2 Julius Axelrod

The discovery of reuptake

It may be of some interest to you that I actually began my research in psychopharmacology working on 5-HT reuptake into platelets and so I came across your work very early on and so I'd love to hear about how it all came about – how you stumbled on the idea of amine reuptake. We probably should begin though with how you entered the field and we can move forward from there to what you've done since.

I was born in New York from immigrant parents. My mother's side came from Vienna and my father's side from Poland. I was raised on the Lower East Side of Manhattan. It was a Jewish ghetto at that time. There had been a tremendous influx of immigrants who arrived around the beginning of the century. I was born in 1912 and I was raised in an impoverished neighbourhood but it was colourful and lively, mostly of a Yiddish culture. My parents were poor. They were barely literate, well at least in English. They were fairly well cultured in Yiddish. I went to a public school where there was a spectrum of students. Some were almost illiterate, some literate, some wound up in jail, some became fairly distinguished. I then went to Seward Park High School on Lower East Side. I wanted to go to Stuyvesant High School where the bright kids went but I didn't make it.

Why not?

Oh I don't know. I just wasn't good enough. The High School I went to though was not too bad. It had a number of interesting graduates, mostly entertainers – Zero Mostel, Walter Matthau and Tony Curtis, who were actors and Sammy Cahn the composer – but no great scholars. I read a great deal when I was young. All kinds of books. The books that interested me most and gave me a feeling of what I'd like to be were two books, one by Sinclair Lewis, Arrowsmith, *and the other was* The Microbe-Hunters *by Paul De Kruif, which was about the lives of the bacteriologists Pasteur, Ross and people like that and how they made their discoveries. My dream was to become a doctor, a research physician. I went to City College, a free college in New York City. If there hadn't been a City College, I don't think I could have afforded to go to College. It was a fairly selective school. You had to have high*

grades. I think it was an important influence on its students, mostly Jewish. It was highly intellectual and it graduated nine future Nobel laureates.

They were poor kids who were very bright. When I graduated from City College, I applied to several medical schools but couldn't get into any. At that time there were quotas for Jewish students; many of them were very bright and there were too many Jewish students applying for the limited number of places. I wasn't in the top echelon. My grades were good but they weren't extraordinarily good. I graduated from City College in 1933, during the depths of the Great Depression.

There were very few jobs and I decided to take an examination for a position with the Post Office, which I passed. At the same time, I was offered a position in a laboratory at New York University, which paid $25 a month, to help a fairly welloknown biochemist, K.G. Falk. I got an offer for the position in the post office and I had to make a fateful decision. I decided to take the laboratory position. That decision was very crucial to me. I assisted Dr Falk in his research on enzymes in malignant tissues.

In 1935 I decided to get married and I needed to make more money. A position opened up for me in a non-profit laboratory to test vitamins in foods. Vitamins were a big thing in the 1930s. I remained there until 1945. The laboratory work was fairly interesting. I thought I was set for life testing for vitamins. I spent most of my time modifying methods which was important to my future career.

At that time very few people worked in research. To do research then you had to be wealthy and smart or a physician who did research in his spare time. I had no idea about doing research but in the laboratory we had periodicals like the Journal of Biological Chemistry, which I read, so I had a sense of what was going on in biochemical research. In 1946, the Head of the laboratory was a retired professor of pharmacology, George Wallace, who was also one of the editors of the *Journal of Pharmacology, Experimental Therapeutics*

That's about the most prestigious journal.

Yes, it was. And he came to me one day and said 'Julie I have an interesting proposition for you. A group of manufacturers of analgesic drugs are having problems. Some people taking the non-aspirin analgesics acetaniline or phenacetin have come down with methaemoglobinaemia. Would you like to work on this problem?' I said 'I'd love to but I have had no experience in research of this kind'. He told me that there was an associate of his, Bernard Brodie, working at Goldwater Memorial Hospital in New York and he advised me to go and see him and discuss the problem.

This was 1946. I remember the day – it was Lincoln's birthday February 12. I telephoned Dr Brodie and he invited me to visit him. He was working at Goldwater Memorial Hospital, in a unit associated with New York University. It had been set up during the war to test antiomalarial drugs. The Japanese had cut off the supply of quinine and the US had to develop new antimalarials. Goldwater was devoted to clinically testing new synthetic antimalarials. The head of antimalarial research at Goldwater

was James Shannon. He was instrumental in later making the NIH what it is now. He was an MD working on secretory mechanisms in the kidney. During the War he was asked to set up a clinical laboratory testing the new antimalarials that were being synthesized. One of his great qualities was that he had a good nose for picking people. What he did was call up the professors of pharmacology throughout the country. 'Send me your best people', he told them. And they did – of course it was either that or going somewhere in the Pacific. So Shannon picked Brodie to do research on the physiological disposition and metabolism in man of the synthetic antimalarials.

Brodie was born in the UK, wasn't he?

He was born in Liverpool. He spent his youth in Canada. He was a graduate of McGill. He was an interesting and colourful person. Somebody told me he had been a boxer and also that at one point he had earned his living by playing poker.

He was 40 years old, when I first met him, 6 years older than me. But to me he was of a different generation. What he did was really revolutionary for that time. He measured plasma levels of drugs. And to do that he devised methods to measure the antimalarial drugs. There was a series of germinal papers that he published in the *Journal of Biological Chemistry*, with his close associate Sidney Udenfriend. To get back to my problem, I called Brodie up. Everyone called him Steve Brodie. There had been a Steve Brodie who lived in Brooklyn and one day he said to some people in a bar that he could jump off the Brooklyn bridge if anyone wanted to bet him. He did and survived. They called Bernard Brodie, 'Steve' because he was always prepared to take a chance.

You have to remember, when I visited him at that time, all I had was a masters degree in chemistry. While I worked in the food testing laboratory, I had taken a masters degree in the evenings after work at New York University. I came to Brodie with the problem of the toxicity of acetanilide. He told me that drugs or foreign compounds are transformed in the body. I vaguely knew this but this was an important piece of information for me. He suggested that it was possible that these analgesic drugs were transformed into toxic metabolites. I put the structure of acetanilide on the blackboard. We conjectured that it was possible that one of the metabolic transformation products would be deacetylation to form aniline. I looked up the literature and found that aniline could cause methaemoglobinaemia.

One of the most important things that I learnt that day was to ask the right questions and not only to ask the right question but know how to answer these questions – to have the right methods. Dr Brodie then invited me to spend some time at Goldwater to find out whether we could find aniline in the blood or the urine after acetanilide. We had to develop very sensitive methods to measure aniline. Brodie was one of the

world's experts in developing methods because of the antimalarial research. We soon developed a method for measuring aniline (Brodie and Axelrod, 1948a) and sure enough when we took acetanilide – myself and others – we found traces of aniline in the urine (Brodie and Axelrod, 1948b).

We also developed a method for measuring it in the blood and we found it in the blood after taking acetanilide. We showed that there was a direct relationship between the amount of aniline in the blood and methaemoglobinaemia. Brodie and I solved that problem – it didn't take us very long. I just loved doing it; I'd never had experience of doing this kind of thing – particularly with a charismatic person like Steve Brodie.

There are mixed views about him.

He had charisma but he also had a lot of other problems but that is something else. He was very stimulating. He was almost magnetic. He fired you up. It wasn't just me, he did it to many people. So here I was really doing important work. We found that aniline only represented a few percent of the metabolic product; most of acetanilide was metabolized to something else. We looked for other metabolites of acetanilide and we found a compound which we identified as N-acetylparaminophenol. Brodie had this compound tested for analgesia and it was just a good an analgesic as acetanilide.

So you guys had a new drug then?

Yes, it is now called acetaminophen, commonly known as Tylenol. We recommended when we first wrote about it in the literature (Brodie and Axelrod, 1948b) that it should be used as an analgesic. Well, it took off. Anyway, I just loved doing research. I worked on the metabolism of antipyrine and phenacetin. I published many papers with Brodie but I got only one senior authorship, although I initiated and did most of our work. And I realized that I had very little chance getting any place in an academic institution with a masters degree. I needed a PhD. I was married with two children. Either I didn't want to or was afraid it would be too difficult to get a PhD. I didn't want to think about it.

I saw an item in the *New York Times* – Dr Shannon had been appointed the Director of the National Heart Institute in Bethesda. I wrote to him for a position and he offered me one. He also persuaded Brodie to come to Bethesda and when I went there I was assigned to Brodie's laboratory. I worked for a year or two and then I was offered a position in a drug company. When I told Brodie I would like to leave, Dr Brodie asked me what would make me stay. I told him that I wanted to do my own research. Brodie agreed and asked me to stay.

The first problem I worked on was the metabolism of caffeine. Nobody knew anything about what happened to caffeine in the body. I published the first report on its fate. I also became interested in a group of compounds called sympathomimetic amines, and I worked on the metabolism

of ephedrine and amphetamine and published the first report on their metabolism.

At that time there was one problem that intrigued pharmacologists, which was how did the body know how to transform all of these synthetic compounds? There must be endogenous enzymes and I became very interested in this problem – this has been written up in a book called *Apprentice to Genius* by Robert Kanigel (see Glossary). It's about Brodie, me and Sol Synder. I also have a written prefatory chapter in the *Annual Reviews of Pharmacology and Therapeutics* in 1988 (Axelrod, 1988). Anything you miss now, you can find in these publications.

So I got interested in enzymes that metabolize drugs. I had a benchmate, a brilliant guy, Gordie Tomkins, who gave me a lot of good advice on enzyme research, which led to me finding a metabolite of amphetamine in a liver slice. I then found that ephedrine was also metabolized by a liver enzyme but in a different way. I wanted to find out more about this enzyme. I won't go into details but I found that there was a new class of enzyme that was present in the microsomes of the liver that required NADPH and oxygen. These enzymes metabolized both ephedrine by demethylation and amphetamines by deamination and I knew then that I was on to something very important (Axelrod, 1955a; Axelrod, 1955b).

I submitted two abstracts on the enzymatic metabolism of amphetamine and ephedrine for the usual meeting of the American Society of Pharmacology and Therapeutics. Brodie saw these later and was upset. He knew it was an important discovery and he set the whole laboratory to work on this problem. I hate to tell you this, I owe a great deal to Brodie, but this was something that upset me very much. Brodie wished to write a paper on this group of enzymes, the microsomal enzymes, as they are called now, with himself as the senior author.

I now thought I had to get my PhD and leave Brodie's lab. To get a PhD I took a year off and went to George Washington Medical School. I knew the professor very well and he said all the work on drug metabolizing enzymes would be very good for a thesis but that I would still have to take courses and pass exams – one of the courses, however, I would have to give myself, the one on drug metabolism. I did. By the time I got my PhD, Shannon had become the head of the entire NIH.

Tell me more about Shannon.

He had very good rapport with two important congressmen. One was Fogarty, the congressman from Rhode Island. And the other one was Lister Hill, a Senator from Alabama. Shannon convinced them that the best way to treat and cure diseases is not to invest large amounts of money on targeted research on diseases but to understand the fundamental process, the biology, etc. Congress were generous to the NIH while he was there. He also recruited some really top flight people to the NIH – Jim Wyngaarden, Don Fredrickson, future directors of the NIH, Christian

Anfinson, who became a Nobel laureate, and a whole lot of other excellent people.

There was considerable scepticism at the time that an arm of government, a bureaucratic institution, could possible be compatible with doing ground-breaking science; why did the NIH track-record turn out so well?

The reason why the intramural NIH and NIMH worked so well was due to Shannon's ability to convince Congress, during the period that he was director, between 1955 and 1968, that basic research was necessary to find treatments and cures for diseases. The generosity of funding meant that little grant writing was necessary and this gave the scientists and bright postdocs a free hand.

So you sent your application . . .

Yes. I sent applications out to both the National Cancer Institute and the National Institute of Mental Health and I received a call from Seymour Kety, who was at that time the Director of the intramural programme of the NIMH. He interviewed me for the position. I knew he was interested in me. He sent my application to several laboratories in the Institute. There was one laboratory I wanted to work in and that was Giulio Cantoni's, a well-known biochemist who discovered S-adenosylmethionine, but I didn't get to work with him. I was hired by Ed Evarts, a neurophysiologist and psychiatrist. I don't know if you know of him?

No, I haven't heard.

Evarts was a lovely man. He was the Head of a Laboratory of Clinical Science and he did a lot of fundamental work on the central control of motion. At that time Evarts was interested in biological psychiatry. He saw my papers on amphetamine and asked me to come and work in his laboratory. That was just as I was taking my PhD. He was working on LSD at that time. In my spare time, while going to class, I was working on the metabolism of LSD. We published a paper in *Nature* on the metabolism of LSD in 1955 (Axelrod, Brady, Witkop and Evarts, 1956). We developed a fluorescent method for measuring it and found that incredibly small amounts of LSD in the brain could cause behavioural effects.

The philosophy of Seymour Kety in the NIMH was to hire the best people you can and leave them alone because they are in the best position to know what problems are important, doable and possibly relevant to the Institution. That was a great philosophy for me. I knew nothing about neuroscience or the brain. I had worked in the Heart Institute and I felt almost intimidated by these bright physiologists and psychiatrists working on these electrical phenomena. They were all very good talkers – especially Kety.

Anyway, I started to work on the microsomal metabolism of morphine.

I had a theory of tolerance which I published in *Science* (Axelrod, 1956), which proposed a downregulation of morphine receptors – the term downregulation hadn't been coined then but in some of my experiments I showed a reduction in the number of receptors with tolerance and I proposed that this led to a need for more morphine. It was criticized at the time but I think the theory and also the experiments were not bad.

Well, anyway, I felt a little guilty because this was work on the liver – even though these were good and highly regarded papers. We used to have weekly seminars in the laboratory and at one of these Seymour Kety gave an account of the experiment by two Canadian psychiatrists, Hoffer and Osmond. Their work hadn't actually been published yet but he had heard from them that when they exposed adrenaline to the air, adrenochrome, an oxidative product of adrenaline, was formed and that when this was ingested it caused schizophrenic-like hallucinations. They proposed that schizophrenia could be caused by an abnormal metabolism of adrenaline to adrenochrome.

Anyway, I was intrigued by this. I searched the literature and there was nothing known about what happened to adrenaline in the body. I thought this would be a good problem for me because I had worked on amphetamine, which is related to adrenaline, one of the sympathomimetic amines – this fascinating group of compounds, worked on by Sir Henry Dale many years before.

First, I tried to look for the enzyme involved in forming adrenochrome. I spent three frustrating months looking for this enzyme and I couldn't find it. Then one day I came across an abstract in the *Proceedings of the Federated Society of Biology* by a biochemist, Marvin Armstrong. He found that patients with tumours of the adrenal gland excreted a large amount of what he called vanillylmandelic acid (VMA). It was a methylated compound and it struck me that this compound had to come from adrenaline. I knew about the deamination of adrenaline by the enzyme monoamine oxidase and VMA looked like it had been formed by the deamination and methylation of adrenaline. I found the methylating enzyme, catechol-ortho-methyl-transferase (COMT), that formed a compound which we called metanephrine – methylated adrenaline. It also methylated noradrenaline to a compound we called normetanephrine and we also found another metabolite called 3-methoxy-4-hydroxyphenylglycol (MHPG).

At that time, in 1955, there were two neurotransmitters known to be present in the central nervous system. One was acetylcholine and the other was noradrenaline. It was known that the mechanism for inactivation for acetylcholine was metabolism by acetylcholinesterase. But experiments showed that monoamine oxidase was not the means of inactivation of noradrenaline. I thought that COMT must, therefore, surely be the mechanism for inactivation for noradrenaline. However, just at that time we found an inhibitor for COMT. An inhibitor for monoamine oxidase,

iproniazid, was also known but Dick Crout found that when both of these enzymes were inhibited, the action of noradrenaline was still rapidly terminated, even though neither of those enzymes were working. Therefore there had to be another mechanism for the inactivation of noradrenaline.

Just at that time Kety wanted to test Osmond and Hoffer's hypothesis that schizophrenia was due to an abnormal metabolism of adrenaline. To do this he commissioned New England Nuclear to synthesize tritium-labelled adrenaline. The idea was to inject it into humans to measure the amounts of radiolabelled adrenaline and its metabolites that resulted. We had identified all the metabolic products of adrenaline by this time. Briefly, no differences were found between the amounts of radiolabelled adrenaline or its metabolites between normal males and subjects with schizophrenia. When he had done this study, I asked him if I could have some of the radiolabelled adrenaline. Hans Weil-Malherbe and I had developed a method for measuring radioactive noradrenaline.

Where did Weil-Malherbe come from?

He was German and then he emigrated to Britain. He was well known at that time. He was one of the pioneers in the study of the biochemistry of mental illness in the 1930s and 1940s. He worked in the mental hospitals in Britain. It was actually Joel Elkes who arranged for him to come to my laboratory. Hans developed a fluorescent method for measuring adrenaline, which was very non-specific but I had radioactive adrenaline which made a difference to the specificity.

Seymour was prepared to give you the radioactive compound. Did he know though how critical it was going to be to your study.

No idea. He knew I worked on the metabolism of adrenaline and was very impressed but he didn't know where it was going to lead. We injected the radioactive adrenaline into cats and we measured it in their tissues afterwards and found that unchanged adrenaline remained in certain tissues for hours, long after its effects were gone. So we knew it was being sequestered someplace. Gordon Whitby came to the lab then from Cambridge. He was doing his PhD. We decided to study the tissue distribution of radioactive noradrenaline and we found the same thing – that it persisted in certain tissues – in those tissues that were very rich in sympathetic nerves. We suspected it was being taken up into sympathetic nerves but we had to prove it.

About this time, 1959, I was attracting postdocs and visiting scientists and one of these was George Hertting from Vienna. He was a classical pharmacologist and a very good one. Hertting and I had many discussions on how to prove that radiolabelled noradrenaline was taken up by the sympathetic nerves. One day we came up with the right experiment. We removed the superior ganglion from one side of the cat. After one week

we had a unilateral denervated cat. When we injected radiolabelled noradrenaline very little was found on the denervated side, while a lot of radiolabelled noradrenaline was localized in tissues on the innervated side (Hertting, Axelrod, Kopin and Whitby, 1961a). This was the first crucial experiment to prove that noradrenaline was taken up into the nerves.

You made a marvellous comment some years later. You wrote an article in 1972 in Seminars of Psychiatry, *which said that because you were outside the field, that you were an enzymologist, you didn't come to this problem with the preconceptions that other people had.*

You have to have an open mind. One thing I tell my students when they are starting is don't read the literature too much, you might be influenced and you won't do experiments which you should do and would do if you have a naive approach.

I think that's almost the classic statement about science.

You have to be naive. You'll probably be frequently wrong but sometimes you will discover something new.

At that point there was no concept at all of a reuptake mechanism.

No. We knew we had it but we had to do further experiments. I did another experiment with George Hertting, where we perfused the spleen with labelled noradrenaline, and stimulated the splenic nerve. Every time we stimulated the nerve, there was an outflow of noradrenaline (Hertting and Axelrod, 1961b). We now knew it was taken up by nerves and released on stimulation. Then we did an experiment, where we gave phenoxybenzamine, and we found a much greater outflow – as Brown and Gillespie had also found. So we proposed that the mechanism of activation of phenoxybenzamine was to block reuptake into the neurone. We missed that one.

In the next experiment, we used radioautography with Keith Richardson, an anatomist, and David Wolfe who did radioautography. I was working on the pineal gland at that time and we knew that the pineal gland was rich in innervation from sympathetic nerves. What we did was to inject radiolabelled noradrenaline and after a few days we found that the sympathetic nerves of the pineal had a high concentration of radiolabelled noradrenaline – all of the radioactivity ended up in sympathetic nerves when we injected it and we knew we had it (Wolfe, *et al.*,1962). The concept of inactivation by reuptake which we proposed was accepted after some initial controversy. It was later confirmed by others.

We then examined the effect of drugs on the uptake of radiolabelled noradrenaline in peripheral tissues. We had to work on peripheral tissues because Weil-Malherbe and I had shown that there is a blood – brain barrier to radiolabelled noradrenaline. Whitby and I showed that cocaine blocked the uptake of noradrenaline in tissues that were heavily innervated

with sympathetic nerves, such as the heart and the spleen (Whitby, Hertting and Axelrod, 1960). The reason we didn't work with dopamine was that there was no convincing evidence at that time that it was a neurotransmitter – it was just seen as a precursor for noradrenaline.

Brodie and co-workers reported a very important finding just around the same time. They gave reserpine to rabbits and showed that reserpine reduced the level of serotonin in the brain. He had a theory about serotonin at the time. A few months later Martha Vogt found that reserpine also depletes noradrenaline in the brain. It was also known that reserpine, if you give too much of it, causes suicidal depression. These experiments with reserpine indicated that noradrenaline and serotonin were involved with mental illness. The thinking was there but when you have the beginning of something, like this, there are all kinds of by-ways and sidetracks before you zero in on the real mechanism.

At that time, I had many bright young postdocs joining my laboratory – Sol Snyder, Dick Wurtman, Les Iversen and Jacques Glowinski. Snyder worked on circadian rhythms in the pineal. Wurtman on the role of glucocorticoids in the regulation of the enzymes that synthesize adrenaline from noradrenaline. Glowinski devised a procedure to introduce radiolabelled noradrenaline into the lateral ventricle of the brain. He also worked on the metabolism of catecholamines in the brain. Glowinski and I showed that imipramine and its chemically effective analogues blocked the reuptake of noradrenaline in the brain (Glowinski and Axelrod, 1964). We got a series of tricyclics, I think from Geigy, some of which were active as antidepressants and some inactive and we found that those that were clinically inactive had no effects on the levels of radioactive noradrenaline. So we knew there was some relationship between clinical effectiveness and an antidepressant's ability to block reuptake.

Later Iversen demonstrated that GABA was taken up in nerves. Joe Coyle, now Chairman of Psychiatry at Harvard, demonstrated that dopamine was taken up into nerve endings and Snyder found that serotonin was also taken up. Later in the 1970s, other labs showed that many amino acid neurotransmitters were similarly taken up by nerves. Recently the transporters that take up neurotransmitters have been cloned – two of them, the dopamine and serotonin transporters, were cloned in our laboratory.

Well, that was that. But I was mainly a biochemist. My interests were in enzymes so I worked on in that area. I found the enzyme that converted noradrenaline to adrenaline, called phenylethanol-N-methyl-transferase (PNMT) in 1962. I was particularly interested in methylating enzymes. Don Brown and I found the enzyme that inactivated histamine, histamine methyltransferase and hydroxyindole-O-methyltransferase, the enzyme that synthesizes the pineal hormone melatonin. I also found a curious enzyme which methylated tryptamine to dimethyltryptamine, which induces psychosis. I found this in both the lung and the brain. There

were some very simplistic ideas around about dimethyltryptamine at the time – that it was responsible for psychosis – but I couldn't believe that. This was just a by-product of metabolism – the theory was too good to be true, too simple. I had learnt working in biology that things aren't as simple as they may appear. If something is too simple, you should distrust it but we published a lot of papers on the psychotomimetics that might be formed in the brain.

Now I was also interested in the enzymes that regulated noradrenaline metabolism. We found two regulatory mechanisms; we found a relationship between the adrenal cortex and the enzyme that makes adrenaline. Coupland, a British anatomist, found that in the dogfish, where the adrenal cortex is separated from the medulla, the principal catecholamine is noradrenaline – unmethylated adrenaline. However, in mammals where the adrenal cortex is contiguous with the medulla, the main catecholamine present is adrenaline. This suggested to Dick Wurtman, a postdoc, and I that the cortex had something to do with the methylation of noradrenaline to adrenaline. Remember I had found the enzyme that methylates noradrenaline to adrenaline (PNMT), so then we removed the pituitary gland from rats – this should deplete glucocorticoids from the adrenal cortex. After several weeks there was a profound drop in the medullary PNMT activity. Injecting glucocorticoids (dexamethasone) or ACTH (which induces the synthesis of glucocorticoids) brought about a restoration of PNMT activity. This was the first demonstration that a substance from the cortex could regulate the medulla (Thoenen, *et al.*, 1969).

The other regulatory mechanism we discovered was with Hans Thoenen, who is now a Director of Neurochemistry, at the Max Planck Institute, in Munich. He's a very distinguished cell biologist, who discovered the ciliary nerve factor and other nerve factors. When he came to me, we found that when we gave reserpine there was an increase in tyrosine hydroxylase in the adrenal gland. We thought about it – what's happening? We realized that what reserpine did was to increase the firing of the nerves and this firing caused an increase in tyrosine hydroxylase. When we denervated the adrenal gland, there was no increase. We called this the trans-synaptic induction of tyrosine hydroxylase (Snyder, *et al.*,1965).

These were the kind of experiments I liked to do. I didn't try to develop drugs – my students, Sol Snyder and Leslie Iversen, did that.

Tell me more about Sol Snyder and Leslie Iversen.

When Whitby went back to Cambridge, Les Iversen was his graduate student. Les did a lot of important work exploring further the details of the reuptake mechanism – how it is regulated, the effects of competition; he showed that sodium was involved in the uptake. He was very good and I think he became a fellow of Trinity when he graduated.

Les came to me with all these credentials and we worked on the

metabolism of noradrenaline in the brain. He wanted to do more detailed neurochemistry and fortunately Glowinski, a neurochemist, was there at the same time. They developed a method for dissecting various parts of the rat brain. Their paper on the Glowinski/Iversen dissection technique is still highly cited. That's how Leslie learnt neurochemistry. He stayed a year and in that year he wrote his book called *The Uptake of Noradrenaline by Sympathetic Nerves.*

That was in 1967

No, in 1965. He was a Rockefeller fellow and they gave him an automobile, so he could travel with his wife Susan across the US. I don't know how he did it. He then went to Harvard for a year to work with Kravitz, where he did the GABA work, and Susan worked with Peter Dews, a psychiatrist in Harvard, on operant conditioning.

Sol Synder, also, wanted to become a psychiatrist. He worked as a graduate student across the hall from my lab with Don Brown, who is now a distinguished molecular biologist. Sol was interested in schizophrenia and he talked to me a lot about my work. I was working on the pineal at that time. After getting his MD, Sol came to my lab as a postdoc. I put him on a project on pineal gland. I won't go into the detail, it's too complicated, but he first worked on histamine metabolism. He says he's a klutz in the lab but he wasn't when he worked with me. He was very good. Sol had a sharp mind; he knew how to do the right experiments.

We developed a very sensitive method for measuring serotonin, the precursor of melatonin. We could measure the serotonin level in a single pineal gland and we found that it was highest during the daytime and lowest at night. When the rats were kept in constant darkness, there was free-running rhythm in serotonin levels which we abolised after denervation of the pineal. These experiments told us that there is a circadian rhythm in pineal serotonin which was controlled by the brain. We knew that there was some internal clock. Well anyway that's what he found. A very fundamental discovery. The assay for serotonin was very important for this; methods are very important.

On the question of methods, how important was Sidney Udenfriend?

Oh, he was very important. Sid was involved in the development of a new type of spectrofluorimeter. He worked with Brodie when they were measuring quinine in the blood in the 1940s. They developed an instrument, with the help of some engineers, that could measure fluorescence – the instrument had two filters, one that measures incoming light at one wavelength and another to measure outgoing light at a different wavelength. They developed this instrument and Sid wrote a book on fluorimetry. They used fluorimetry for their antimalarial work.

Who was the crucial person there, would you say?

Udenfriend and Brodie together. I owe Brodie a great deal despite everything else I've mentioned. Udenfriend and Brodie developed a fluorimeter using filters on the antimalarial project, during the War in 1943–1945. This enabled them to measure blood levels of atrabrine and other antimalarials. It was very important that they got this right because the Japanese had cut off the supply of quinine used to treat malaria. So atrabine was used instead but the troops found atrabrine unpalatable and they didn't want to take it because of side effects. Using the fluorimeter to measure blood levels, Udenfriend and Brodie developed a dosage regime for atrabine that was more palatable.

The spectrophotofluorimeter was the next development; this was developed by Bob Bowman, also at NIH. He also came from Goldwater. In 1955, Bowman improved on the original fluorimeter by using prisms instead of filters. They named the new fluorimeter after him – the Aminco-Bowman fluorimeter. It was more sensitive and easier to use and its introduction made it possible to measure blood and tissue levels of serotonin, noradrenaline and dopamine and this revolutionized catecholamine research. I used it in 1955, when I was measuring LSD. Bowman allowed me to use it when it was still in development. I was lucky to have it because I could then measure very tiny amounts of LSD in the brain.

Where did he come from, Bowman?

Bowman was a physician. He came from Goldwater and worked on the antimalarial project. He loved tinkering with instruments. He also developed an instrument called the flame photometer to measure sodium levels in plasma. People forget this – how important instruments are.

I agree completely. The instruments are absolutely critical. So much so that you wonder about the theories. You have people who say that science is all about theories, having the right kind of theories, trying to suss the theory out.

It's all about the right methods and asking the right questions. The introduction of radioactive noradrenaline and other radioactive neurotransmitters also had a great impact on neuropharmacology and on neurochemistry research. This was how fluoxetine was developed. They used labelled serotonin and tried out thousands of drugs to see what blocked the uptake. People often don't realize how critical technical developments like these are.

I agree completely with you.

Some of these young people have no idea where some of these developments come from and how important they are. Anyway, talking about Sol Synder, he took a residency in psychiatry but he was hooked on research. His early work demonstrated the importance of dopamine in schizophrenia, showing the relationship between binding to dopamine receptors

and clinical effectiveness of drugs in the treatment of schizophrenia. These were important experiments. Seeman also did a lot of work in this area.

Sol Synder, I think, did more for receptorology than anybody. He revolutionized the field by using radioactive ligands of high specific activity to measure the binding constants of ligands to receptors. The grind and bind approach. He showed, for example, that there are two serotonin receptors – these were important experiments – and also the existence of an opiate receptor. They sound very crude experiments now but they were germinal at the time. The whole field of receptorology exploded.

He seems to keep on coming up with things – for instance, the work on nitric oxide recently.

With all kinds of things, yes. He did and still does a lot of very good experiments. He's a brilliant guy. He has a skill at picking the right things at the right time. One thing I am very pleased about are the people who worked with me – almost all of them became distinguished in different fields – pharmacology, physiology, psychiatry. I have a very small laboratory. I never have more than two or three postodocs at any one time. I feel a great sense of pride in the type of people who work with me and in getting them involved in research. I don't know what it was but I tried to make it as pleasurable an experience as I could. Most of them came out of the grind of studying medicine and I said 'Relax, no more exams, just enjoy yourself, let your mind explore things'. With my help and their intelligence and enthusiasm, it worked out very well.

One thing about psychopharmacology is that these drugs are such powerful tools biochemically as well as pharmacologically. Drugs like reserpine, the monoamine oxidase inhibitors and the uptake inhibitors, they were really important tools. Well let's see, from 1970 I became . . .

Before you go onto 1970, let me ask you about a few people whose careers began during the 1960s and you might like to comment on. There's Arvid Carlsson.

Arvid was trained as a pharmacologist. He came to Brodie's lab just around the time I left–1956. Brodie had a tremendous influence on Arvid, as well as on Pletscher who was working there in the lab at the time. Brodie had many brilliant people working with him. Costa was there. There was a real ferment about that time. Soon after Carlsson left Brodie's lab, he got into the dopamine field. He showed that dopamine was present in the brain and he did the preliminary experiments showing that rats can develop a Parkinson-like syndrome by giving reserpine which reduced brain dopamine. This influenced the thinking of Hornykiewicz who examined dopamine levels in patients who had died of Parkinson's and found that it was decreased in the striatum.

I have nominated Arvid for a Nobel prize many times. It's a pity he didn't get it. I think he deserves it. He has done so much important work. Not only the work I've just mentioned but work showing that dopamine

might be involved in schizophrenia. He was the one who started to make dopamine what it finally became. He tells me he owes a great deal to Brodie.

There really are very many people who would say that he was extremely important. Silvio Garattini, for instance, would say he had the pharmacological 'attitude'.

Well, Brodie wasn't a pharmacologist at first. He was a biochemist. He was very imaginative. What a fund of ideas he had and he really swept you up with his ideas and . . .

Are you saying that even when he was wrong he was convincing?

Very convincing. He had a theory of the inhibitory action of serotonin in the brain which had considerable influence even though it was incorrect. But you know, in order to be a productive scientist you have to have lots of ideas which you can try out. Even if only one or two of them work out, it will have been worth it. If you have no novel ideas, nothing happens – you can do incremental work – that's just improving on something already known. But to do something original you have to have really bold ideas which Brodie had and he was also convincing. He was very stimulating and you wanted to rush to the lab to try out his ideas.

The other thing you hear about though was that he used to work by night, sleep by day.

Well, yes, he used to come to the lab about noon. He would then talk a lot to the people in the lab and sometimes he wouldn't get home until late. Sometimes he would call me at two in the morning if he had an idea.

He also seemed, in the mid 1960s, to vanish from the scene.

He always complained about his health when I worked with him. He led a life which wasn't very healthy. He ate hamburgers and stayed up late. It finally caught up with him in the 1960s. He had all kinds of medical problems in the 1960s and he just faded away because of that.

I think he had a great influence on all the people who worked under him. He was one of the father figures in psychopharmacology. His fame could rest just on the reserpine experiments. I shall tell you how that started. Sid Udenfriend and Herb Weissbach described the metabolism of serotonin to 5-hydroxy-indole-acetic acid (5-HIAA). Park Shore, then, discovered that if you gave reserpine to rats there was an elevation in 5-HIAA levels in the brain. Pletscher and Brodie started to theorize about that and came up with the idea that maybe reserpine was doing something to serotonin in the brain. So it was Park Shore who made the initial observation but it was Brodie . . .

Who really picked it up and ran with it.

Yes, that's how it started. You needed the imaginative bold thinking by someone like Brodie to really drive something like that forward. Sometimes it may not work out but sometimes it does and it happened to work in this case. But then his idea about the function of serotonin in the brain was wrong. He was very disappointed when Vogt and Carlsson found that reserpine also did the same thing to catecholamines. His theory was shattered. But anyway, it didn't matter. You forget the things that don't work but you remember the things that do.

If we move on to the 1970s. When did you get conferred with the Nobel prize?

In 1970. I knew I was nominated by Seymour Kety and Irv Kopin but it was a surprise.

What role did Irv Kopin play?

Irv Kopin came to the NIMH as a clinical associate but he had a nose for laboratory research. He happened to be in my laboratory when we were doing the crucial experiments on denervation with Hertting. Every time we did an experiment Irv Kopin showed up to help so we made him a co-author on some of the papers. Kopin and I discovered MHPG. He shifted from clinical research and wound up working in my lab most of the time. It was a very crucial period with the uptake experiments and in metabolism of catecholamines. He was a co-author on many of the papers. He remained in the catecholamine field longer than I did and he still is in the field. He's now the Director of the Neurological Disease Institute.

And after the Nobel prize?

In the 1970s, I mainly worked on the pineal gland, on methylation reactions, and started work on signal transduction. We discovered a new transduction pathway, in which arachidonic acid was a second messenger. I continued with this during the 1980s with the G-proteins which are heterotrimers – with alpha, beta and gamma units. When a receptor is occupied by a ligand, the G-proteins dissociate to alpha and beta-gamma subunits. The thinking at that time was that it was the alpha subunit that activates adenylate cyclase and phospholipases. But Carol Jelsema and I found that the beta-gamma subunits of the G-proteins can activate phospholipase A2 in the retina. We sent the paper to *Nature* in 1986 and it was rejected.

But they don't reject things from a Nobel prize winner.

They sure do. Our manuscript was published in the *Proceedings of the National Academy of Sciences* in 1987. About that time a paper appeared in *Nature* showing that the beta-gamma subunit can activate a potassium ion channel. A few years later more than a dozen papers were published in *Nature* showing that the beta-gamma subunits of G-proteins can activate

adenylate cyclase, phospholipase C, kinases, etc. Evidently, by then, even the reviewers for *Nature* had started to believe it. But I have to say that almost all of our papers (about 30) that we submitted to *Nature* were accepted.

Why do you think they'd turn down a paper like that?

Well, they did it because it was too revolutionary. Any time a dogma is challenged, it meets with scepticism. The criticisms were just lousy and nit-picking. They just didn't believe it. They questioned lots of things but it was true and it was confirmed later on.

You said that you were surprised to get the Nobel prize.

Most scientists dream about getting a Nobel prize. In the 1960s, catecholamines and neurotransmitters were hot – they still are. There were several people working in the area at that time that were likely candidates for the prize – von Euler, Carlsson, Bernard Katz, Hillarp, who was working on mapping catecholamine nerve pathways; Vogt and Blaschko. Von Euler, Katz and I got it. They decided to give it on neurotransmitters. So they gave it to Bernard Katz for his work on release of acetylcholine. They gave it to von Euler because he discovered noradrenaline as a neurotransmitter and they gave it me for inactivation. So I just happened to be doing the right thing at the right time.

Has it changed your life?

Not much. You become a minor celebrity. You get called up by news reporters. You get many honorary degrees and a lot of important lectureships. People recognize you – it makes me feel uncomfortable. But it hasn't changed my life very much. Of course, I'm delighted to have it. It's a great honour. I think I deserve it, but a lot of other people do too and don't get it.

What about your more recent work?

To continue with the rest of my work, in the 1980s I was beginning to wind down. I still loved to do research. Most of my work in the 1980s was on signal transduction, mainly phospholipase A2.

In 1984, I officially retired from government and became a unpaid guest worker in the laboratory of my former postdoc Mike Brownstein. I am still active and I am presently working on anatomized, the endogenous ligand for the cannabinoid receptor. The cannabinoid receptor was cloned by Mike Brownstein and Lisa Matsuda, a postdoc in Mike's laboratory. This meant that there must be an endogenous ligand for the receptor and Bill Devane and Raphael Mechoulen found it and called it anandamide. Bill and I described the enzyme that synthesizes anandamide. We have preliminary evidence that it is a neurotransmitter. Anandamide has a bright future I think – it has a receptor, it has an enzyme that synthesizes

it in nerves and we know a few of the things that it does. That's a very exciting project and I have really got caught up with it.

Let me pick up two things – radiolabelled antidepressant binding and of course the whoe SSRI story with fluoxetine and all that. Now that Steven Paul, who worked with you, has moved to Lilly, you have close links in a sense with both of these developments

Yes, Steven Paul was a postdoc in my lab. He was a very bright guy and he's done a lot of work on antidepressant mechanisms.

But was the radiolabelling of the antidepressant binding site, which he played a part in making fashionable with his early reports that there was decreased binding in people who were depressed, a mistake? It seems to me that the earlier work looking at altered uptake in people who were depressed was more promising in a sense but the field was seduced by the glamour of this new hi-tech approach and a great number of groups became bogged down in trying to sort out what has not been methodologically sorted out.

No, I don't think it was a mistake. It led to the next great development which was the cloning of the noradrenaline, dopamine, serotonin, GABA and glutamate transporters. It now appears that labelled antidepressant drugs do bind to these transporters.

I agree with what you say from the point of view of the basic sciences but do you not think that clinical research went down the wrong path, when they radiolabelled the antidepressants? So many groups got involved with this assay expecting it to be a diagnostic marker and it has led nowhere.

You have to try. If you do nothing, nothing will happen. As long as you're able to recognize you are on the wrong path. Some people become a prisoner of their ideas. They put so much work in it, that it must be true and they can't stop. You have to know when to stop and cut your losses. I've made a lot of mistakes but I found out fairly soon and I didn't waste my time. Things don't always work out the way you hoped they would but you have to try out your ideas. The binding of antidepressants indicated that there must be something there. It didn't pick up the transporter but it showed that there must be something there. It was the revolution in molecular biology that made the cloning of transporters possible.

Costa was someone who was into this area as well as GABA and other things.

Yes, he was mainly into GABA. He and his co-workers discovered a natural compound that inhibits benzodiazepine binding. Costa is very bright. He's done a lot of work on GABA and benzodiazepines, a lot of important work. Nothing germinal but very influential I think. He was greatly influenced by Brodie. Brodie was his hero. At the very end, when Brodie died, he took care of his wife. He's a warm-hearted person and

he has trained a lot of good people, particularly Italians. He is the guru of Italian neuropharmacology.

How do the 5-HT reuptake inhibiting drugs look from your perspective?

I think they were an important development but there has been a lot of hype about what these drugs can do.

As I understand it when they were introduced first, there were at least two groups, and maybe more, which appear to have been involved. One was the group with Arvid Carlsson who thought it would be a good idea to make the 5-HT reuptake inhibitor as an antidepressant . . .

I didn't know that. I thought there were several but I thought it was the Lilly group who were first. I don't know the history other than what I read in the book by Kramer (see Glossary). But you know the old saying: there are a lot of fathers to success and a lot of orphans to failure. You can never pin these things down. Take the discovery of dopamine; Carlsson had an important role and so did Seeman and so did Snyder. All of these things build up – it isn't any one individual that does it. There are several people contributing and it becomes compelling after a while. I'm sure Brodie and Carlsson had a lot of ideas that didn't turn out, but when they do, they're remembered. You have to have a lot of ideas and Carlsson had many.

What role do you think Seymour Kety had in everything?

Seymour Kety was a germinal figure in neuroscience. A statesman of neuroscience. He was the one who set up the NIMH in a way to do solid science. There had been some psychoanalysis research at the NIMH but he wanted basic science included as well. And he also had a nose in hiring good people.

He also had the ability to enthuse people.

Well, no, not in the way Brodie did. Kety had an analytical mind and he wrote an influential review in *Science* critical of the sloppy research in biological psychiatry – the pink spot and the Akerfelt test, for example. Kety believed that without sufficient basic knowledge doing targeted research on mental illness would be a waste of time and money. He did pioneering research on cerebral blood flow. His work and that of Lou Sokoloff provided the underpinning for PET scan imaging today.

What was the Akerfelt test?

Akerfelt reported that he had a blood test for schizophrenia. It was later shown that the Akerfelt test was a test for vitamin C deficiency. It so happened that schizophrenics in mental institutions were lacking in vitamin C. At the time there were many psychiatrists and others who were looking for abnormal metabolites in the urine of schizophrenics using

paper chromatography. Some did find abnormal metabolites but they were later shown to be artefacts. This was the kind of thing Kety was very critical about. This was very different from Brodie who was very enthusiastic.

Pink Spots were a big industry at one time.

Yes, you have these fashions which peter out after a while. We found that in a group of schizophrenics and controls, schizophrenics always had two spots and the controls never did. So we couldn't believe that. It was too good to be true. So we analysed the diet of our subjects and found that our controls were Mennonites – they didn't drink coffee. That was Kety, that type of thinking. A great analytical mind. He was a very nice person. And the thing was he never took advantage of you. He left you alone. But if you did something important he really pushed you, recognized it.

I've had two or three people who've talked about you at length – particularly Merton Sandler.

I always found Merton stimulating and amusing. It's interesting, in his interview he talked about a meeting in 1958 where he met me; actually I was never at that meeting. It was at a meeting in 1961 that I met him.

Well, this says something about history in a sense – maybe the way we remember things is in one sense more important than the way they actually were

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