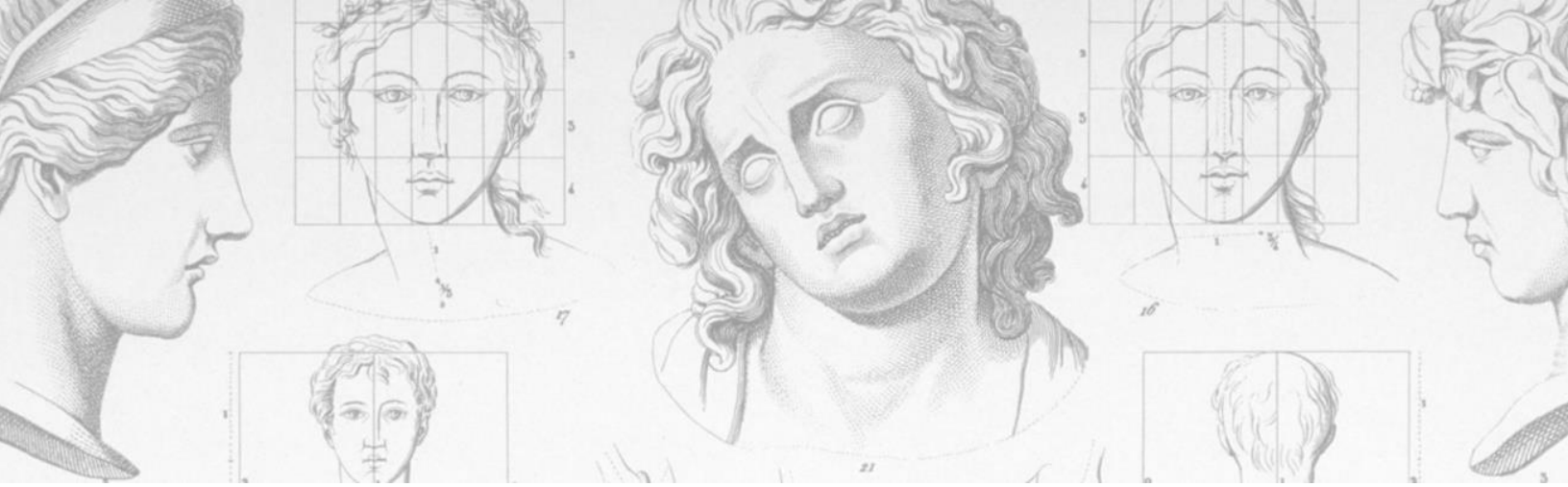

The History of Neuroscience in Autobiography
Volume 1

Edited by Larry R. Squire
Published by Society for Neuroscience
ISBN: 0-12-660301-4

Julius Axelrod

pp. 50–78

https://doi.org/10.1016/S1874-6055(98)80004-2

Julius Axelrod

BORN:

New York, New York
May 30, 1912

EDUCATION:

College of the City of New York, B.S., 1933
New York University, M.A., 1941
George Washington University, Ph.D., 1955

APPOINTMENTS:

Goldwater Memorial Hospital, Third New York University Research Division (1946)
National Heart Institute (1949)
National Institute of Mental Health; Chief, Section on Pharmacology (1955)
National Institute of Mental Health Guest Researcher (1984)
Scientist Emeritus of the National Institutes of Health (1996)

HONORS AND AWARDS (SELECTED):

Nobel Prize for Physiology or Medicine (1970)
American Academy of Arts and Sciences (1971)
National Academy of Sciences USA (1971)
Foreign Member of the Royal Society of London (1979)
Leibniz Medal, Academy of Sciences, East Germany (1984)
Mahoney Award "Decade of the Brain" (1991)
Ralph W. Gerard Prize, Society for Neuroscience (1992)

Julius Axelrod has carried out extensive, fundamental research on a wide range of topics, including biochemical mechanisms of drug and hormone actions and metabolism; enzymology; pineal gland membranes; and transduction mechanisms. He is most well known for his Nobel Prize-winning elucidation of the storage, release, and inactivation of catecholamine neurotransmitters and the effect of psychoactive drugs.

Julius Axelrod

Beginnings*

Successful scientists are generally recognized at a young age. They go to the best schools on scholarships, receive their postdoctoral training fellowships at prestigious laboratories, and publish early. None of this happened to me.

My parents emigrated at the beginning of this century from Polish Galicia. They met and married in America, and eventually settled in the Lower East Side of New York, then a Jewish ghetto. My father, Isadore, was a basketmaker who sold flower baskets to merchants and grocers. I was born in 1912 in a tenement on East Houston Street in Manhattan.

I attended PS22, a school built before the Civil War. Another student at that school before my time was I.I. Rabi, who later became a world-renowned physicist. After PS22 I attended Seward Park High School. I really wanted to go to Stuyvesant, a high school for bright students, but my grades were not good enough. Seward Park High School had many famous graduates, mostly entertainers: Zero Mostel, Walter Matthau, and Tony Curtis. My real education was obtained at the Hamilton Fish Park Library, a block from my home. I was a voracious reader and read through several books a week, from Upton Sinclair, H.L. Mencken, and Tolstoy to pulp novels such as the Frank Merriwell and Nick Carter series.

After graduating from Seward Park High School, I attended New York University in the hope that it would give me a better chance to get into medical school. After a year my money ran out, and I transferred to the tuition-free City College of New York in 1930. City College was a proletarian Harvard, which subsequently graduated seven Nobel Laureates. I majored in biology and chemistry, but my best grades were in history, philosophy, and literature. Because I had to work after school, I did most of my studying during the subway trip to and from uptown City College. Studying in a crowded, noisy New York subway gave me considerable powers of concentration. When I graduated from City College, I applied to several medical schools but was not accepted by any.

*A major portion of this article has been reproduced, with permission, from *Ann Rev Pharmacol Toxicol* 1988;28:1–23, by Annual Reviews, Inc.

In 1933, the year I graduated from college, the country was in the depths of a depression. More than 20 percent of the working population was unemployed, and there were few jobs available for City College graduates. I had heard about a laboratory position that was available at the Harriman Research Laboratory at New York University, and although the position paid $25 a month, I was happy to work in a laboratory. I assisted Dr. K.G. Falk, a biochemist, in his research on enzymes in malignant tumors. I also purified salts for the preparation of buffer solutions and determined their pH. The instrument used to measure pH at that time was a complex apparatus; the glass electrode occupied almost half a room. In 1935 the laboratory ran out of funds and I was fortunate to get a position as a chemist in the Laboratory of Industrial Hygiene. This laboratory was a nonprofit organization and was set up by New York City's Department of Health to test vitamin supplements added to foods. I worked in the Laboratory of Industrial Hygiene from 1935 to 1946.

My duties there were to modify published methods for measuring vitamins A, B, B_2, C, and D so that they could be assayed in various food products that city inspectors randomly collected. Vitamins had just been introduced at that time, and the New York City Department of Health wanted to establish that accurate amounts of vitamins were added to milk and other food products. The methods used for measuring vitamins then were chemical, biological, and microbiological. It required some ingenuity to modify the methods described in the literature to assays of food products. This experience in modifying methods was slightly more than routine, but it proved to be useful in my later research. The laboratory subscribed to the *Journal of Biological Chemistry*, which I read with great interest. Reading this journal made it possible to keep up with advances in enzymology, nutrition, and methodology. During the time I was in the Laboratory of Industrial Hygiene, I received an M.S. degree in chemistry at New York University in 1942 by taking courses at night. My thesis was on the ester-hydrolyzing enzymes in tumor tissues. Because of the loss of one eye in a laboratory accident, I was deferred from the draft during World War II. In 1938 I married Sally Taub, a graduate of Hunter College, who later became an elementary school teacher. We had two sons, Paul and Alfred, born in 1946 and 1949.

First Experience in Research: Goldwater Memorial Hospital

I expected that I would remain in the Laboratory of Industrial Hygiene for the rest of my working life. It was not a bad job, the work was moderately interesting, and the salary was adequate. One day early in 1946 the Institute for the Study of Analgesic and Sedative Drugs approached the president of the Laboratory of Industrial Hygiene with a problem. The president of the

laboratory at that time was George B. Wallace, a distinguished pharmacologist who had just retired as chairman of the department of pharmacology at New York University. Many analgesic preparations contained nonaspirin analgesics, such as acetanilide or phenacetin. Some people who became habituated to these preparations developed methemoglobinemia. The Institute for the Study of Analgesic and Sedative Drugs offered a small grant to the Laboratory of Industrial Hygiene to find out why acetanilide and phenacetin taken in large amounts produced methemoglobinemia. Dr. Wallace asked me if I would like to work on this problem. I had little experience in this kind of research, and he suggested that I consult Dr. Bernard "Steve" Brodie. Dr. Brodie was a former member of the department of pharmacology at New York University and was doing research at Goldwater Memorial Hospital, a New York University division.

I met with Brodie in February 1946 to discuss the problem of analgesics. It was a fateful meeting for me. Brodie and I talked for several hours about what kind of experiments could be done to find out how acetanilide might produce methemoglobinemia. Talking to Brodie about research was one of my most stimulating experiences. He invited me to spend some time in his laboratory to work on this problem.

One of a number of possible products of acetanilide that would cause the toxic effects was aniline. It had previously been shown that aniline could produce methemoglobinemia. Thus, one approach was to find out whether acetanilide could be deacetylated to form aniline in the body. With the help and guidance of Steve Brodie, I developed a method for measuring aniline in nanogram amounts in urine and plasma. After the administration of acetanilide to human subjects, aniline was found to be present in urine and plasma. A direct relationship between the level of aniline in blood and the amount of methemoglobin present was soon observed (Brodie and Axelrod, 1948). This was my first taste of real research, and I loved it.

Very little acetanilide was found in the urine, suggesting extensive metabolism in the body. As acetanilide was almost completely transformed in the body, we looked for other metabolic products. Methods to detect possible metabolites, *p*-aminophenol and N-acetyl-*p*-aminophenol, were developed that were specific and sensitive enough to be used in the plasma and urine. Within a few weeks, we identified the major metabolite as hydroxylated acetanilide N-acetyl-*p*-aminophenol and its conjugates. This metabolite was also found to be as potent as acetanilide in analgesic activity. By taking serial plasma samples, acetanilide was shown to be rapidly transformed to N-acetyl-*p*-aminophenol (Brodie and Axelrod, 1948). After the administration of N-acetyl-*p*-aminophenol, negligible amounts of methemoglobin were produced. As a result of these studies, Brodie and I stated in our paper (Brodie and Axelrod, 1948), "the results are compatible with the assumption that acetanilide exerts its action

mainly through N-acetyl-*p*-aminophenol [now known as acetaminophen]. 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." This was my first paper, and I was determined to continue doing research.

Soon after Brodie and I examined the physiological disposition and metabolism of acetanilide, we turned our attention to a related analgesic drug, phenacetin (acetophenetidin). I spent some time developing sensitive and specific methods for the identification of phenacetin and its possible metabolite, *p*-phenetidine. Brodie and I soon found that in humans, the major metabolic product was also N-acetyl-*p*-aminophenol arising from the deethylation of the parent compound (Brodie and Axelrod, 1949). A minor metabolite was *p*-phenetidine, which we found was responsible for the methemoglobinemia formed after the administration of large amounts of phenacetin to dogs. After the administration of phenacetin to human subjects, N-acetyl-*p*-aminophenol was rapidly formed. The speed and the amount with which N-acetyl-*p*-aminophenol was formed in the body suggested that the analgesic activity resided in its deethylated metabolite.

The laboratories at Goldwater Memorial Hospital where I began my research career were set up during World War II to test newly synthesized antimalarial drugs for their clinical effectiveness. Early in the war, the Japanese had cut off most of the world's supply of the antimalarial quinine. James Shannon, then a renal physiologist at New York University, was put in charge of this program. Shannon had the remarkable capacity to pick the bright young people to carry out research on the antimalarial project. Members of the team that worked at Goldwater in addition to Steve Brodie were Sid Udenfriend, Robert Berliner, Bob Bowman, Tom Kennedy, and Gordon Zubrod. The atmosphere at Goldwater was highly stimulating, and an outpouring of important new findings resulted. It was in this atmosphere that, in a period of a few years, I became a researcher.

After completion of the studies on acetanilide and phenacetin, Brodie invited me to stay on at Goldwater to study the fate of other analgesic drugs. We received a small grant from the Institute for the Study of Analgesic and Sedative Drugs, and the Laboratory of Industrial Hygiene paid my salary. Another drug we investigated was the analgesic antipyrine. A sensitive method for the detection of this drug was developed, which has since been used by other investigators as a marker to determine the activity of drug-metabolizing enzymes *in vivo*. We identified 4-hydroxyantipyrine and its sulfate conjugate as metabolites of antipyrine. We also observed that antipyrine distributed in the same manner as body water. Because of this property, antipyrine has been used for the measurement of body water. Another analgesic we studied was aminopyrine. Many of the drugs whose fate Brodie and I studied were

later used by many investigators as substrates for the microsomal drug-metabolizing enzymes: aminopyrine for N-demethylation, phenacetin for O-dealkylation, and aniline for hydroxylation. Together with Jack Cooper, we developed a method for measuring the anticoagulant dicoumerol in plasma. In a study on the disposition of dicoumerol in humans, an exceedingly wide difference in the plasma levels of this drug was found, suggesting genetic differences in drug metabolism.

Move to the National Heart Institute

Because I did not have a doctorate degree, I realized that I would have little chance for advancement in any hospital attached to an academic institution. I had neither the inclination nor the money to spend several years getting a Ph.D., so I decided to join the National Heart Institute as a research chemist. In 1949, Shannon was chosen as the director of the newly organized National Heart Institute in Bethesda, and he offered me a position. Also coming to the National Institutes of Health (NIH) at that time were many members of the Goldwater staff—Brodie, Sidney Udenfriend, Robert Berliner, Thomas Kennedy, and Robert Bowman.

At the National Heart Institute from 1950 to 1952, I collaborated with Brodie and his staff on the metabolism of analgesics and adrenergic blocking agents and the actions of ascorbic acid on drug metabolism. After a while, I became dissatisfied with working with a large team and was allowed to work independently. The first problem I chose was an examination of the physiological disposition of caffeine in humans. Very little was known about the physiological disposition and metabolism of this widely used compound. A method for measuring caffeine in biological material was developed, and the plasma half-life and distribution were determined (Axelrod and Reichenthal, 1953). Because of my work on analgesics and caffeine, I was delighted to be elected without a doctorate as a member of the American Society of Pharmacology and Experimental Therapeutics in 1953. K.K. Chen and Steve Brodie were my sponsors.

At that time, I became intrigued with the sympathomimetic amines. In 1910, George Barger and Henry Dale reported that numerous ß-phenylethanolamine derivatives simulated the effects of sympathetic nerve stimulation with varying degrees of intensity and precision. They coined the term *sympathomimetic amines*. Sympathomimetic amines such as amphetamine, mescaline, and ephedrine also produced unusual behavioral effects. In 1952 very little information concerning the metabolism and physiological disposition of these amines was known. Because of my experience in drug metabolism, I decided to undertake a study on the fate of ephedrine and amphetamine. In retrospect, this was an important decision.

The first amine that I studied was ephedrine. Ephedrine, the active principle of *Ma Huang*, an herb used by ancient Chinese physicians, was introduced to modern medicine by Chen and Schmidt in 1930. I soon found that ephedrine was transformed in animals by two pathways (demethylation and hydroxylation) to yield metabolic products that had pressor activity. Various animal species showed considerable differences in the relative importance of these two metabolic routes. The next sympathomimetic amines I examined were amphetamine and methylamphetamine. These compounds were shown to be metabolized by a variety of metabolic pathways including hydroxylation, demethylation, deamination, and conjugation. Marked species variations in the transformation of these drugs were also observed.

The Discovery of the Microsomal Drug Metabolizing Enzymes

When amphetamine was given to rabbits, it disappeared without a trace. This puzzled me, so I decided to look for enzymes that metabolized this drug. I had no experience in enzymology, but there were many outstanding enzymologists in Building 3 on the NIH campus where my laboratory was located. Gordon Tomkins, who occupied the lab bench next to mine, offered me good advice. Gordon had the capacity of demystifying enzymology and told me that all I needed to start *in vitro* experiments was a method of measuring amphetamine, an animal liver, and a razor blade. I did my first *in vitro* experiment with rabbit liver in January 1953. When rabbit liver slices were incubated in Krebs-Ringer buffer solutions with amphetamine, the drug was almost completely metabolized. On homogenization of the rabbit liver, amphetamine was not metabolized unless cofactors such as DPN (NAD), TPN (NADP), and ATP were added. I then decided to examine which subcellular fraction was responsible for transforming amphetamine. Hogeboom and Schneider had just described a reproducible method for separating the various subcellular fractions by homogenizing tissue in isotonic sucrose and subjecting the homogenate to differential centrifugation. After separation of nuclei, mitochondria, microsomes, and the cytosol, none of these fractions were able to metabolize amphetamine, even in the presence of added cofactors. However, when the microsomes and cytosol were combined, amphetamine rapidly disappeared on the addition of DPN, TPN, and ATP. At that time Bert La Du, a colleague at the NIH, observed that the demethylation of aminopyrine in a dialyzed rat liver whole homogenate required TPN. In a subsequent experiment I found that amphetamine was metabolized in a dialyzed preparation of microsomes and cytosol in the presence of TPN, but not DPN or ATP. However, when microsomes and cytosol were separately incubated, little or no drug was metabolized, despite the addition of TPN. I realized then that I was dealing with a unique enzymatic reaction.

Before I went further, I decided to identify the metabolic products of amphetamine produced when the combined microsomes and cytosolic fraction were incubated with TPN. One of the possible metabolic pathways might be deamination, leading to the formation of phenylacetone. After incubation of amphetamine with the above preparations, phenylacetone and ammonia were identified. These results indicated that amphetamine was deaminated by an oxidative enzyme requiring TPN either in the microsomes or cytosol to form phenylacetone and ammonia. Because of its properties and the structure of its substrate, it was apparent that this enzyme differed from another deaminating enzyme, monoamine oxidase.

Where was the enzyme located, in the microsomes or the soluble supernatant fraction? An approach that I used to locate the enzyme was to heat each fraction for a few minutes at 55°C, a temperature that would destroy heat-sensitive enzymes. When the cytosol was heated to 55°C and then added to unheated microsomes and TPN, amphetamine was deaminated. When the microsomes were heated and added to the cytosolic fraction together with TPN, amphetamine was not metabolized. This was a crucial experiment, which demonstrated that a heat-labile enzyme that deaminated amphetamines was localized in the microsomes and that the cytosol provided factors involving TPN necessary for this reaction.

Bernard Horecker, then working in Building 3, prepared several substrates for the TPN-requiring dehydrogenase for his classic work on the pentose phosphate pathway. He generously supplied me with these substrates, which I could test on my preparation. I found that the addition of glucose-6-phosphate, isocitric acid, or phosphogluconic acid, together with TPN, to unwashed microsomes transformed amphetamine. A reaction common to these substrates is the generation of TPNH, suggesting that the enzymes in the cytosol fraction were reducing TPN. Incubating microsomes with a TPNH-generating system using glucose-6-phosphate and glucose-6-phosphate dehydrogenase resulted in the deamination of amphetamine. On incubation of chemically synthesized TPNH, microsomes, and oxygen, amphetamine was deaminated. At about the same time, I also found that ephedrine was demethylated to norephedrine and formaldehyde by enzymes present in rabbit microsomes that required TPNH and oxygen. By the end of June 1953, I felt confident that I had described a new enzyme that was localized in the microsomes, required TPNH and oxygen, and could deaminate and demethylate drugs. I reported these findings at the 1953 fall meeting of the American Society of Pharmacology and Experimental Therapeutics (Axelrod, 1954).

After the description of the TPNH-requiring microsomal enzymes that deaminated amphetamine and demethylated ephedrine, several members of the Laboratory of Chemical Pharmacology at the NIH described similar enzyme systems that could metabolize other drugs by a variety of pathways: N-demethylation of aminopyrine (La Du, Gaudette, Trousof, and

Brodie), oxidation of barbiturates (Cooper and Brodie), and the hydroxylation of aniline (Mitoma and Udenfriend) as reviewed in the *Annual Review of Biochemistry* (Brodie et al., 1958). In a study of the N-demethylation of narcotic drugs that I made soon after, it became apparent that there were multiple microsomal enzymes that required TPNH and O_2 (Axelrod, 1956a). Research on the microsomal enzymes (now called cytochrome-P450 mono-oxygenases) has expanded enormously and has had a profound influence on biomedical science, ranging from studies of metabolism of normally occurring compounds to carcinogenesis. In retrospect, the discovery of the microsomal enzymes is among the best work I did.

Brodie and I were struck by the findings of investigators at Smith Kline & French that SKF525A, a compound with little pharmacological action of its own, prolonged the duration of action of a wide variety of drugs. We conjectured that the compound might exert its effects by inhibiting the metabolism of drugs. The effects of SKF525A on the metabolism of ephedrine in dogs and on the metabolism and duration of action of hexabarbital in the plasma and the sleeping time in rats and dogs was examined. We found that SKF525A slowed the metabolism of ephedrine in dogs. It prolonged the presence of hexabarbital in the plasma and sleeping time in rats and dogs. Thus, the ability of SKF525A to prolong the action of drugs could be explained by its ability to slow their metabolism. As soon as the microsomal enzymes were described, it was observed that SKF525A inhibited this class of enzymes. Subsequently, SKF525A was widely used as an inhibitor of the microsomal enzymes.

The effect of the microsomal enzymes on the duration of drug actions was examined with the collaboration of Gertrude Quinn, a graduate student at George Washington University, and Steve Brodie. Because sleeping time of hexabarbital was easy to measure, we chose that drug to make this study. Jack Cooper and Brodie had found that hexabarbital was metabolized by microsomal enzymes in the liver. The sleeping time of a given dose of hexabarbital was compared with its plasma half-life and with the activity of a liver enzyme preparation using the barbiturate as a substrate in a number of mammalian species. There were considerable differences in the plasma half-life, sleeping time, and enzyme activity among the various species (Quinn et al., 1958). A high correlation was observed between the plasma half-life and sleeping time of the barbiturate. There was also an inverse relationship between the duration of action of hexabarbital and its ability to be metabolized by the microsomal enzymes.

In 1956, I reported that narcotic drugs such as morphine, meperidine, and methadone were N-demethylated by the liver, requiring TPNH and O_2 (Axelrod, 1956a). Differences in the rate of N-demethylation of various narcotic drugs in several species made it apparent that more than one enzyme was involved in their demethylation. There was also a marked sex difference in N-demethylation of narcotic drugs by rat liver microsomal enzymes.

Microsomes obtained from male rats were found to N-demethylate narcotic drugs much faster than those from female rats. When testosterone was administered to oophorectomized female rats, the activity of the demethylating enzyme was markedly increased. Estradiol given to male rats decreased the enzyme activity. Subsequent work by many investigators found similar sex differences in microsomal enzyme activity for many metabolic pathways.

While working on the metabolism of narcotic drugs, I observed that repeated administration of narcotic drugs not only produced tolerance to these drugs, but also markedly reduced the ability to N-demethylate them enzymatically (Axelrod, 1956b). There was also a correlation between the rate of demethylation of opiate substrates and their cross-tolerance to morphine. Opiate antagonists not only blocked the development of tolerance, but also prevented the reduction of enzyme activity. On the basis of these observations, a mechanism for tolerance to narcotic drugs was proposed. In a paper reporting these experiments (Axelrod, 1956b) the following statement was made: "The changes in enzyme activity in morphine-treated rats suggests a mechanism for the development of tolerance to narcotic drugs. If one assumes that enzymes which N-demethylate narcotic drugs and the receptors for these drugs are closely related, then the continuous interaction of narcotic drugs with the demethylating enzymes inactivates the enzymes. Likewise, the continuous interaction of narcotic drugs with their receptors may inactivate the receptors. Thus, a decreased response to narcotic drugs may develop as a result of unavailability of receptor sites." This hypothesis stimulated considerable critical reaction, mostly negative.

Although I had just described the physiological disposition of caffeine, demonstrated the variety of metabolic pathways of amphetamine and ephedrine, and independently described the microsomal enzymes and their role in drug metabolism, it was difficult for me to obtain a promotion to a higher rank at the National Heart Institute because I had no doctorate. I decided to get a Ph.D. degree at George Washington University, because few courses were required if a candidate already had an M.S. degree. However, it would be necessary to take demanding comprehensive examinations in several subjects. Paul K. Smith, then chairman of pharmacology, accepted me as a graduate student in his department. He allowed me to submit my work on the metabolism of sympathomimetic amines and the microsomal enzymes for my dissertation. I took a year off to attend courses at George Washington University, and I found going back to school pleasant and challenging. A few of the medical students did better than I did on the pharmacology examinations. On one occasion a question was asked on a multiple-choice examination on antipyrine, a compound on which I published several papers, and I gave the wrong answer. After a year's study, I passed a tough comprehensive examination, and my thesis, "The Fate of Phenylisopropylamines," was accepted. In 1955, at the age of 42 years, I received my Ph.D.

Setting Up A Laboratory at the National Institute of Mental Health

While studying for my Ph.D., I was invited by Edward Evarts to set up a Section of Pharmacology in his Laboratory of Clinical Sciences at the National Institute of Mental Health (NIMH). To get started in my new position at the NIMH I took a few afternoons off my classes at George Washington University to do laboratory work. I thought that a study of the metabolism and distribution of LSD would be an appropriate problem for my new laboratory at the NIMH. LSD was then used as an experimental drug by psychiatrists to study abnormal behavior. Bob Bowman at the NIH was in the process of building a spectrofluorometer. He was kind enough to let me use his experimental model, which allowed me to develop a very sensitive fluorometric assay for LSD. This made it possible to measure the nanogram amounts found in brain and other tissues. This instrument later became the well-known Aminco Bowman spectrofluorometer. The availability of this instrument made it possible for many laboratories to devise sensitive methods for the measurement of endogenous epinephrine, norepinephrine, dopamine, and serotonin in brain and other tissues. These newly developed methods for biogenic amines were crucial in the subsequent rapid expansion in neurotransmitter research.

Just before I left the Heart Institute, I read a report in the literature that uridine diphosphate glucuronic acid (UDPGA) was a necessary cofactor for the formation of phenolic glucuronide in a cell-free preparation of livers. Jack Strominger, a biochemist then at the NIH, and I discussed the possible mechanism for the enzymatic synthesis of UDPGA. We suspected that it would arise from the oxidation of uridine diphosphate glucose (UDPG) by either TPN or DPN. We obtained a sample of UDPG from Herman Kalckar and did a preliminary experiment in which I measured the disappearance of morphine in guinea pig liver. When morphine was incubated with guinea pig liver microsomes and the soluble fraction with DPN and UDPG, morphine was metabolized; TPN had no effect. When either DPN or UDPG, soluble fraction, or liver was omitted, the disappearance of morphine was negligible. After a period of incubation during which the mixture was heated in 1N HCl, the morphine that disappeared was recovered. These experiments suggested that morphine was enzymatically conjugated in the presence of UDPG and DPN, presumably by the formation of UDPGA followed by morphine glucuronide formation. I had little time to continue this problem because I was in the process of getting my Ph.D. Strominger and co-workers then went on to purify an enzyme UDPG dehydrogenase that formed UDPGA from UDPG and DPN.

After completion of my Ph.D., I returned to the glucuronide problem in my new laboratory at the NIMH. As expected from my preliminary experiment with morphine, I found that morphine and other narcotic

drugs formed glucuronide conjugates by an enzyme present in liver microsomes that required UDPGA. Working together, Joe Inscoe, a graduate student at George Washington University, and I showed that glucuronide formation could be induced by benzpyrene and 3-methylcholanthrene.

The work on glucuronide conjugation led to a study on the role of glucuronic acid conjugation on bilirubin metabolism. Rudi Schmid, then at the NIH, made the interesting observation that bilirubin was transformed to a glucuronide. Schmid and I then went on to describe the enzymatic formation of bilirubin glucuronide by enzymes in the liver requiring UDPGA. This conjugating enzyme served as a mechanism for inactivating bilirubin. This finding led to an interesting clinical observation concerning a defect in glucuronide formation. In congenital jaundice there is a marked elevation of free bilirubin in the blood. This fact suggested to us that something might be wrong with glucuronide formation in this disease. The availability of a mutant strain of rats (Gunn rats) that exhibited congenital jaundice made it possible to examine whether the glucuronide-forming enzyme was defective. We then went on to demonstrate that these rats showed a marked defect in the ability to synthesize glucuronides from UDPGA (Axelrod et al., 1957). Glucuronide formation was also examined in humans with congenital jaundice by measuring the rate and magnitude of plasma acetaminophen glucuronide after the administration of the acetaminophen. A defect in glucuronide formation in this disease was demonstrated.

Catecholamine Research

When I joined the NIMH, I knew very little about neuroscience. My impression of neuroscience then was that it was concerned mainly with electrophysiology, brain anatomy, and behavior. To me these subjects were somewhat strange and esoteric and concerned with complicated electronic equipment. I believed that an investigator had to be a gifted experimentalist and theorist to do research in the neurosciences. Ed Evarts, my lab chief, assured me that I could work on whatever problem I thought would be likely to yield new information. The philosophy of Seymour Kety, then head of the Intramural Programs of the NIMH, was to allow investigators working in the laboratories of the NIMH to do their research on whatever was potentially productive and important. Kety believed that without sufficient basic knowledge about the life processes, doing targeted research on mental illness would be a waste of time and money.

Instead of working on a neurobiological problem, I thought it would be best to work on one that I knew something about, and that might be appropriate to the mission of the NIMH. I began to experiment on the metabolism and physiological disposition of LSD and the enzymes

involved in the metabolism of narcotic drugs. I also worked on the enzymatic synthesis of glucuronides described above.

Although the NIMH administrators were supportive of the type of research I was doing, I still felt guilty that I was not working on some aspect of the nervous system or mental illness. Dr. Kety, in a seminar to our laboratory, gave a fascinating account of the findings of two Canadian psychiatrists. They reported that adrenochrome produced schizophrenic-like hallucinations when it was ingested. Because of these behavioral effects, they proposed that schizophrenia could be caused by an abnormal metabolism of epinephrine to adrenochrome. I was intrigued by this proposal. In searching the literature, I was surprised to find that little was known about the metabolism of epinephrine at that time, in 1957. In view of the provocative hypothesis about the abnormal metabolism of epinephrine in schizophrenia, I decided to work on the metabolism of epinephrine. Epinephrine was then believed to be metabolized and inactivated by deamination by monoamine oxidase. However, with the introduction of monoamine oxidase inhibitors by Albert Zeller and co-workers, it was observed that, after the inhibition of monoamine oxidase *in vivo*, the physiological actions of administered epinephrine were still rapidly ended. This finding indicated that enzymes other than monoamine oxidase metabolized epinephrine. A possible route of metabolism of epinephrine might be via oxidation. I spent several months looking at oxidative enzymes for epinephrine without any success.

An abstract in the March 1957 *Federation Proceedings* gave me an important clue regarding a possible pathway for the metabolism of epinephrine. In this abstract, Armstrong and McMillan (1957) reported that patients with norepinephrine-forming tumors (pheochromocytomas) excreted large amounts of an O-methylated product, 3-methoxy-4-hydroxymandelic acid (VMA). This finding suggested that this metabolite could be formed by the O-methylation and deamination of epinephrine or norepinephrine. The O-methylation of catecholamines was an intriguing possibility that could be experimentally tested. A potential methyl donor could be *S*-adenosylmethionine. That afternoon I incubated epinephrine with a homogenate of rat liver, ATP, and methionine. I did not have *S*-adenosylmethionine available, but Cantoni (1953) had shown that an enzyme in the liver could convert ATP and methionine to adenosylmethionine. I found that epinephrine was rapidly metabolized in the presence of ATP, methionine and liver homogenate. When either ATP or methionine was omitted or the homogenate was heated, there was a negligible disappearance of epinephrine. This experiment suggested that epinephrine was O-methylated in the presence of a methyl donor, presumably *S*-adenosylmethionine. In a subsequent experiment, I obtained *S*-adenosylmethionine and observed that incubating liver homogenate with the methyl donor resulted in the metabolism of epinephrine. The most likely site of methylation would be on

the *meta* hydroxyl group of epinephrine to form 3-O-methylepinephrine. I prevailed on my colleague Bernhard Witkop, an organic chemist, to synthesize the O-methyl metabolite of epinephrine. A few days later Sheroh Senoh, a visiting scientist in Witkop's laboratory, synthesized meta-O-methylepinephrine. After incubating liver and *S*-adenosylmethionine, the metabolite formed from epinephrine was identified as meta-O-methylepinephrine, which we named *metanephrine*, indicating the existence of an O-methylating enzyme. The O-methylating enzyme was purified and found to O-methylate catechols, including norepinephrine, dopamine, L-DOPA, and synthetic catechols, but not monophenols (Axelrod, 1971). In view of the substrate specificity, the enzyme was named *catechol-O-methyltransferase* (*COMT*). The enzyme was found to be widely distributed in tissues, including the brain.

Injecting catecholamines into animals resulted in the excretion of the respective O-methylated metabolites. We soon identified normally occurring O-methylated metabolites such as normetanephrine, metanephrine, 3-methoxy tyramine, and 3-methoxy-4-hydroxyphenylglycol (MHPG) in liver and brain. As a result of the discovery of the O-methylated metabolites, the pathways of catecholamine metabolism were clarified (Axelrod, 1971). Catecholamines were metabolized by O-methylation, deamination, glycol formation, oxidation, and conjugation. As a result of these findings, I then considered myself a neurochemist. This work also gave me a long-lasting interest in methylation reactions that I describe later. The metabolites of catecholamines, particularly MHPG, have been used as a marker in many studies in biological psychiatry.

A major problem in neurobiology research is the mechanism by which neurotransmitters are inactivated. At the time I described the metabolic pathway for catecholamines in 1957, it was believed that the actions of neurotransmitters were terminated by enzymatic transformation. Acetylcholine was already known to be rapidly inactivated by acetylcholinesterase. However, when the principal enzymes for the metabolism of catecholamines, catechol-O-methyltransferase and monoamine oxidase, were almost completely inhibited *in vivo*, the physiological actions of injected epinephrine were rapidly ended. These experiments indicated that there were other mechanisms for the rapid inactivation of catecholamines.

The answer to the question of the inactivation of catecholamines came in an unexpected way. When the metabolism of catecholamines was described, Seymour Kety and co-workers set out to examine whether or not there was an abnormal metabolism of epinephrine in schizophrenic patients. To carry out this study, Kety asked the New England Nuclear Corporation to prepare tritium-labeled epinephrine and norepinephrine of high specific activity. The first batch of ^{3}H-epinephrine that arrived in late 1957 was labeled on the 7 position, which we found to be stable. Kety was kind enough to give me some of the ^{3}H-epinephrine for my studies. I

thought it would be a good idea to examine the tissue distribution and half-life of ^{3}H-epinephrine in animals.

About that time, Hans Weil-Malherbe spent three months in my laboratory as a visiting scientist, and together we developed methods of measuring ^{3}H-epinephrine and its metabolites in tissues and plasma. To our surprise, when ^{3}H-epinephrine was injected into cats, it persisted unchanged in the heart, spleen, and the salivary and adrenal glands long after its physiological effects were ended. This phenomenon puzzled us. We also found that ^{3}H-epinephrine did not cross the blood-brain barrier. Just about this time Gordon Whitby, a graduate student from Cambridge University, came to our laboratory to do his Ph.D. thesis. I suggested that he use methods for assaying ^{3}H-norepinephrine similar to those we used for ^{3}H-epinephrine to study its tissue distribution. As in the case of ^{3}H-epinephrine, ^{3}H-norepinephrine persisted in organs rich in sympathetic nerves (heart, spleen, salivary gland). These studies gave us a clue regarding the inactivation of catecholamine neurotransmitters: uptake and retention in sympathetic nerves.

The crucial experiment that established that catecholamines were selectively taken up in sympathetic neurons was suggested by George Hertting from the University of Vienna, who joined my laboratory as a visiting scientist. In the next experiment, the superior cervical ganglia of cats were taken out of one side, resulting in a unilateral degeneration of sympathetic nerves in the salivary gland and eye muscles. On the injection of ^{3}H-norepinephrine, radioactive catecholamine accumulated on the innervated side, but very little appeared on the denervated side (Hertting et al., 1961). This simple experiment clearly showed that sympathetic nerves take up and store norepinephrine. In another series of experiments, Hertting and I found that injected ^{3}H-norepinephrine taken up by sympathetic nerves was released when these nerves were stimulated (Hertting and Axelrod, 1961). As a result of these experiments, we proposed that norepinephrine is rapidly inactivated by reuptake into sympathetic nerves. Other slower mechanisms for the inactivation of catecholamines proposed were removal by the bloodstream, metabolism by O-methylation, and/or deamination by liver and kidney.

In 1961, the first postdoctoral fellow, Lincoln Potter, joined my laboratory via the NIH Research Associates Program. The NIH Research Associates Program and the Pharmacology Research Associates Program provided an opportunity for recent Ph.D. and M.D. graduates to spend two or three years in Bethesda doing full-time research. Because of the number of applicants for this program, the investigators in the Intramural Program at the NIH would get the best and brightest postdoctoral fellows. During the past 25 years more than 60 postdoctoral fellows joined my laboratory to do full-time research. With one or two exceptions, most of the postdocs who worked in my laboratory went on to productive careers in research.

When Linc Potter joined my laboratory, we directed our attention to the sites of the intraneural storage of norepinephrine. We suspected that ^{3}H-norepinephrine, already shown to be taken up by sympathetic neurons, would label intracellular storage sites. ^{3}H-norepinephrine was injected into rats, and their hearts were homogenized in isotonic sucrose. The various cellular fractions were then separated in a continuous sucrose gradient. There was a sharp peak of radioactive norepinephrine in a fraction that coincided with endogenous catecholamines and dopamine-ß-hydroxylase, the enzyme that converts dopamine to norepinephrine. The norepinephrine-containing particles exerted a pressor response only when they were lysed. In another experiment, ^{3}H-norepinephrine was injected, and the pineal gland, an organ rich in sympathetic nerve terminals, was subjected to radioautography and electron microscopy. Photographic grains of ^{3}H-norepinephrine were highly localized over dense core-granulated vesicles of about 500 angstroms (Axelrod, 1971). All these experiments indicated that norepinephrine in sympathetic nerves was stored in small, dense core vesicles.

Subsequent studies with another postdoc, Dick Weinshilboum, showed that on stimulation of the hypogastric nerve of the vas deferens, both norepinephrine and dopamine-ß-hydroxylase were discharged from the nerve terminals. This finding suggested that norepinephrine and dopamine-ß-hydroxylase were colocalized in the catecholamine storage vesicles of sympathetic nerves and were then discharged together by exocytosis (Weinshilboum et al., 1971). These findings led us to the postulation that the released dopamine-ß-hydroxylase would appear in the blood, which was soon confirmed. Later, our laboratory and others found abnormally low levels of plasma dopamine-ß-hydroxylase in familial dysautonomia and Down's syndrome, and high levels in patients with torsion dystonia, neuroblastoma, and certain forms of hypertension.

As soon as it was found that catecholamines could be taken up and inactivated by reuptake into sympathetic nerve terminals, my co-workers and I turned our attention to the effect of adrenergic drugs on this process. We designed relatively simple experiments for this study, injecting the drug into rats and then measuring the uptake of injected ^{3}H-norepinephrine in tissues. Cocaine was the first drug we examined. It had been postulated that cocaine causes supersensitivity to norepinephrine by interfering with its inactivation. After pretreatment of cats with cocaine, there was a marked reduction of ^{3}H-norepinephrine in tissues that were innervated by sympathetic nerves after the injection of the radioactive catecholamine (Whitby et al., 1960). This experiment indicated that cocaine blocked the reuptake of norepinephrine in nerves and thus allowed large amounts of catecholamine to remain at the synaptic cleft and act on the postsynaptic receptors for longer periods of time. Using a similar approach, we observed that antidepressant drugs amphetamine and other

sympathomimetic amines also blocked the uptake of norepinephrine (Axelrod, 1971). In another type of experiment, using an isolated perfused beating rat heart whose nerves had previously been labeled with ^{3}H-norepinephrine, we found that the physiological action of sympathomimetic amines, such as tyramine, was mediated by releasing the norepinephrine from sympathetic nerves (Axelrod et al., 1962). After repeated treatment of the isolated heart with tyramine, the heart rate and amplitude of contraction were gradually reduced, presumably by the depletion of the releasable stores of the neurotransmitters. After replenishing the isolated heart with exogenous norepinephrine, the heart rate and amplitude of contraction of the isolated heart were restored. Amphetamine also released norepinephrine, and it was later shown by others that the physiological effects of the amine were due to the release of dopamine.

Most of my early work in catecholamines was done in the peripheral sympathetic nervous system. Hans Weil-Malherbe and I had found that catecholamines did not cross the blood-brain barrier. This finding made it impossible to study the metabolism, storage, and release of norepinephrine in the brain by peripheral administration of ^{3}H-norepinephrine. It was Jacques Glowinski, a visiting scientist from France, who circumvented this problem. He devised a technique to introduce ^{3}H-norepinephrine directly into the brain by injection into the lateral ventricle. Subsequent experiments showed that ^{3}H-norepinephrine was mixed with the endogenous catecholamines in the brain. As in the peripheral nervous system, the ^{3}H-norepinephrine was found to be metabolized by O-methylation and deamination. In a series of experiments we established that ^{3}H-norepinephrine could serve as a useful tool in studying the activity of brain adrenergic nerves (Axelrod, 1971).

After labeling adrenergic neurons in the brain (Glowinski and Axelrod, 1964), we examined the effect of psychoactive drugs on brain biogenic amines. We found that only the clinically effective antidepressant drugs block the reuptake of ^{3}H-norepinephrine in adrenergic nerve terminals. This finding, together with the observation that monoamine oxidase inhibitors have antidepressant actions and that reserpine, a depleter of biogenic amines, sometimes causes depression, led to the formulation of the catecholamine hypothesis of depression (Schildkraut, 1965). We also found that amphetamines block the reuptake as well as the release of ^{3}H-norepinephrine in the brain. Other investigators later showed the paranoid psychosis caused by excessive ingestion of amphetamines is due to the release of the catecholamine dopamine. One of the reasons that Les Iversen came to my lab as a postdoctoral fellow was to learn about the brain and its chemistry. Iversen and Glowinski worked extensively together in my laboratory on the effects of drugs on the adrenergic system in different areas of the brain. To conduct this study they devised a method of dissection of various parts of the brain that has become a classic procedure.

For several years our laboratory was concerned with the adaptive mechanism of the sympathoadrenal axis. One such mechanism, the induction of the catecholamine's biosynthetic enzyme, tyrosine hydroxylase, was observed in an unexpected manner, as often happens in research. Hans Thoenen, then working in Basel, asked to spend a sabbatical year in my laboratory. He and Tranzer had observed that injected 6-hydroxydopamine selectively destroys catecholamine-containing nerve terminals (Thoenen and Tranzer, 1968). I invited Thoenen to join my laboratory and bring 6-hydroxydopamine. The first experiment that Thoenen tried was to examine the effects of the destruction of peripheral sympathetic nerves on tyrosine hydroxylase. As expected, after the injection of 6-hydroxydopamine, tyrosine hydroxylase almost completely disappeared from sympathetically innervated nerves. A surprising observation was a marked elevation of tyrosine hydroxylase in the adrenal medulla. 6-Hydroxydopamine was known to cause persistent firing of nerves. We suspected that tyrosine hydroxylase was elevated in the adrenal medulla by continuous firing of the splanchnic nerve innervating the adrenals. This supposition was confirmed when other drugs that caused prolonged nerve firing, such as reserpine and α_2-adrenergic blocking agents, also increased tyrosine hydroxylase (Thoenen et al., 1969). Subsequent experiments showed that increased nerve firing induced the synthesis of new tyrosine hydroxylase molecules in nerve cell bodies and the adrenal medulla in a transsynaptic manner. Similar results were obtained with another catecholamine biosynthetic enzyme, dopamine-ß-hydroxylase.

Another regulatory mechanism for catecholamine synthesis was found by asking the right questions rather than by serendipity. The ratio of epinephrine to norepinephrine in the adrenal medulla was known to be dependent on how much of the medulla was enveloped by the adrenal cortex. In species in which the cortex is separated from the medulla, norepinephrine is the predominant catecholamine. In species in which the medulla is surrounded by the adrenal cortex, the methylated catecholamine, epinephrine, is by far the major amine. Dick Wurtman, a research associate in my laboratory, suggested an elegant experiment to determine the role of the adrenal cortex in regulating the synthesis of epinephrine. He removed the rat pituitary, a procedure that depleted glucocorticoid in the adrenal cortex, and then measured the effect on the levels of the epinephrine-forming enzyme, phenylethanolamine-N-methyltransferase (PNMT), in the medulla. I had just characterized PNMT and found that it was highly localized in the adrenal medulla. The ablation of the pituitary caused a profound decrease in PNMT in the medulla after several days (Wurtman and Axelrod, 1966). The administration of adrenocorticotropic hormone (ACTH), a peptide that increases the formation of glucocorticoids in the adrenal cortex, or the injection of the synthetic glucocorticoid, dexamethasone, increased PNMT in hypophysectomized rats almost to normal values.

Methyltransferase Research

After the description of catechol-O-methyltransferase (COMT), I became very much involved with methyltransferase enzymes (Axelrod, 1981). I spent most of my time at the lab bench working on methylating enzymes for many years. Soon after describing COMT, I turned my attention to the enzymatic N-methylation of histamine. A major pathway for histamine metabolism occurs via N-methylation. This finding prompted a search for a potential histamine-methylating enzyme. As is the case with other methyltransferases, I suspected that the most likely methyl donor would be *S*-adenosylmethionine. To make the identity of the histamine-methylating enzyme possible, Donald Brown, a postdoc in the lab of a colleague, and I synthesized [^{14}C-methyl]-*S*-adenosylmethionine enzymatically from rabbit liver with ^{14}C-methylmethionine and ATP. Because of its ability to label the O or N groups of potential substrates by the transfer of ^{3}H-methylmethionine, the availability of ^{14}C-*S*-adenosylmethionine led to the discovery of a number of methyltransferase enzymes. Histamine N-methyltransferase was soon found and purified and its properties described. The enzyme is highly localized in the brain, and it also has an absolute specificity for histamine. Other methyltransferases soon discovered using [^{14}C-methyl]-*S*-adenosylmethionine were PNMT, hydroxyindole O-methyltransferase, the melatonin-forming enzyme, a protein carboxymethyltransferase enzyme, and a nonspecific N-methyltransferase. This latter enzyme was found to convert tryptamine, a compound normally present in the brain, to N-N-dimethyltryptamine, a psychotomimetic agent.

These methyltransferase enzymes, together with [^{3}H-methyl]-*S*-adenosylmethionine of high specific activity were used in developing very sensitive methods for the measurement of trace biogenic amines. We were able to detect, localize, and measure octopamine, tryptamine, phenylethylamine, phenylethanolamine, and tyramine in the brain and other tissues. The methyltransferases and [^{3}H-methyl]-*S*-adenosylmethionine also made it possible to measure norepinephrine, dopamine, histamine, and serotonin in 130 separate brain nuclei. Because of the sensitivity of the enzymatic micromethods, my colleagues and I were able to show the coexistence of several neurotransmitters in single identified neurons of Aplysia (Brownstein et al., 1974). Later, Thomas Hokfelt et al. (1980), using immunohistofluorescent techniques, demonstrated the coexistence of neurotransmitters in many nerve tracts.

The Pineal Gland

I was struck by an article from Aaron Lerner's laboratory, published in 1958, that described the isolation of 5-methoxy-N-acetyltryptamine (mela-

tonin) from the bovine pineal gland, a compound that had powerful actions in blanching the skin of tadpoles (Lerner et al., 1958). This compound attracted my attention for two reasons: it had a methoxy group and a serotonin nucleus. The methoxy group of melatonin had a special attraction for me. Also, at that time, serotonin was believed to be involved in psychoses because of its structural resemblance to LSD. I thought it would be fun to spend some time working on the pineal gland, an organ that was a mystery to me. The best way to start was to concentrate my efforts on aspects of the problem that I was familiar with, such as O-methylation.

Herbert Weissbach expressed an interest in collaborating with me to work out the biosynthetic pathway for melatonin. Weissbach had already made important contributions on the metabolism of serotonin. The availability of *S*-adenosyl-l-methionine with a radioactive methyl group provided an opportunity to examine whether the pineal gland could form labeled melatonin from potential precursor compounds. When we incubated bovine pineal extracts with N-acetylserotonin and [^{14}C-methyl]-*S*-adenosyl-l-methionine, a radioactive product that we soon identified as melatonin was found (Axelrod and Weissbach, 1961). Weissbach and I then purified the melatonin-forming enzyme, which we named hydroxyindole-O-methyltransferase (HIOMT), from the bovine pineal gland. We also found another enzyme that converted serotonin to N-acetylserotonin in the rat pineal gland. From these observations, we proposed that the synthesis of melatonin in the pineal proceeds as follows: tryptophan → 5-hydroxytryptophan → serotonin → N-acetylserotonin → melatonin (Axelrod, 1974). Irwin Kopin, Weissbach, and I also found that melatonin was metabolized mainly by a microsomal enzyme via 6-hydroxylation. In a study of the tissue distribution of HIOMT we observed that the enzyme was highly localized in the pineal. This finding convinced me that the pineal was a biochemically active organ containing an unusual enzyme and product and was worth further study.

During 1960 to 1962 I spent little time doing pineal research. Most of my efforts were directed toward the biochemistry of catecholamines and the effect of psychoactive drugs. In 1962, when Wurtman joined my laboratory, I thought that he should devote most of his time to catecholamine research. As a medical student Wurtman had already made an important finding that bovine pineal extracts blocked gonadal growth in rats induced by light. Although pineal research was not a fashionable subject for research then, Wurtman and I were caught up by the romance of this organ, so we decided to spend our spare time working on the pineal. We thought that a good place to start was the isolation of the gonad-inhibitory factor of the pineal. Neither of us wanted to go through a tiresome isolation and bioassay procedure, and we decided to take a chance and examine the effects of melatonin. We found that melatonin reduced ovarian weight and decreased the incidence of estrus in the rat (Wurtman and Axelrod, 1965).

Wurtman and I turned our attention to the effects of light on the biochemistry of the pineal. We found that keeping rats in the dark for a period of time increased HIOMT activity, compared to those kept in continuous light. This experiment gave Wurtman and me a biochemical marker to study how light transmits its message to an internal organ. Ariens Kappers had found that the pineal is innervated by sympathetic nerves arising from the superior cervical ganglia. This finding suggested an experiment to determine the effects of light on the pineal by removing the superior cervical ganglia and examining the effects of light and dark on the HIOMT. When the superior cervical ganglia were removed, the effects of light on HIOMT were abolished. This experiment told us that the effects of light on melatonin synthesis were mediated via sympathetic nerves arising from the superior cervical ganglia.

In 1964, Sol Snyder joined my laboratory as a postdoc, and he too was fascinated by pineal research. Quay had just made an important observation that the levels of serotonin, a precursor of melatonin, in the pineal are high during the day and low at night. Snyder and I developed a very sensitive assay for measuring serotonin in a single pineal. This gave us the opportunity to study how the serotonin rhythm, which can serve as a marker for the melatonin rhythm, is regulated by light in a tiny organ such as the pineal. We found that in normal rats in continuous darkness, or in blinded rats, the daily serotonin rhythm in the pineal persisted (Snyder et al., 1965). This finding indicated that the indoleamine rhythms in the pineal were controlled by an internal clock. Keeping rats in constant light abolished the circadian serotonin rhythm, showing that light somehow stopped the biological clock. These experiments were the first demonstration that the rhythms of indoleamines in the pineal were endogenous and that they were synchronized by environmental light stimuli. We found that the circadian serotonin rhythm was abolished after ganglionectomy and also after decentralization of the superior cervical ganglion, indicating that the circadian clock for the serotonin and presumably the melatonin rhythm resided somewhere in the brain. Wurtman and I published an article in *Scientific American* in which we suggested that the pineal serves as a neuroendocrine transducer, converting light signals to hormone synthesis via the brain and noradrenergic nerves (Wurtman and Axelrod, 1965).

Harvey Shein, a psychiatrist at McLean Hospital, Wurtman, who was then at the Massachusetts Institute of Technology (MIT), and I decided to see whether the rat pineal in organ culture metabolized tryptophan to melatonin, and it did. This finding provided an opportunity to examine whether the neurotransmitter of the sympathetic nerve, norepinephrine, could affect the synthesis of melatonin in pineal organ culture. The addition of norepinephrine to rat pineals in organ culture increased the synthesis of melatonin from tryptophan. Shein and Wurtman then showed that noradrenaline specifically stimulated the ß-adrenergic receptor.

For two years after 1970 I did little work on the pineal until Takeo Deguchi, a biochemist from Kyoto, joined my laboratory. Because interest in receptors was beginning to grow at that time, we decided that the pineal gland would be a good model to study the regulation of the ß-adrenergic receptor. The activity of the ß-adrenergic receptor could be determined by measuring changes in serotonin N-acetyltransferase (NAT). David Klein previously showed that pineal serotonin NAT had a marked circadian rhythm that was controlled by a ß-adrenergic receptor (Klein and Weller, 1970). Deguchi and I devised a rapid assay for NAT and soon confirmed Klein's findings. We then found that the nighttime rise in NAT was abolished by ß-adrenergic blocking agents, reserpine, decentralization, ganglionectomy, and agents that inhibit protein synthesis (Axelrod, 1974). This finding told us that noradrenaline released from sympathetic nerves innervating the pineal gland stimulated the ß-adrenergic receptor, which then activated the cellular machinery for the synthesis of NAT. Blocking the ß-adrenergic receptor with propranolol at night or exposing rats to light also caused a rapid fall in NAT. These results indicated that unless the ß-adrenergic receptor is stimulated by norepinephrine at a relatively high frequency, NAT rapidly decays. We thought that the rapid synthesis and decrease in NAT would provide a useful model to study the molecular events in receptor-linked synthesis of a specific protein (NAT) leading to the formation of a hormone (melatonin).

The regulation of supersensitivity and subsensitivity of receptors is an important biological problem. The rapidly changing pineal NAT provided a productive approach to study the mechanism of super- and subsensitivity of the ß-adrenergic receptor (Axelrod, 1974). Procedures that depleted the neuronal input of noradrenaline in the rat pineal (denervation, constant light, or reserpine) caused a superinduction of NAT when rat pineals were cultured and treated with the ß-adrenergic agonist, 1-isoproterenol. When pineal ß-adrenergic receptors were repeatedly stimulated by injections of 1-isoproterenol into rats, the cultured pineals became almost unresponsive to the ß-adrenergic agonist. In collaboration, my postdoctoral fellows Jorge Romero and Martin Zatz and I showed that the regulation of NAT and subsequent melatonin synthesis consists of a complex series of steps involving ß-adrenergic receptors, cyclic AMP, cyclic GMP, protein kinases, specific activation of mRNA for NAT, and synthesis of NAT. Decreased nerve activity induced by light caused an increase in receptor number and adenylate cyclase and kinase activity. This cascade of events then explained why a small change in release of noradrenaline from nerves causes a large change in pineal NAT. With the onset of darkness, there is an increase in sympathetic nerve activity that acts on the supersensitive receptor, cyclase, kinase, etc. This, we believed, considerably amplifies the signal (norepinephrine) to cause the large nighttime rise in NAT formation. Klein

later showed that norepinephrine acting on an α1-adrenergic receptor further amplified the NAT levels.

Later, most of the research in my laboratory was concerned with how neurotransmitters transmit their specific messages. Fusao Hirata, a visiting scientist in my laboratory, and I observed that the occupation of certain receptors stimulated the methylation of phospholipids. On the basis of these findings we proposed a mechanism for the transduction of biological signals (Hirata and Axelrod, 1980). This proposal generated considerable controversy, and the role of phospholipid methylation in signal transduction still remains to be resolved. With the collaboration of several postdoctoral fellows, we reported on the interaction of stress hormones (catecholamines, ACTH, and glucocorticoids) and the multireceptor release of ACTH (Axelrod and Reisine, 1984).

Recent Research

In 1984, I retired from government service at the age of 72. I had no intention to stop doing research. Fortunately I was invited to join the Laboratory of Cell Biology at the NIMH as a visiting scientist by my former postdoc, Mike Brownstein. Mike generously gave me laboratory space, a small office, and funds to continue my research.

About the time I retired, an explosive growth occurred in our knowledge concerning neurotransmitter receptors and how they transduce their specific messages into the cell. It was observed that ligands bind to receptors and activate GTP binding proteins (G proteins). G proteins are heterotrimers composed of α, ß, and γ subunits. On activation, the G protein dissociates into α and ßγ subunits (Birnbaumer, 1990). The α subunit then stimulates effector systems to generate second messengers. My interest in phospholipase A_2 as an effector enzyme and arachidonic acid as a second messenger stemmed from a previous observation that the chemotactic peptide f-met-leu-phe liberated arachidonic acid from neutrophils (Hirata et al., 1979). A direct association between the amount of arachidonic acid released by f-met-leu-phe and the extent of chemotaxis was found.

Using a thyroid cell line, FRTLS, we (Burch et al., 1986) found that noradrenaline via an α_1-adrenergic receptor stimulated the release of arachidonic acid and the two second messengers of phospholipase C, inositol triphosphate and diacylglycerol. This finding provided an opportunity to examine whether arachidonic acid arises from phospholipase A_2 or phospholipase C. The belief at that time was that arachidonic acid is released by diacylglycerol generated from phospholipase C. In a series of experiments using inhibitors of α_1-noradrenergic agonists, phospholipase C activators and inhibitors of G proteins, we demonstrated that the noradrenaline via an α_1-adrenergic receptor can release arachidonic acid by the activation of

phospholipase A_2 and that this phospholipase is linked to G proteins. The characterization of the G protein associated with phospholipase A_2 remains to be determined. Subsequently, my postdocs and I have found that several neurotransmitter receptors such as bradykinin; muscarinic m_1, m_3, and m_5 receptors; and the cytokine interleukin-1 activate phospholipase A_2 and release arachidonic acid as a second messenger via G proteins (Axelrod, 1990). We and others have shown that stimulation of a single receptor can activate G proteins linked to many effectors such as adenylate cyclase, phospholipases A_2 and C, and ion channels.

The most direct evidence showing that phospholipase A_2 can activate G proteins was found by examining the effect of light on isolated rod outer segments of the bovine retina. The G protein present in rod outer segments is transducin. Like all G proteins, transducin is a heterotrimer consisting of $\alpha\beta\gamma$ subunits. In 1986, Carole Jelsema and I found that the $\beta\gamma$ dimer of transducin can activate phospholipase A_2 in the rod outer segments. These observations contradicted the dogma at that time that only the α subunit can activate effector systems. The $\beta\gamma$ dimer also serves to reassociate with the α subunit to terminate the actions of the receptor ligand (Birnbaumer, 1990). We submitted our findings to *Nature* and after many months of review our manuscript was rejected. Our findings on the effect of the $\beta\gamma$ dimer were subsequently published in the *Proceedings of the National Academy of Sciences* (Jelsema and Axelrod, 1987). Subsequently, many papers were published showing that the $\beta\gamma$ dimers can activate many effectors (Clapham and Neer, 1993) such as phospholipase C, receptor kinase, yeast mating factor, adenylate cyclase, and ion channels.

Because of my long-standing interest in psychoactive drugs, my coworkers and I have been involved for the past few years in an investigation of cannabinoids. The hemp plant *Cannabis sativa*, the source of marijuana and hashish, has been used for thousands of years for its medicinal and euphoric effects. The psychoactive principle of marijuana was isolated and identified as delta-9-tetrahydrocannabinol (THC). About 35 years ago my colleagues and I reported on the physiological disposition and metabolism of ^{14}C-THC in humans (Lemberger et al., 1970). We found that ^{14}C-THC and its metabolites were excreted for more than eight days. THC was then shown to be stored in body fat (Kreuz and Axelrod, 1973).

My interest in cannabinoids was recently revived by the identification and cloning of the cannabinoid receptor in the brain by my colleagues in the Laboratory of Cell Biology (Matsuda et al., 1990). This receptor was found to be a member of the G protein superfamily that spans the plasma membrane seven times. The cannabinoid receptor is functionally coupled to the inhibition of adenylate cyclase and N-type calcium channels (Felder et al., 1993). The presence of a cannabinoid receptor in the brain indicated the existence of a natural ligand for this receptor. The endogenous ligand for the cannabinoid receptor was isolated from the brain, identified as arachi-

donylethanolamide and named anandamide (Devane et al., 1992), derived from ananda, the Sanskrit word for bliss. Anandamide was found to bind to the transfected human cannabinoid receptor with high affinity and to inhibit adenylate cyclase and N-type calcium channels (Felder et al., 1993). When injected into rodents, anandamide induces hypomotility and hypothermia (Crawley et al., 1993).

The enzyme that synthesizes anandamide was found in brain membranes (Devane and Axelrod, 1994). This enzyme, anandamide synthase, acts by conjugating arachidonic acid and other fatty acids with ethanolamine. Arachidonic acid was found to be the best substrate for this enzyme.

Anandamide synthase activity was found to be highest in the hippocampus followed by thalamus, cortex, and striatum, and lowest in the cerebellum, pons, and medulla. The ability of brain tissues to synthesize anandamide enzymatically, and the presence of specific receptors for this compound suggest the presence of anandamide-containing (anandaergic) neurons. Experiments with cultured brain cells demonstrated a receptor-evoked synthesis and release of anandamide from neurons, suggesting that anandamide is a novel neurotransmitter.

Little is known about the physiological role of anandamide and its pharmacological effects at high doses. Recent experiments using hippocampal slices showed that anandamide can inhibit long-term potentiation, a form of memory. Anandamide also blocks long-term transformation of GABAergic synaptic inhibition to synaptic excitation (Collin et al., 1995). Research on anandamide has a promising future. It has the potential to become a member of a new class of neurotransmitters (fatty acid amides) and I hope to be occupied with research on this compound for some time.

Afterword

F. Scott Fitzgerald once stated that there are no second acts in American lives. After a mediocre first act, my second act was a smash. So far the third act has not been so bad. I often reflect on why I succeeded in research. For someone with my educational, social, and economic background it would be unlikely that I would have made it. In today's climate of intense competition for positions and funds it would have been almost impossible for a late bloomer like myself to get started. 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. 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.

References

Armstrong MD, McMillan A. Identification of a major urinary metabolite of norepinephrine. *Fed Proc* 1957;16:146.

Axelrod J. An enzyme for the deamination of sympathomimetic amines. *J Pharmacol Exp Ther* 1954;110:2.

Axelrod J. The enzymatic N-demethylation of narcotic drugs. *J Pharmacol Exp Ther* 1956a;117:322–330.

Axelrod J. Possible mechanism of tolerance to narcotic drugs. *Science* 1956b; 124:263–264.

Axelrod J. Noradrenaline: fate and control of its biosynthesis. In: *Les Prix Nobel.* Stockholm: Imprimerieal Royal P.A. Norstedt and Soner, 1971;189–208; Science 1971;173:598–606.

Axelrod J. The pineal gland: a neurochemical transducer. *Science* 1974; 184:1341–1348.

Axelrod J. Following the methyl group. In: Matthysee S, ed. *Psychiatry and the biology of the human brain.* A symposium dedicated to S.S. Kety. New York: Elsevier/North Holland, 1981;5–14.

Axelrod J. The discovery of the microsomal drug-metabolizing enzyme. *Trends Pharmacol Sci* 1982;3:383–386.

Axelrod J. Receptor-mediated activation of phospholipase A_2 and arachidonic acid release in signal transduction. *Biochem Soc Trans* 1990;503–508.

Axelrod J, Reichenthal J. The fate of caffeine in man and a method for its estimation in biological material. *J Pharmacol Exp Ther* 1953;107:519–523.

Axelrod J, Reisine T. Stress hormones: their interaction and regulation. *Science* 1984;224:452–459.

Axelrod J, Weissbach H. Purification and properties of hydroxyindole-O-methyl transferase. *J Biol Chem* 1961;236:211–213.

Axelrod J, Schmid R, Hammaker L. A biochemical lesion in congenital, nonobstructive, non-hemolytic jaundice. *Nature* 1957;180:1426–1427.

Axelrod J, et al. On the mechanism of tachypylaxis to tyramine in the isolated rat heart. *Br J Pharmacol* 1962;19:56–63.

Birnbaumer L. G proteins in signal transduction. *Ann Rev Pharmacol Toxicol* 1990;30:675–705.

Brodie BB, Axelrod J. The fate of acetanilide in man. *J Pharmacol Exp Ther* 1948;94:429–438.

Brodie BB, Axelrod J. The fate of acetophenetidin (phenacetin) in man and methods for the estimation of acetophenetidin and its metabolites in biological materials. *J Pharmacol Exp Ther* 1949;97:58–67.

Brodie BB, Gillette JR, LaDu B. Enzymatic metabolism of drugs and other foreign compounds. *Ann Rev Biochem* 1958;27:427–484.

Brownstein MJ, et al. Coexistence of several putative neurotransmitters in single identified neurons of Aplysia. *Proc Natl Acad Sci USA* 1974; 71:4662–4665.

Burch RM, Luini A, Axelrod J. Phospholipase A_2 and phospholipase C are activated by distinct GTP binding proteins in response to α_1-adrenergic stimulation. *Proc Natl Acad Sci USA* 1986;83:7201–7205.

Cantoni GL. Adenosyl methionine: a new intermediate formed enzymatically from 1-methionine and adenosine triphosphate. *J Biol Chem* 1953;187:439–452.

Clapham DE, Neer E. New roles for G-protein ßγ dimers in transmembrane signalling. *Nature* 1993;365:403–406.

Collin D, Devane WA, Dahl D, Lee DS, Axelrod J, Alkon DE. Long term synaptic transformation of hippocampal CA1 γ-aminobutyric synapses and the effect of anandamide. *Proc Natl Acad Sci USA* 1995;92:10167–10171.

Crawley JN, Corwin RL, Robinson JK, Felder CC, Devane WA, Axelrod J. Anandamide, an endogenous ligand of the cannabinoid receptor induces hypomotility and hypothermia *in vivo* in rodents. *Pharmacol Biochem Behav* 1993;46:967–972.

Devane WA, Axelrod J. Enzymatic synthesis of anandamide, the endogenous ligand for the cannabinoid receptor, by brain membranes. *Proc Natl Acad Sci USA* 1994;91:6698–6701.

Devane WA, Hanuş L, Breuer A, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992;258:1946–1949.

Felder CC, et al. Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor mediated signal transduction. *Proc Natl Acad Sci USA* 1993;90:7612–7660.

Glowinski J, Axelrod J. Inhibition of uptake of tritiated-noradrenaline in the intact brain by imipramine and structurally-related compounds. *Nature* 1964;204:1318–1319.

Hertting G, Axelrod J. The fate of tritiated-noradrenaline at the sympathetic nerve endings. *Nature* 1961;192:172–173.

Hertting G, Axelrod J, Kopin IJ, Whitby LG. Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. *Nature* 1961;189:66.

Hirata F, Axelrod J. Phospholipid methylation and biological signal transmission. *Science* 1980;209:1082–1090.

Hirata F, Corcoran BA, Venkatasubramanian K, Schiffman E, Axelrod J. Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. *Proc Natl Acad Sci USA* 1979;76:2640–2643.

Hokfelt T, Johansson A, Lundberg HM, Schultzberg M. Peptidergic neurons. *Nature* 1980;284:515–521.

Jelsema C, Axelrod J. Stimulation of phospholipase A_2 activity in bovine rod outer segments of the ßγ subunits. *Proc Natl Acad Sci USA* 1987; 84:3623–3627.

Klein DC, Weller JL. Indole metabolism in the pineal gland: a circadian rhythm in N-acetyltransferase. *Science* 1970;169:348–353.

Kreuz D, Axelrod J. Delta 9-tetrahydrocannabinol: localization in body fat. *Science* 1973;179:391–393.

Lemberger L, Silverstein SD, Axelrod J, Kopin IJ. Marijuana: studies on the disposition and metabolism of delta 9-tetrahydrocannabinol in man. *Science* 1970;170:561–564.

Lerner B, et al. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J Am Chem Soc* 1958;80:2587.

Matsuda LA, et al. Structure of the cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990;346:561–564.

Quinn GP, Axelrod J, Brodie BB. Species, strain and sex differences in metabolism of hexobarbital, amidopyrine, antipyrine and aniline. *Biochem Pharmacol* 1958;1:152–159.

Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of the supportive evidence. *Am J Psychiatry* 1965;122:509–522.

Snyder SH, Zweig M, Axelrod J, Fischer JE. Control of the circadian rhythm in serotonin content of the rat pineal gland. *Proc Natl Acad Sci USA* 1965;53:301–305.

Thoenen H, Tranzer JP. Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. *Naunyn Schmiedebergs Arch Pharmacol* 1968;261:271–288.

Thoenen H, Mueller RA, Axelrod J. Increased tyrosine hydroxylase after drug induced alteration of sympathetic transmission. *Nature* 1969;221:1264.

Weinshilboum R, Thoa NB, Johnson DG, Kopin IJ, Axelrod J. Proportional release of norepinephrine and dopamine ß-hydroxylase from sympathetic nerves. *Science* 1971;174:1349–1351.

Whitby LG, Hertting G, Axelrod J. Effect of cocaine on the disposition of noradrenaline labeled with tritium. *Nature* 1960;187:604–605.

Wurtman RJ, Axelrod J. The pineal gland. *Sci Am* 1965;213:50–60.

Wurtman RJ, Axelrod J. Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. *J Biol Chem* 1966;241: 2301–2305.