I graduated from tuition-free College of the City of New York (CCNY) in 1933. At that time the country was in the depths of the Great Depression, and there were few positions available for City College graduates. Its students were mainly children of immigrants who were bright and highly motivated. CCNY graduates included eight future Nobel Prize awardees and several distinguished biochemists.

After graduating from City College, I obtained a position at the Harriman Research Laboratories at New York University. Though it paid $25 a month, I was delighted to work in a laboratory. I assisted Dr. K. G. Falk in his research on enzymes in malignant tumors. Dr. Falk was trained as a biochemist in the laboratories in Europe. In 1924, Falk wrote a monograph on “The Chemistry of Enzyme Action.” Falk was greatly influenced by Richard Willstätter, an eminent German biochemist. Willstätter believed that enzymes were catalysts of low molecular weight adsorbed on colloidal carriers such as proteins.

In 1935 I was lucky to get a position as a chemist in the Laboratory of Industrial Hygiene for $40 a week. This laboratory was a nonprofit organization and was set up by the New York City Department of Health to test the amount of vitamin supplements added to foods. In the 1930s vitamins were a hot subject in biochemistry. My duties were to modify published methods for vitamins so that they could be assayed in various food products. It required some ingenuity to modify methods for the measurement of vitamins from the literature. This experience proved useful in my later research.

First Experience in Research: The Metabolism of Analgesic Drugs

In 1946, there was an unexpected change in my career. At that time, analgesic drugs such as acetanilide and phenacetin were widely used. Some people who used excessive amounts of these drugs became habituated and developed methemoglobinemia. An independent organization, the Institute for the Study of Analgesic and Sedative Drugs, approached Dr. George B. Wallace, then president of the Laboratory of Industrial Hygiene and retired Chairman of the Department of Pharmacology at New York University, for advice. Dr. Wallace asked me if I would like to work on this problem and he suggested that I consult Dr. Bernard B. Brodie.

Bernard Brodie (Fig. 1) was a professor in the Pharmacology Department at New York University doing research at Goldwater Memorial Hospital in New York City. Goldwater Memorial Hospital was set up during World War II to clinically test newly synthesized antimalaria drugs. Brodie was responsible for developing methods for measuring blood levels of these drugs to establish the most effective dosage regimen. Soon after the end of the war, Brodie and his assistant Sidney Udenfriend published a series of influential papers in the Journal of Biological Chemistry on “The Estimation of Basic Organic Compounds in Biological Materials.”

I met with Brodie in February 1946 to discuss the cause of toxicity of acetanilide. It was a fateful meeting for me. Brodie invited me to spend time in his laboratory to work on this problem. One of the possible transformation products of ingested acetanilide causing the toxic
effects could be aniline (Fig. 2). One important lesson I learned from my discussions with Brodie was to ask the right questions at the right time and devise the means to answer these questions.

From my previous experience, I learned how to develop methods. Within a few weeks I developed a colorimetric method to measure aniline in blood and urine by diazotizing the amino group and coupling with a dye. After taking acetanilide orally, I identified aniline in my urine. This was one of the most exhilarating experiences in my life, making an important new discovery.

Experiments in dogs showed that there was a direct relationship between the concentration of plasma aniline and methemoglobinemia after the administration of acetanilide. After the oral administration of acetanilide to humans acetanilide was almost completely metabolized. Aniline represented only about 4% of the ingested acetanilide.

A common transformation of compounds containing a benzene ring is hydroxylation on the para position. Thus a possible metabolic product after the ingestion of acetanilide could be $N$-acetyl-$p$-aminophenol. Within a few weeks we identified the major metabolic product in humans after the oral administration of acetanilide as $N$-acetyl-$p$-aminophenol and its conjugates, sulfate and glucuronide. The route of metabolism of acetanilide in humans was found to proceed as shown in Fig. 2. $N$-Acetyl-$p$-aminophenol was found to be as potent as acetanilide in analgesic activity. By taking serial plasma samples, acetanilide was rapidly transformed to $N$-acetyl-$p$-aminophenol. After the administration of $N$-acetyl-$p$-aminophenol, negligible amounts of methemoglobin were formed.

In our paper published in 1948 (1), Brodie and I stated “the results are compatible with the assumption that acetanilide exerts its actions through $N$-acetyl-$p$-aminophenol (now known as acetaminophen); it is possible, therefore, that it might have distinct advantages over acetanilide as an analgesic.” This was my first taste of research and I loved it. “The Fate of Acetanilide in Man” (1) was my first paper and I was determined to continue doing research.

Several pharmaceutical companies subsequently began to sell products containing $N$-acetyl-$p$-aminophenol. However, aspirin still dominated the analgesic market. In the early 1970s Johnson & Johnson marketed $N$-acetyl-$p$-aminophenol as Tylenol. Because aspirin might produce gastrointestinal ulcers, Tylenol became one of the best selling analgesics.

Brodie invited me to stay on at Goldwater Memorial Hospital to study the fate of other analgesic drugs. We received a small grant from the Institute for the Study of Analgesic and Sedative Drugs. Another analgesic drug we studied was antipyrine. We found that this drug distributes like body water. The first paper I published as coauthor in the Journal of Biological Chemistry in 1949 was “The Use of the Antipyrine in the Measurement of Total Body Water in Man” (2).
Move to the National Institutes of Health

Because I did not have a doctoral degree, advancement was unlikely in a hospital associated with an academic institution. In 1949, James Shannon was appointed director for intramural research at the newly formed National Heart Institute in Bethesda, Maryland. I applied for a position at the National Heart Institute and Shannon accepted me. In 1949 the government expanded the original National Institute of Health to a number of medical institutes to form the National Institutes of Health.

At that time, many scientists believed that medical research in government laboratories was mediocre. When Sid Udenfriend, a postdoctoral fellow at Washington University, was offered a position in the National Heart Institute, he asked Carl Cori, his laboratory chief, for advice. Cori told Udenfriend that working in a government laboratory would be the end of his research career.

Shannon persuaded Brodie to come to Bethesda as the Chief of the Laboratory of Chemical Pharmacology at the National Heart Institute. I was assigned to the Laboratory of Chemical Pharmacology in Building 3. This three-story building on the Bethesda campus of the NIH became one of the most fertile research settings in the world. Among the scientists working in Building 3 in the early 1950s, more than half became members of the National Academy of Science, five became Nobel laureates, and three were appointed directors of the NIH.

The ambience in Building 3 was highly stimulating. Everyone knew each other and their research. As the immunologist and essayist Lewis Thomas so eloquently stated, "The National Institutes of Health is not only the largest institution for biomedical science on earth, it is one of the nation’s great treasures. As social interventions for human betterment go, this is one standing proof that, at least once in a while, government possesses the capacity to do something unique, imaginative, useful, and altogether right."

Metabolism of Caffeine, Amphetamines, and Ephedrine

The first problem I chose was the physiological disposition of the widely used compound caffeine in man. I developed a sensitive and specific method for measuring caffeine in biological material. The plasma half-life of caffeine in man and the distribution in dog tissues were determined (3).

I soon became intrigued with the sympathomimetic amines. In 1910, Barger and Dale (4) found that $\beta$-phenylethanolamine derivatives simulated the effects of sympathetic nerve stimulation with varying degrees of intensity and precision, and they coined the term sympathomimetic amines. Some sympathomimetic amines produced unusual behavioral effects. Amphetamine and methamphetamine in large doses produced symptoms of paranoia. Mescaline, the active principle of peyote, caused hallucinations.

In 1952 little was known about the metabolism of these amines. Because of my experience in drug metabolism, I decided to study the metabolism of ephedrine and amphetamine in a number of animal species. The first amine I examined 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 (5) in 1930 to elevate blood pressure. I soon found that ephedrine was metabolized in animals (dogs, guinea pigs, rats) by two pathways, demethylation and hydroxylation on the benzene ring, to yield metabolites that had pressor activity (6).
I then examined the metabolism of amphetamine and methamphetamine (7). These compounds were transformed by a variety of pathways including hydroxylation, demethylation, deamination, and conjugation. Marked species variations in the transformation of these drugs were also found.

**Microsomal Drug-metabolizing Enzymes**

Over the past 150 years biochemists and pharmacologists have observed that almost all chemical compounds ingested are metabolized by a variety of biochemical changes. Depending on the chemical structure, the body can inactivate or activate drugs and foreign compounds by chemical transformation. In some cases toxic as well as pharmacologically active metabolites can be formed. In 1953 little was known about the enzymes involved in metabolizing drugs and foreign compounds. The ability of animals to metabolize amphetamines and ephedrine by a variety of metabolic pathways stimulated my interest in finding the enzymes involved in these transformations.

I was hesitant to do enzymology; I believed it required special training and aptitude. Gordon Tomkins, then a postdoctoral fellow who shared my laboratory, gave me good advice. He told me all I needed to start was a method for measuring amphetamine and ephedrine, an animal liver, and a razor blade.

In January, 1953 I did my first *in vitro* experiment. To my great pleasure, amphetamine almost completely disappeared when I incubated this drug with rabbit liver slices in a Krebs-Ringer solution. In the following experiment I homogenized the rabbit liver and found that the metabolism of amphetamine was increased when a cofactor, TPN (NADP), was added. I decided to examine which subcellular fraction was transforming amphetamine. Schneider (8) had developed a method for separating the various subcellular fractions by homogenizing tissues in isotonic sucrose and subjecting the homogenate to differential centrifugation. After separation of the nuclei, mitochondria, microsomes (homogenized endoplasmic reticulum), and the cytosol, none of these fractions were able to metabolize amphetamine even in the presence of added TPN. However, when microsomes and the cytosolic fractions were combined, amphetamine rapidly disappeared upon the addition of TPN.

Before going any further I decided to identify the metabolic products of amphetamine. When the combined microsomal and cytosolic fractions were incubated with amphetamine and TPN, ammonia and phenylacetone were identified (9). These results indicated that amphetamine was deaminated by an oxidative enzyme in rabbit liver requiring TPN. This experiment also suggested that the enzyme was present either in the microsomes or the cytosol, and one subcellular fraction supplied factors to the other fraction containing the enzyme. Because of the structure of amphetamine and the requirement of TPN, it was apparent that this enzyme was different from monoamine oxidase.

An approach that I thought was likely to give me a clue to the intracellular location of the amphetamine-deaminating enzyme was to subject each subcellular fraction to elevated temperatures. When I heated the cytosolic fraction to 55 °C for 10 min and then added unheated microsomes, amphetamine, and TPN, amphetamine was metabolized. However, after heating the microsomes to 55 °C and then adding unheated cytosolic fraction and TPN, amphetamine was no longer metabolized. This experiment told me that a heat-labile enzyme was located in the microsomes, and the cytosol provided factors necessary for the deamination of amphetamine.

I suspected that the cytosolic fraction was involved in the action of TPN. Bernard Horecker (then at the NIH) had prepared several substrates for his classic experiments on the pentose phosphate pathway. These enzymes required TPN. He generously supplied me with several substrates that I could test. The addition of glucose 6-phosphate, isocitric acid, or phosphogluconate together with TPN and dialyzed cytosol fraction to microsomes resulted in the metabolism of amphetamine. These substrates had one thing in common: they all generated TPNH (NADPH) even in the presence of oxygen. It became obvious that the cytosolic fraction was supplying a dehydrogenase and substrates to reduce TPN to TPNH. When microsomes were incubated in air with glucose-6-phosphate dehydrogenase, glucose 6-phosphate, TPN, and amphetamine, ammonia and phenylacetone were generated. To confirm that the deaminating enzyme was in the microsomes and that TPNH was a necessary cofactor, I incubated TPNH, microsomes, and amphetamine. Ammonia and phenylacetone were formed in stoichiometric amounts. DPNH could not be substituted for TPNH. In the absence of air there was no
metabolism of amphetamine. By 1954 I felt quite confident that I had found an enzyme localized in rabbit liver microsomes that deaminated amphetamine in the presence of TPNH and O₂.

At about the same time I also found that ephedrine was demethylated to norephedrine and formaldehyde by an enzyme present in rabbit microsomes that required TPNH and oxygen. I reported these findings in 1954 at the fall meeting of the American Society of Pharmacology and Experimental Therapeutics. Complete papers appeared in the *Journal of Biological Chemistry* (amphetamine) and the *Journal of Pharmacology and Experimental Therapeutics* (ephedrine) in 1955 (9, 10). Soon after my report on the TPNH-dependent microsomal enzymes was published many drugs that are metabolized by a similar enzyme system were described.

In 1957, it was found that enzymes in the microsomes that required TPNH and oxygen could also catalyze the oxidative metabolism of normally occurring compounds such as androgens to estrogen (11). In studies on the N-demethylation of narcotic drugs in a variety of species, it became apparent that there were several microsomal enzymes involved in the metabolism of foreign and normally occurring compounds (12, 13).

In 1965 Omura *et al.* (14) reported that cytochrome P450 was present in liver rat microsomes, and about the same time Estabrook and co-workers demonstrated that this hemoprotein was responsible for the oxidative metabolism of drugs and steroids (14). After many years of intensive work in several laboratories, many cytochrome P450 enzymes were purified (15).

By 1954 I had published about 25 papers, most of them independently and many of them as a solo author. I applied for a promotion at the National Heart Institute and was turned down because I did not have a doctorate. I decided to get a Ph.D. in pharmacology at George Washington University and in 1955 at the age of 42, I received a Ph.D. I decided to leave the National Heart Institute and soon was appointed to a position at the National Institute of Mental Health.

**Glucuronides and Jaundice**

Just before I left the National Heart Institute, I was intrigued by a paper that found that uridine diphosphate glucuronic acid (UDPGA) was a necessary cofactor for the formation of phenolic glucuronides in a cell-free preparation of liver (16). In a chance meeting with Jack Strominger, then a biochemist at the NIH, we discussed the possible mechanisms for the enzymatic synthesis of UDPGA. We suspected it would be formed by the oxidation of uridine diphosphate glucose (UDPG) by either TPN or DPN. In a preliminary experiment we measured the formation of morphine glucuronide after incubating microsomes and the cytosolic fraction of rat liver with UDPG and either TPN or DPN. Morphine glucuronide was formed in the presence of DPN but not TPN. An enzyme, uridine diphosphate glucose dehydrogenase (17), was purified 180-fold which carried out the following reaction: $\text{UDPG} + 2\text{DPN}^+ \rightarrow \text{UDPGA} + 2\text{DPNH} + \text{H}^+$. The work on glucuronide conjugation led to studies on the role of bilirubin glucuronide formation in jaundice. Rudi Schmidt, then at the NIH, and I observed that bilirubin was detoxified by enzymatic transformation to bilirubin glucuronide in the liver, a reaction requiring UDPGA. This led to an interesting clinical observation relating glucuronide formation and jaundice. In patients with congenital jaundice there is a marked increase in free bilirubin in the blood. This suggested to us that there must be a defect in a glucuronide-forming enzyme in this disease. The availability of a mutant strain of rats (Gunn rats) that were jaundiced made it possible to examine whether these animals had a defective glucuronide-forming enzyme (18). We showed that the Gunn rats had a low glucuronide-forming enzyme activity as compared with normal rats. Brodie and I previously showed the N-acetyl-p-aminophenol was mainly metabolized by glucuronide formation in humans (1). Plasma levels of N-acetyl-p-aminophenol and its glucuronide were then examined in patients with jaundice. Plasma levels of free N-acetyl-p-aminophenol were markedly elevated in jaundiced patients as compared with normal subjects (18).

**Move to the National Institute of Mental Health (NIMH)**

In 1955 I changed the direction of my research to the biochemistry of the nervous system and the effect of psychoactive drugs. The advice and encouragement of Seymour Kety (Fig. 3) influenced my subsequent career to a considerable degree. Seymour Kety was the first director of the intramural program of the NIMH in 1951. He established a program in Bethesda that was world class. His vision, wisdom, and integrity had an important impact on the develop-
ment of basic neuroscience and biological psychiatry. In 1948 at the University of Pennsylvania, Kety developed the nitrous oxide method for the measurement of cerebral blood flow. This germinal contribution had a profound influence on our understanding of how the brain uses oxygen and glucose in a variety of normal and abnormal conditions. Together with his student Louis Sokoloff, Kety developed methods for the measurement of brain regional blood flow based on exchanges of non-metabolized diffusible molecules between capillaries and tissues. These investigations led to the development of brain imaging by positive emission tomography.

In the mid-sixties, Kety and co-workers initiated a major study on the genetics of schizophrenia. Using the Danish population registry, Kety and Fini Schulsinger used adoption as a means of separating environmental and genetic factors in the transmission of schizophrenia among family members. From these studies they concluded that about 50% of schizophrenia was of genetic origin, and the mode of transmission appeared to be polygenic.

In addition to his scientific achievements, Kety was an outstanding administrator. When Kety stepped down as director, he became head of the Laboratory of Clinical Science at the NIMH in 1956. This laboratory became a fertile scientific organization integrating basic and clinical research related to psychiatric problems. Scientific excellence and freedom rather than conspicuous relevance were the guiding principles of the Laboratory of Clinical Science under Kety’s leadership.

When I joined the Laboratory of Clinical Science in 1955, there was no such discipline as neuroscience. There had been an intellectual and technical separation among scientists working in neurophysiology, neurochemistry, neuroanatomy, neuroparmacology, psychiatry, and neurology. During the 1960s, the barriers among these fields began to break down. Neuroscientists borrowed heavily from molecular biology, biochemistry, biophysics, genetics, and immunology. The ingenious application of new technologies made it possible to ask more sophisticated and penetrating questions regarding the nervous system and the brain. In 1968 a Society of Neuroscience was established. At the first national meeting in 1968 there were about 700 attendees; in 2001 there were 29,000.
When I joined the NIMH, I was given a small laboratory with a technician. Ed Evarts, my laboratory chief, allowed me to work on any problem that was potentially productive and important. I thought that a study on the metabolism and tissue distribution of LSD would be an appropriate problem for my new laboratory. LSD was then used as an experimental drug by psychiatrists to produce abnormal behavior. Bob Bowman at the NIH was in the process of building a spectrofluorimeter. He was kind enough to let me use his experimental model, which made it possible to develop a sensitive fluorometric assay for LSD. This instrument later became the well known Aminco-Bowman spectrofluorimeter. 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 the brain and other tissues. These newly developed methods for monoamines played an important role in the subsequent rapid expansion in neurotransmitter research.

In a seminar, Kety gave an account of the observations of two Canadian psychiatrists. They found that when epinephrine was exposed to air it was converted to adrenochrome. When adrenochrome was ingested, it produced schizophrenia-like hallucinations. Because of these behavioral effects, they proposed that schizophrenia might be caused by an abnormal metabolism of epinephrine to adrenochrome.

In searching the literature, I was surprised to find that little was known about the metabolism of epinephrine at that time (1957). Because of the provocative hypothesis about the abnormal metabolism of epinephrine in schizophrenia and my previous experience in research on compounds related in structure to epinephrine such as amphetamine, I decided to work on the metabolism of epinephrine and norepinephrine. Epinephrine was then believed to be metabolized and inactivated by monoamine oxidase. However, it was previously shown that after the administration of a monoamine oxidase inhibitor the blood pressure elevation induced by epinephrine to cats was still rapidly reversed. This indicated that enzymes other than monoamine oxidase metabolize and inactivate epinephrine. An abstract in the March 1957 issue of the *Federation Proceedings* gave me a clue. Armstrong and McMillan (19) reported that patients with epinephrine-forming tumors (pheochromocytomas) excreted large amounts of an O-methylated product 3-methoxy-4-hydroxyvanillic acid (VMA). This suggested that VMA could be formed by O-methylation and deamination of epinephrine or norepinephrine. A potential methyl donor could be S-adenosylmethionine. When I incubated S-adenosylmethionine, epinephrine, and homogenized rat liver, epinephrine was metabolized.

According to the structure of VMA the most likely site of methylation would be in the metahydroxy group of epinephrine to form 3-O-methylepinephrine (metanephrine). The metabolite formed by incubating epinephrine with S-adenosylmethionine was identified as metanephrine (20). The O-methylating enzyme was purified and was found to O-methylate norepinephrine, dopamine, L-DOPA, and catechol but not monophenols. Because of its substrate specificity we named the enzyme catechol-O-methyltransferase (COMT). The enzyme was widely distributed in tissues including the brain. Pyrogallol was found to inhibit COMT both in vitro and in vivo (21). It was observed that COMT mainly O-methylated norepinephrine that had been released from nerves. Injecting catecholamines into animal resulted in the excretion of the respective O-methylated metabolites.

Irv Kopin, then a postdoctoral fellow, and I soon identified normally occurring metabolites of catecholamines such as normetanephrine, metanephrine, 3-methoxytyramine, and 3-methoxy-4-hydroxyphenylglycol in liver and brain. As a result of the discovery of the O-methylated metabolites, metabolic pathways of catecholamine metabolism were clarified. Catecholamines were metabolized by deamination, O-methylation, glycol formation, oxidation, and conjugation to glucuronides and sulfates.

The work on COMT gave me a long lasting interest in methylation reactions. The metabolites of catecholamines have been used as a marker for many studies in biological psychiatry. Inhibitors of COMT are used in the treatment of Parkinson’s disease with L-DOPA.

**Methyltransferase Enzymes**

With the discovery of catechol-O-methyltransferase I became involved in describing several methyltransferase enzymes. To make methyltransferases more easy to detect, Donald Brown, then a postdoctoral fellow in the laboratory of a colleague, and I synthesized [14C-methyl]S-adenosylmethionine enzymatically from rabbit liver. Thus the transfer of the labeled methyl group would make the product of a potential methyltransferase radioactive. The first enzyme described by this procedure was histamine N-methyltransferase (22).
Other methyltransferases discovered using $[^{14}\text{C-}\text{methyl}]S$-adenosylmethionine were phenylethanolamine $N$-methyltransferase (the enzyme that converts norepinephrine to epinephrine), protein carboxyl methyltransferase, and tryptamine $N$-methyltransferase. This latter enzyme was found to convert tryptamine, a compound found in the brain, to $N,N$-dimethyltryptamine, a psychotomimetic agent.

The methyltransferase enzymes together with $[^{3}\text{H-}\text{methyl}]S$-adenosylmethionine of high specific activity were used to develop sensitive methods to measure trace biogenic amines in tissues. We were able to detect, localize, and measure octopamine, tryptamine, phenylethylamine, phenylethanolamine, and tyramine in the brain and other tissues (23). Because of the sensitivity of the enzymatic micromethods, my colleagues and I were able to show the coexistence of several neurotransmitters in single identified neurones of *Aplysia* (24). Later Tomas Hökfelt, using immunohistofluorescent techniques, demonstrated the coexistence of more than one neurotransmitter in single neurons in mammalian brain (25).

**Chemical Neurotransmission**

The experiments on epinephrine stimulated my subsequent research on neurotransmitters and chemical neurotransmission. Chemical neurotransmission has a colorful and unusual history. In 1895 the British physician George Oliver injected an extract of the adrenal medulla into a dog and noted a market elevation in blood pressure (26). When the British physiologist J. N. Langley, in 1901, injected an extract of the adrenal medulla *in vivo*, organs responded as if they were stimulated by the sympathetic nerves. John Jacob Abel in 1897 isolated the active principle of the adrenal medulla as L-epinephrine (61). Abel was one of the founders of the *Journal of Biological Chemistry*.

Langley’s observation prompted his student T. R. Elliot at the Cambridge University to inject epinephrine into a dog. Elliot noted that epinephrine produced a response in organs that was similar to that evoked by electrical stimulation of the sympathetic nerves. In an abstract to the British Physiological Society in 1904 (27), Elliot made the brilliant suggestion that epinephrine is released from sympathetic nerves to induce a physiological response in organs with which the nerves form junctions. Elliot was thus the first to propose that nerves communicate by the release of a chemical. Langley, who disliked theories, discouraged Elliot from publishing this idea until more facts were available. The concept of chemical neurotransmissions was not mentioned in the paper published by Elliot in the British *Journal of Physiology* (28).

The idea of chemical neurotransmission influenced the thinking of Otto Loewi, a pharmacologist at the University of Graz. In an autobiographical sketch, Loewi describes a dream, which he had in 1921, of an experiment that would prove chemical neurotransmissions (29). It was an elegant and crucial experiment. Loewi placed a frog heart with an attached vagus nerve in a bath in which the heart could be kept beating and then stimulated the vagus nerve (a nerve that reduces the heart rate). When he collected the fluid of the stimulated heart and transferred it to a denervated second heart, it slowed its beating (30). This demonstrated that the stimulated heart released a compound from the vagus nerve that slowed the unstimulated heart beat. The substance was later identified by Henry Dale as acetylcholine, the first neurotransmitter to be identified (31).

In a similar experiment, Loewi stimulated the accelerans nerve (the sympathetic nerve that increases the heart rate), and the fluid increased the beat of an unstimulated frog heart. For many years it was believed that this neurotransmitter was epinephrine. In 1946, the Swedish physiologist Ulf Von Euler isolated the neurotransmitter of the sympathetic nervous system and identified it as norepinephrine.

**Inactivation of Neurotransmitters by Uptake in Nerve Terminals**

For many years it was believed that the actions of neurotransmitters are terminated by enzymatic transformation. The neurotransmitter acetylcholine was known to be rapidly inactivated by acetylcholinesterase. Because monoamine oxidase had already been shown not to inactivate norepinephrine, I thought that norepinephrine might be inactivated by COMT. When pyrogallol, a COMT inhibitor (21), was administered to a dog it almost completely inhibited this enzyme. Despite the inhibition of COMT, the blood pressure-elevating action of injected norepinephrine was rapidly ended. This experiment indicated that there were other mechanisms for the inactivation of norepinephrine.
The answer to how catecholamines and other neurotransmitters are inactivated came in an unexpected way. When the metabolic pathway for epinephrine was described, Seymour Kety and co-workers were in a position to test the hypothesis of an abnormal metabolism of epinephrine in patients with schizophrenia. Because of the low endogenous levels of epinephrine and norepinephrine in urine, it was necessary to use [3H]epinephrine and [3H]norepinephrine of high specific activity. Using [3H]epinephrine, Kety and co-workers found no difference in the metabolism of epinephrine between schizophrenic and normal subjects.

Kety was kind enough to give me some [3H]epinephrine for my studies. At that time Hans Weil-Malherbe, a British biochemist, spent several months in my laboratory, and we developed methods for measuring [3H]epinephrine and its metabolites in tissues and plasma. When we injected [3H]epinephrine into cats, it persisted unchanged for at least 2 h in heart, spleen, and salivary gland long after the physiological effects were ended (32). We also found the [3H]epinephrine did not cross the blood-brain barrier (33). [3H]Epinephrine and [3H]norepinephrine were concentrated in organs rich in sympathetic nerves (heart, spleen, and salivary gland). This unexpected phenomenon puzzled us, but it gave us a clue regarding the inactivation of the neurotransmitter norepinephrine. This catecholamine might be taken up and sequestered in sympathetic nerves.

The crucial experiment proving that norepinephrine was taken up and inactivated by sympathetic nerves was suggested by Georg Hertting, a visiting scientist in my laboratory. In this experiment, the superior cervical ganglia of cats were removed on one side resulting in the unilateral degeneration of nerves in the eye muscles and salivary gland. Upon the injection of [3H]norepinephrine the radioactive catecholamine accumulated on the innervated side, but very little appeared on the denervated side (34). This experiment clearly indicated that the neurotransmitter norepinephrine was inactivated by uptake into sympathetic nerves.

The ability of sympathetic nerves to take up [3H]norepinephrine made it possible to label these nerves with the radioactive norepinephrine and to examine whether it can be released on nerve stimulation. Hertting and I found that injected [3H]norepinephrine taken up by sympathetic nerves was released when these nerves were stimulated (35). As a result of these experiments, we proposed that norepinephrine is inactivated by reuptake into sympathetic nerves. Other slower mechanisms for inactivation of catecholamines were by removal by the bloodstream, metabolism by O-methylation, and deamination in effector tissues, liver, or kidney.

In another experiment demonstrating uptake of norepinephrine, we injected rats with [3H]norepinephrine. Pineal glands, which are rich in sympathetic nerves, were prepared for electron microscopic autoradiography. Electron microscopy showed a striking localization of photographic grains in non-myelinated axons (36). Grain concentrations appeared only in non-myelinated axons containing dense core 500-Å vesicles. Soon after the reuptake of norepinephrine by nerves was established, the monoamine neurotransmitters, serotonin (37) and dopamine (38), were also found to be inactivated by uptake into nerves. Later it was reported that the amino acid neurotransmitters GABA (γ-aminobutyric acid), glycine, proline, and L-glutamate are taken up by neurons and inactivated (39).

About 40 years after the discovery of the uptake of neurotransmitters in nerves, the uptake sites were cloned and characterized as sodium/chloride-dependent transporters (40). Sodium provides the thermodynamic energy to pump neurotransmitters from low concentrations outside the cell to the much higher concentrations inside the cell. Chloride ions prevent changes in the resting potential of the cell. The molecular characteristics of several neurotransmitter transporter families have been described (40).

Soon after the phenomenon of reuptake was demonstrated, a study on the effect of psychoactive drugs on the uptake of norepinephrine was initiated (41). To do these experiments, we first administered the drug to cats and then measured the concentration of injected [3H]norepinephrine in various tissues. Amphetamine, imipramine, and reserpine block the uptake of [3H]norepinephrine. We also examined the effect of cocaine because this drug was found to cause supersensitivity to epinephrine. After pretreatment of cats with cocaine there was more than 80% reduction of injected [3H]norepinephrine in tissues innervated by sympathetic nerves. This experiment indicated that cocaine blocked the uptake of norepinephrine in sympathetic nerves (42). This allowed greater amounts of the neurotransmitter to remain in the synaptic cleft after cocaine and act on the post-synaptic receptors more intensely and for
longer periods of time. It was later shown that cocaine, amphetamines, and antidepressants also blocked the uptake of dopamine and serotonin (43).

Most of the early work on uptake was done in the peripheral nervous system. Because catecholamines do not cross the blood-brain barrier (33) it was impossible to study the uptake, metabolism, storage, and release of catecholamine in the brain. Jacques Glowinski, a postdoctoral fellow, devised a technique to introduce $[^3\text{H}]$norepinephrine directly into the rat brain by injection into the lateral ventricle. It was shown that injected $[^3\text{H}]$norepinephrine entered the endogenous pool of catecholamines in the brain.

With the labeling of brain noradrenergic neurons with $[^3\text{H}]$norepinephrine, Glowinski and I examined the effect of antidepressant drugs. We found that only the clinically effective tricyclic antidepressant drugs block the uptake of $[^3\text{H}]$norepinephrine into brain neurons (44). Amphetamine blocked the uptake as well as the release of $[^3\text{H}]$norepinephrine in the brain (45). The discovery of the reuptake of neurotransmitters into nerve terminals and the ability of antidepressant drugs to block reuptake of monoamine neurotransmitters led to the rapid development of more effective drugs to treat depression.

About twenty million Americans suffer from depression in any one year. It is one of the most disabling diseases, causing considerable suffering, and one of the major causes of suicide. There was no effective treatment for depression until the Swiss psychiatrist Roland Kuhn observed that imipramine, a tricyclic compound, can relieve depression in many patients (46). These findings led to the rapid screening of potential antidepressant drugs by measuring the ability of a compound to block the uptake of tritium-labeled dopamine, serotonin, and norepinephrine into synaptosomes (pinched off nerve endings) (47).

The discovery process to find an effective drug by a pharmaceutical industry takes about 6 years. Numerous potential synthetic antidepressant compounds can now be measured in a day for their ability to block the uptake of $^3\text{H}$-labeled monoamine neurotransmitters. This procedure saves the pharmaceutical industry many millions of dollars and several years of development. The most well known drug developed in this manner is fluoxetine (Prozac), an SSRI (specific serotonin uptake inhibitor). There are many other SSRI drugs available as well as antidepressant drugs that inhibit the uptake of norepinephrine.

**The Pineal Gland as a Neurochemical Transducer**

In 1958 Aaron Lerner and co-workers described the isolation of melatonin (5-methoxy-N-acetyltryptamine), a compound that blanched the skin of tadpoles, from the bovine pineal gland (48). Lerner, a dermatologist and biochemist, undertook this problem because he thought that melatonin might be useful in treating skin diseases. Melatonin attracted my attention because it had a methyl group and a serotonin nucleus.

I decided to work on the biosynthetic pathway to melatonin in collaboration with Herb Weissbach. The availability of radioactive $^3\text{S}$-adenosylmethionine provided an opportunity to examine whether the pineal gland can form melatonin from a potential precursor compound. When we incubated bovine pineal extracts with $[^{14}\text{C}-\text{methyl}]S$-adenosylmethionine and N-acetylserotonin, a radioactive product was formed that we identified as melatonin. We purified the melatonin-forming enzyme, hydroxindole-0-methyltransferase (HIOMT) from the bovine pineal gland (49). N-Acetylserotonin was found to be the best substrate for HIOMT. We also found the enzyme that converts serotonin to N-acetylsertotonin in the pineal gland (50). We proposed that the synthesis of melatonin in the pineal gland proceeds as follows: serotonin → N-acetylsertotonin → melatonin.

HIOMT was found to be highly localized in the pineal gland. Small amounts of HIOMT were also found in the retina. These observations convinced me that the pineal gland is a biochemically active organ containing an unusual enzyme and product and was worth further study.

In 1962, Richard Wurtman joined my laboratory as a postdoctoral fellow. As a medical student Wurtman had found that bovine pineal extracts blocked the growth of the rat gonads induced by environmental light. Because research on the pineal gland was a neglected subject and because of our mutual interest in this organ, Wurtman and I decided to spend some time working on the pineal gland. We thought that a good place to start was to isolate the gonad inhibitory factor from the pineal gland. Neither of us wanted to go through the tiresome isolation and bioassay procedure and we decided to study the effects of melatonin. We soon found that melatonin reduced ovary weight and decreased the incidence of estrus in the rat.

The next question that our laboratory was concerned with was how does information about environmental lighting reach the pineal gland located between the two cerebral hemispheres.
In 1960, Virginia Fiske found that exposure to environmental light for several weeks caused a decrease in pineal gland weight (51). We found that keeping rats in continuous light decreased HIOMT activity as compared with those kept in continuous darkness.

Ariens-Kappers reported that the pineal gland is innervated by sympathetic nerves arising from the superior cervical ganglia (52). When the superior cervical ganglia of rats were removed, the effect of light on HIOMT was abolished. This experiment told us that the action of light on melatonin synthesis in the pineal gland was mediated via sympathetic nerves. Quay had just made an important observation that levels of serotonin, a precursor of melatonin, are high during daylight and low at night (53). Together with Sol Snyder, a postdoctoral fellow in my laboratory, we developed a highly sensitive method to measure serotonin in a single pineal gland. This gave us the opportunity to examine the serotonin rhythm. Using serotonin as a marker, we could examine how the melatonin rhythm is regulated by light and dark in a tiny (1 mg) rat pineal gland. We found that in rats kept in continuous darkness or in blinded rats, the 24-h serotonin rhythm persisted (54). This indicated that the indoleamine rhythms are controlled by an internal clock. Keeping rats in constant light abolished the serotonin circadian rhythm. These experiments demonstrated that rhythms of indoleamines in the pineal gland were endogenous and were regulated by environmental light. We found that the circadian rhythm of serotonin was abolished after ganglionectomy or by cutting the nerves from the brain to the superior cervical ganglia. These experiments indicated that the source of messages for the circadian rhythm of melatonin resides somewhere in the brain.

In 1970, Klein and Weller (55) described a robust circadian rhythm in pineal gland serotonin N-acetyltransferase, which is 180° out of phase with that of serotonin. One hour after the onset of darkness there was a 30–50-fold rise in serotonin N-acetyltransferase activity. A circadian rhythm in pineal gland melatonin had the same phasing as that of serotonin N-acetyltransferase: high during darkness and low during daytime. Like the serotonin rhythm the serotonin N-acetyltransferase rhythm was abolished by denervation of the sympathetic nerves to the pineal gland or by interrupting nerve impulses from the brain. Bilateral lesions in the suprachiasmatic nucleus in the hypothalamus abolished the circadian rhythm of serotonin N-acetyltransferase and melatonin (56). This and other experiments established that the suprachiasmatic nucleus is the biological clock of the brain. Brownstein and I found a 24-h rhythm in the turnover of norepinephrine in the sympathetic nerves innervating the pineal gland (57). This rhythm in turnover of norepinephrine was abolished in continuous light but persisted in darkness or in blinded rats. This indicated that circadian rhythm in the pineal gland is generated by the rhythmic release of the neurotransmitter norepinephrine. This rhythm in sympathetic nerve activity is driven by the circadian pacemaker in the suprachiasmatic nucleus of the hypothalamus.

Experiments indicated that norepinephrine released from sympathetic nerves innervating the pineal gland stimulated a β-adrenergic receptor, which then activated the cellular machinery for the synthesis of serotonin N-acetyltransferase. Martin Zatz, a postdoctoral fellow, and I (58) showed that the regulation of serotonin N-acetyltransferase and the subsequent synthesis of melatonin consist of a complex series of steps involving the β-adrenergic receptor, cyclic AMP, protein kinase, and synthesis of serotonin N-acetyltransferase mRNA and protein (58).

The link whereby environmental lighting sends its message to the pineal gland and organs outside the brain has been recently clarified. Provencio and co-workers (59) described an unusual opsins-like photosensitive pigment, melanopsin, in the mouse retina residing in a small subset of retinal ganglia cells. The stimulation of melanopsin by light does not require the visual photoreceptors of the rods and cones of the retina.

In 2002 (60), it was reported that the retinal ganglion cells containing the photosensitive pigment melanopsin are connected through the retinohypothalamic tract to the suprachiasmatic nucleus, the primary circadian pacemaker of the brain. Then, as described above, the suprachiasmatic nucleus sends its signal to the superior cervical ganglia, which in turn innervates the pineal gland via norepinephrine-containing (sympathetic) nerves.

Afterthoughts

Despite a late start in my research career, it spanned 50 years. Forty-six of these years were spent at the NIH. The NIH supported my research and gave me full independence in choosing my problem and provided excellent physical and intellectual resources. It spared me the time...
writing grant proposals and relieved me of the anxieties of soliciting financial support. My freewheeling style might have made it difficult to obtain grants had I been at a university instead of the NIH.

I always maintained a small laboratory with no more than three postdoctoral fellows and an occasional visiting scientist. It gave me great pleasure to interact with my postdoctoral fellows. Joining the NIMH in 1955 was propitious. The field of neuroscience was beginning to explode. This gave me an opportunity to apply a biochemist’s approach to study the nervous system and brain. I soon learned that the brain cannot be treated like a liver. The brain has numerous areas with special chemistry and functions. This made the study of the biochemistry of the brain highly complex and challenging.

During my career in biomedical research, the work was largely its own reward. In retrospect, however, a great satisfaction that I have about my work is that it led to treatments for the relief of pain and depression.

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