he found that the drugs failed to cross the blood-brain barrier (which prevents some substances from entering the brain's circulatory system) and that they tended to concentrate in organs richly innervated by the sympathetic nervous system (such as the heart and the salivary glands). This result suggested to Axelrod that epinephrine and norepinephrine might be taken up by sympathetic nerves. To test this hypothesis, he and Georg Hertting (a visitor in the laboratory) made unilateral lesions to the superior cervical ganglia of cats, causing the nerves innervating the eyes and the salivary glands to die. They then injected the cats with [^{3}H]norepinephrine. They found that the radiolabeled neurotransmitter concentrated on the intact (innervated) side and not on the lesioned side (Hertting et al., 1961a), that it is released from nerves when they are stimulated (Hertting and Axelrod, 1961), and that drugs such as cocaine block its reuptake (Hertting et al., 1961b). Research quickly revealed that other transmitters, such as dopamine and serotonin, are also taken up into nerve terminals and that their use could also be regulated with drugs.

Axelrod's work on the synthesis, metabolism, and regulation of drugs and neurotransmitters provided lasting insights into the mechanisms of the chemical synapse. These insights had direct implications for the use of drugs to change the activities of the central and peripheral nervous systems. By the end of the millennium, drugs that inhibit serotonin reuptake, such as fluoxetine (brand name, Prozac), were widely used to treat depression. Axelrod predicted that in the new millennium, drugs would be available to cure mental illness, eliminate prejudice, enhance intelligence, suppress unwanted memories, and make all psychedelic trips pleasant. Asked if Freud was "dead," he replied, "Not for people who want to spend their money on psychoanalysis, but for the treatment of severe mental illness, yes, he is."

Melatonin and the Pineal Gland. A final major component of Axelrod's career as a neurochemist involved work on the synthesis of melatonin (5-methoxy-N-acetyltryptamine) and its regulation in the pineal gland in accordance with circadian rhythms. In 1958, Aaron Lerner isolated melatonin from the pineal gland of cows. Axelrod was attracted to melatonin because it has a methoxy group, as do many other catecholamine metabolites, and because it has a nucleus that resembles serotonin, which is structurally similar to LSD and was then believed to be involved in psychosis.

In 1958 and 1959, Axelrod and the biochemist Herbert Weissbach used radiolabeled enzymes to establish that melatonin is synthesized in the pineal gland from tryptophan and serotonin. The synthesis required the essential enzyme hydroxyindole-O-methyltransferase (HIOMT), which was soon found to be highly localized to the pineal gland in mammals.

When Richard J. Wurtman joined Axelrod's laboratory in 1962, he reawakened Axelrod's interest in the pineal. Wurtman had shown that rats in constant illumination enter a persistent state of estrus (commonly known as heat) and that this state can be reversed with injections of bovine pineal extract. Axelrod surmised that environmental lighting (that is, the light/dark cycle) might control HIOMT activity. In 1963, he and Wurtman found that rats raised in constant light have reduced HIOMT activity in the pineal gland relative to those raised in constant dark. Given that injections of melatonin also prevent the effects of light on the estrus cycle, Axelrod concluded that the effect of illumination on estrus is mediated by the

pathway for the synthesis of melatonin. Knowing that norepinephrine-containing sympathetic nerves innervate the pineal gland, Axelrod and his colleagues killed these nerves by removing the superior cervical ganglia. The effect of light on HIOMT activity (and so melatonin synthesis) was eliminated.

In 1963, W. B. Quay showed that serotonin and melatonin levels have regular daily fluctuations; they are high during daylight and low at night. Axelrod and his postdoctoral fellow, Solomon H. Snyder, found that the circadian rhythm in serotonin and melatonin persisted even when animals were placed in continuous darkness; there is an internal clock (Axelrod, Wurtman, and Snyder, 1965). Constant illumination, however, abolished the serotonin rhythm, as did destroying the nerves that connect the brain to the superior cervical ganglia. This result indicated that the internal clock is in the brain. Subsequent work identified regions in the hypothalamus (particularly, the suprachiasmatic nucleus) as plausible locations for the biological clock. Axelrod developed a theoretical perspective according to which the pineal gland is a transducer of information about the day/light cycle into hormonal signals for the control of bodily functions.

Nobel Prize and Axelrod's Political Turn. Axelrod shared the 1970 Nobel Prize with Bernard Katz and Ulf von Euler for "fundamental research into the nature of the chemical neurotransmission process." He recognized that this award gave him political clout, and he used it to promote several causes. Axelrod repeatedly made and joined public statements against governmental attempts to target biomedical research funding to specific large-scale projects, such as finding a cure for cancer. In this domain, and in his laboratory, Axelrod stressed the importance of knowing what problems can and cannot be solved with the tools available at the time. He also stressed the importance of intellectual freedom in the development of science as a whole and in the development of individual scientific careers.

Although he became an atheist early in life and resented the strict upbringing of his parents' religion, he identified with Jewish culture and joined several international fights against anti-Semitism. Alexandr Solzhenitsyn won the Nobel Prize for Literature in the same year that Axelrod won his own, but Solzhenitsyn was not allowed to attend the ceremony. After returning home, Axelrod repeatedly called on the Soviet Union to free such Jewish scholars as Benjamin G. Levich, Ilye Glezer, and Andrei D. Sakharov, who were imprisoned and had been refused the right of emigration and/or imprisoned on grounds that Axelrod and many others believed to be based in an anti-Semitic Soviet policy. (These Soviet scientists came to be known as "Refuseniks.")

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Axelrod also fought political battles on behalf of Israel. In 1975, he joined an international group of scientists who threatened to resign from the International Brain Research Organization, which is affiliated with UNESCO, until UNESCO resolutions banning Israeli participation in the organization were lifted. In 1979, he was among those who advocated pulling the United States out of the World Health Organization (WHO) if the WHO failed to allow Israel a vote in its decision-making process.

Learning of these pursuits, however, should not cause one to lose sight of the fact that Axelrod was first and foremost a researcher who believed that long hours of free inquiry using the right tools with the right colleagues can result in fundamental discoveries that have the power to shape the future of humankind. Although he officially retired in 1984, he continued to work in a small lab at NIMH until he died of a heart attack in his home in Rockville, Maryland, in 2004.

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