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Molecular Mechanisms of Neuronal Communication

A Tribute to Nils-Åke Hillarp

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Molecular Mechanisms of Neuronal Communication

A Tribute to Nils-Åke Hillarp

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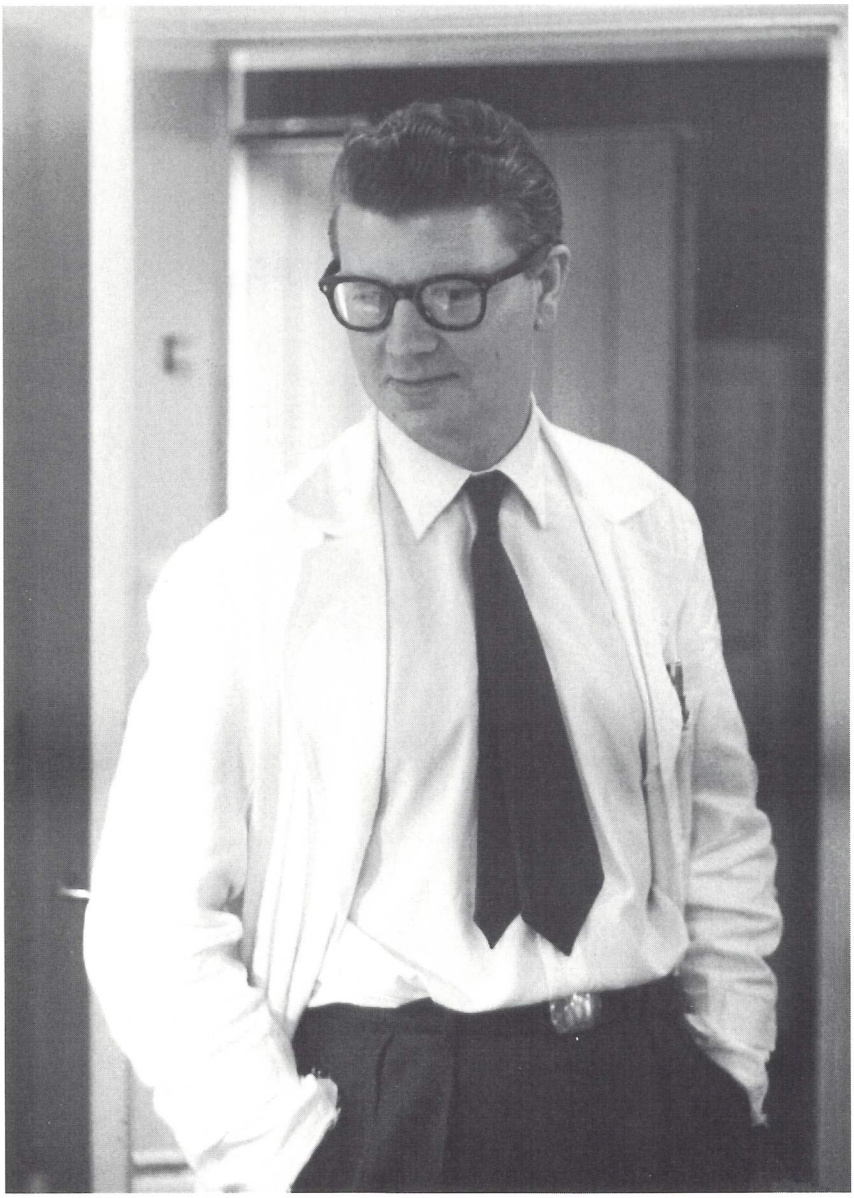

Fig. 1. Nils-Åke Hillarp in the corridor at the Pharmacology Department, Göteborg University, 1960. (Photograph by G. Thieme.)

Nils-Åke Hillarp: The Life of a Great Scientist

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Introduction

In 1962 it became possible, for the first time in the history of science, to visualize with the microscope the exact tissue localization of a neurotransmitter. At the time this was truly revolutionary and formed the basis for an unsurmounted and explosive development in the field of neuroscience. The scientist behind this was Nils-Åke Hillarp (Fig. 1), a person of remarkable character whose vast knowledge in the field and broad overview in this area of science was coupled with a rare generosity in all his activities. Owing to this unusual combination of characteristics he was able to form a group of young followers that has continued to grow and developed into an internationally recognized membership of scientists: "The Amine Group".

Today as we pay homage to Hillarp, 30 years after his untimely death, we who had the great privilege to work closely with him want to share our experience with those who did not have our luck to be in the right place at the right time. It is a strange feeling to realize that it is now more than 30 years since we all worked together at the old location of the Histology Department at the Karolinska Institute. It seems quite recent. However, that is, of course, a common feeling when reflecting on an especially intense period in one's life that is now past. This chapter is intended to describe Nils-Åke Hillarp's life and his development as a scientist. I will take you through his life, mentioning important events in chronological order, as he lived them.

Hillarp's Childhood and Youth

Nils-Åke was born on the 24th of July 1916 in the small town of Hässleholm in the very south of Sweden. His father, Nils Bengtsson, was a hardware dealer, chairman of the city council and a minister in the Swedish Missionary church. He was 62 years of age when Nils-Åke was born. Nils-Åke's mother, Hulda Johansson, came from Knäred in Halland and was a poor peasant girl (Fig. 2). She was constantly knitting stockings, even while walking, as was the practice amongst peasant women in order to earn some extra money. For some time she worked as a walking evangelist before joining a school to study home economics. She was then appointed as housekeeper at the farm of the old bachelor Nils Bengtsson. Within a couple of years they realized that her proper place would be as a wife in the family and they married in 1912, much to the upset of Nils Bengtsson's relatives who had planned on inheriting his estate.

Nils-Åke had an older sister, Ruth, who was born in 1914 (Fig. 2). Ruth Hillarp is still an active writer and produces very interesting photo-montages. This year, at the age of 81, she has just finished an exhibition which was much praised by art critics. She recalls that Nils-Åke was a very difficult brother who always demanded that she should play with him. When she was happily playing with her dolls, Nils-Åke wanted a play-

Fig. 2. The Bengtsson family in Hässleholm in 1918. From the left, father Nils, sister Ruth, Nils-Åke and mother Hulda. (Photograph by M. Piil, Hässleholm.)

mate and made such noise that finally his mother ordered Ruth to play with her brother. As revenge she then made him be the princess, while she herself played the role of the prince who rode out in the world for adventures. In school he was considered a rascal. This was possibly as a reaction against his older sister, who was very clever, pleasant in the classroom and the teacher's favourite. This type of reaction, as sociological studies have shown, is a common reason for impishness in small boys.

When Nils-Åke was 12 his father died of pneumonia after an operation. He was 76 years old (Nils-Åke's mother lived until the age of 90). The farm had to be sold and the family moved to the city of Lund, where Nils-Åke continued his education at the "Cathedral School" in Lund (Fig. 3). Although he had already spent 6 years at school, 2 years had to be repeated in his new school. In Hässleholm no German had been taught and German was a prerequisite for later high-school studies. This made Nils-Åke 2 years older than his classmates. According to Ruth, his sister, this probably worked for the best as Nils-Åke was unusually late in maturing! All of a sudden a change occurred. From being a rascal at school and the plague of the teachers, Nils-Åke now took his studies seriously and became an ideal pupil. During his high-school years he became a Christian Marxist who believed in sharing with other members of the community. His conviction was that a man needed only one pair of socks, shoes, trousers, etc. The effect of this could be noticed on the young Nils-Åke. He was often seen walking around in the winter without socks or stockings, having just given them away to some poor fellow who needed them more than he. He ate very spartanly and never had cakes at gatherings, only dry rusks or biscuits. Later, he left the Swedish state church and

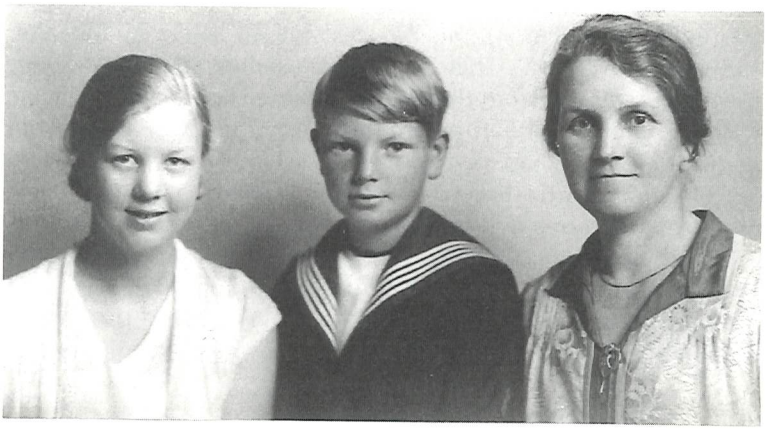

Fig. 3. The Bengtsson family after the death of father Nils and after the move to Lund. From the left, sister Ruth, Nils-Åke and mother Hulda. (Photograph by H. Cederberg, Lund, 1928.)

became 'simply' a Marxist. He was chairman of the Historical Society of the school and wrote an essay, under the supervision of his history teacher Hadar Vesby, entitled "The Capital". His teacher found this essay so excellent that he made efforts to publish it in a prestigious monthly journal but for some reason this first manuscript by the young Nils-Åke Bengtsson was never published.

Nils-Åke wanted to invite the quite left-wing Doctor of Economy, Per Nyström, to give a marxistic analysis of the community situation. Later Per Nyström was to become a highly esteemed governor in Göteborg (he died recently at the age of 91). Josua Mjöby, the Rector of the Cathedral School, became furious. He would never allow a 'Marxist sermon' or political propaganda in his school. Later, however, he himself invited a well-known nationalist to lecture! One must remember that this was at a time when nationalism and Nazism had a considerable grip on Swedish society and inviting a reputed Marxist to the school was indeed asking for trouble! When Per Nyström was informed about the situation within the school he wrote a very sarcastic and elegant article in the Göteborg newspaper *Göteborg's Handels och Sjöfartstidning* (1936). This was the most liberal and anti-nationalistic medium in Sweden at the time, famous for its constant needling of the German Nazi government. The article was simply entitled "The Cathedral School" but its content made the Rector raging mad and he demanded that Nils-Åke kneel and ask forgiveness in public "for having dragged the school's name in the dirt". Åke refused (of course!) and was close to being expelled. However, all the teachers who had had Åke as pupil stood by his side and he won his battle against the Rector.

Hillarp—the Idealistic, Celebrating Student

After high-school (Fig. 4) Nils-Åke became an active member of the 'Brotherhood movement'. This was a social-democratic-based organization for people, mostly from the social working classes, who believed in political and practical solidarity with the poor. After graduation, when all young men in Sweden had to go into compulsory army training, Nils-Åke managed to escape education in the use of weapons to kill an enemy. He became a conscientious objector, along with many Jehovah's witnesses and other religious boys. He, and other weapon-free soldiers, were sent to restore an old historic site in the south of Sweden called 'Glimmingehus' (Fig. 5). I was told that he made a movie during this work but unfortunately this appears to be lost.* After his year of military service

*Just before submitting this manuscript Hillarp's granddaughter informed me that she found the film in the attic.

Fig. 4. Nils-Åke Bengtsson as 'newly hatched' graduate ('student') in his white graduation cap, 1938. (Unknown photographer.)

Fig. 5. Group of conscientious objectors photographed outside the main gate of 'Glimminge-hus' after restoration work. Nils-Åke in the middle of the front row with crossed hands. (Unknown photographer, probably 1939.)

he decided to study medicine. It is likely that this was inspired by his social conscience and he planned to become a 'real doctor'. This is when he changed his last name to Hillarp, after the village where he grew up (Fig. 6). He had a good time as a medical student. He was very active in the student life and was 'Toddy-general', writing humourous essays in *Toddybladet*, the students' journal. He was full of humour and good spirit and never 'spitted in the glass', as the Swedish expression translates. This means that he liked good wine and drink and was very keen that everyone around should join him in celebration.

In 1941 he married a young nurse, Gunnel, whom he met during his early hospital training (Fig. 7). The couple had two children. Jan-Åke, born

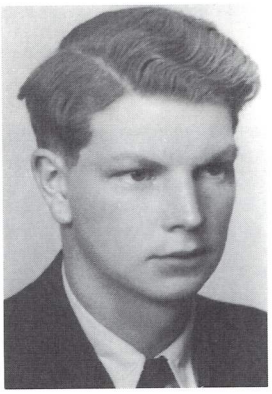

Fig. 6. Nils-Åke Hillarp at the beginning of his medical studies, now after change of his family name. (Unknown photographer, 1940.)

Fig. 7. Gunnel, the young nurse who became Hillarp's first wife. (Unknown photographer, 1941.)

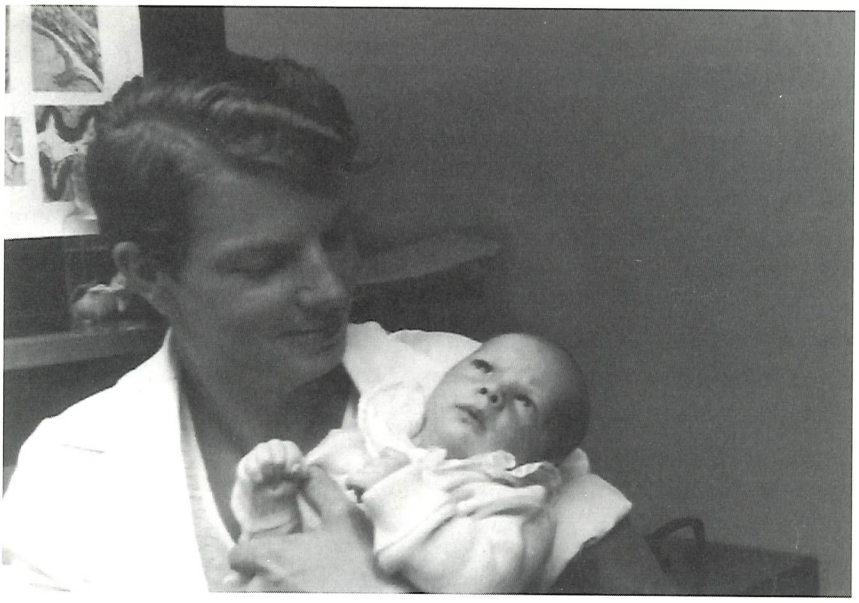

Fig. 8. Nils-Åke with his newly born son, Jan-Åke, in 1943. (Unknown photographer.)

in 1943 (Fig. 8), is now a biology teacher and an expert in birds who is active in, amongst other issues, the protection of sea birds from oil spill hazards. Birgitta, born in 1945, is now a radiologist at the Malmoe Hospital. The marriage lasted until 1949 but was not really happy, mainly, according to Jan-Åke, because of interference from Nils-Åke's mother.

Histology-apprentice (Amanuensis); the Start of a Scientific Career

During the first 2 years of medical education histology is taught and Nils-Åke, in parallel with his medical studies, began to work as an apprentice (amanuensis), under Professor Glimstedt. Professor Glimstedt suggested the endocrine system as a suitable area for research. Nils-Åke's first publication dealt with the effect of oestrogen on strips of squamous epithelium but gradually he 'drifted' via the hypothalamus towards the adrenal medulla and the nervous system, as we shall see later.

During the early days, when he was working on his thesis, Hillarp and his young family were far from well off and he had to work as a radiologist doctor every summer to earn money. In fact, he never completed his medical studies—science had too firm a grip on his mind. Later, the same 'fate' befell many of his young students. Few of them bothered to get a medical degree.

Hillarp was very fond of animals. He let the white rabbits in the laboratory play in the grass and he studied the albino rats, enchanted by the intelligent behaviour they demonstrated. Many times he was seen walking in the streets of Lund with a big white rat on his shoulder. He claimed that it jumped from one shoulder to the opposite one on command. Another characteristic of Nils-Åke was his love of the sun. He preferred to sit outdoors in the summer and work at his typewriter in the sunshine (Fig. 9). Unfortunately, this was clearly not the thing to do for a red-haired person. Since he had the pale complexion of red-blonde people, he was convinced that he looked nicer and more handsome with a suntan. He was a little vain and not uninterested in the opposite sex.

In 1946 Hillarp defended his thesis, "Structure of the Synapse and the Peripheral Innervation Apparatus of the Autonomous Nervous System", published in English in the prestigious *Acta Anatomica*. In this work he was

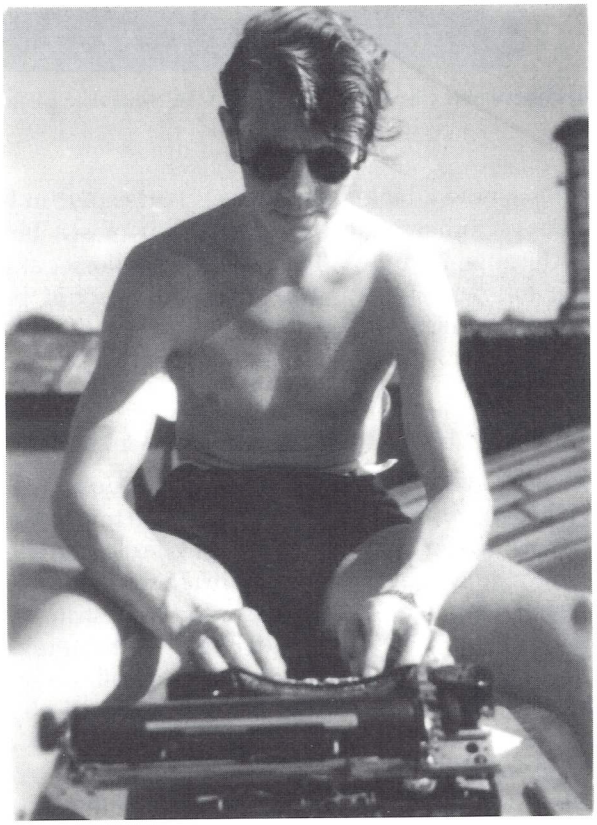

Fig. 9. Nils-Åke typing on the roof of the Histology Department in Lund, on a sunny day in the summer of 1944.

able, for the first time, to obtain a comprehensive and beautiful picture of the autonomic nervous system. He used methylene-blue staining, a method that he was able to refine until it could demonstrate the fine delicate and varicose nerve fibres which constituted the autonomous innervation apparatus. He thus challenged the view, favoured at that time, that the autonomic nerve plexus was organized as a syncytium. This pioneering work, which was an important step towards our present view of the construction of the nervous system, was later elaborated further and summarized in a monograph he published in 1959. In this monograph he gave a detailed analysis of the different components of the autonomic nerves and introduced the term "the autonomic ground plexus" with its different components. Hillarp had a preference for hands-on laboratory work (Fig. 10) and during these years he acquired a technical skill and experience that proved to be very useful in his later work, when he developed the histofluorescence method for the localization of tissue catecholamines and serotonin.

In 1950 Hillarp met his second wife, Ulla. They married on New Year's Eve in 1951 and had two children (Fig. 11). Eva, born in 1952, is now a district nurse with four children. Andreas, born in 1957, is now chief hospital chemist at the Malmoe Hospital. Andreas successfully defended his M.D. thesis in 1991 and has spent a post-doctoral year in Oxford.

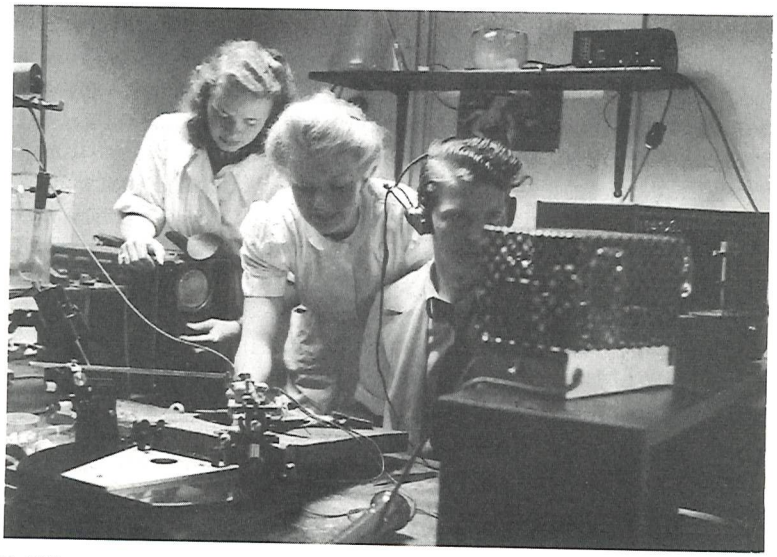

Fig. 10. Hillarp at full action using the stereotactic lesion equipment, built by himself, in the laboratory of the Histology Department in Lund, in 1944. (Photograph by G. Thieme.)

Fig. 11. Hillarp's second wife, Ulla, with their two children Eva (to the right) and Andreas, just after the move to Stockholm in 1963. (Unknown photographer.)

In Search of a Histochemical Method to Demonstrate the Adrenergic Transmitter

In 1953 Hillarp, Lagerstedt and Nilsson (Hillarp *et al.*, 1953) discovered the 'amine storage granules' in the adrenal medulla, organelles which were responsible for the storage of catecholamines (CA) in this endocrine gland. The adrenal medulla is in fact an extension of the sympathetic nervous system. That year, Blaschko in England made the same discovery (Blaschko and Welch, 1953). In collaboration with Arvid Carlsson, at that time associate professor in the Pharmacology Department of the University of Lund, Hillarp investigated the nature of the CA storage mechanism and found that reserpine caused the CA to disappear (Carlsson and Hillarp, 1956). In 1959 Hillarp used reserpine treatment to test the claim that osmic acid–sodium iodine staining was due to the presence of noradrenaline (NA) in adrenergic nerve endings (Coujard, 1943, 1950; Champy *et al.*, 1946). The picture he obtained in reserpine-treated, CA-depleted tissues was the same as in control tissues. Thus, OsO_4–NaI staining was not due to the presence of CA.

Hillarp and Carlsson were impressed by the potential of fluorescence for the detection of small amounts of tissue constituents. Arvid Carlsson had returned from an exciting stay at the Laboratory of Clinical Pharmacology of the National Heart Institute with Bernard Brodie and Sidney Udenfriend, who had developed the first prototype of a spectrophoto-

Furthermore, he rarely wanted his own name to be included in the author list, except concerning large review articles, "I have had my career, now it is the future of you all that we must consider!" Very few scientific leaders have demonstrated this kind of genuine generosity and responsible concern for their younger colleagues!

After a full day in the laboratory Hillarp would say, when the evening was already born, "Now, let us call this a day, let's all go home to Ulla and have some wine and talk about life!" We rarely had other commitments that prevented us from accepting this invitation. We started sometimes by rounding off some scientific discussions that had not been solved in the lab but then lab-talk was banned. Only matters of private life and life in general were discussed, the conversation growing more intense and vivid with the number of bottles that were emptied. Then Hillarp's shyness vanished and he was glowing in his discussions (Fig. 15a–c). What a wonderful tutor he was for us, in every respect!

Hillarp has been dead for 30 years now but his spirit is indeed alive. For those of you who have not inspected the impressive rows of theses front pages hanging in the corridor of the Histology/Neurobiology departments, I strongly suggest that you make a visit. Not only has he scientific children and grandchildren, soon he will probably have scientific greatgrandchildren. I am convinced that the impact of his short but intensely productive life is, and will remain, unmatched in the history of science.

Fig. 15. Nils-Åke during one of last intense discussions on life and science in his and Ulla's home, late in the autumn of 1964. We were all aware that Hillarp had only a limited time left, and on this occasion his intense personality was perhaps even more evident than usual. (Photograph by G. Thieme.)

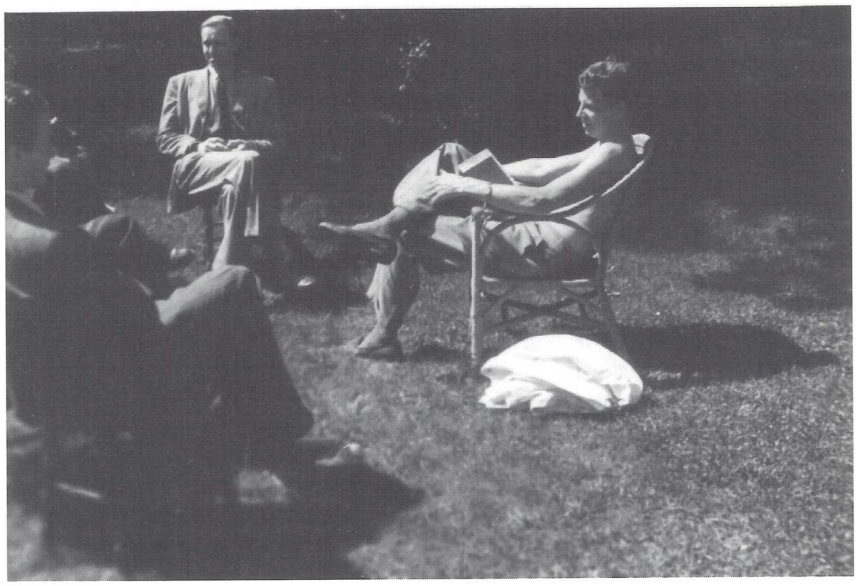

Fig. 14. Entertaining visitors in the backyard of histology building in Lund. Note that the visiting scientists are properly dressed in suits, while Nils-Åke, having unloaded his lab coat, enjoys the sunshine.

one's own equipment could you have exactly what your experiments needed. By building it yourself, you had full command over every step in the procedure.

Hillarp was demanding and could at times be impatient (usually with good reason!). My first solo article (which was printed in 1965) dealt with the exciting observations of pronounced accumulations of fluorescent material proximal to a crush of a peripheral nerve and proximal to a cut in the spinal cord (indicating the phenomenon of fast axonal transport). I had written the article in as sophisticated a way as I could. I received it back from Nils-Åke with more red than typescript on the pages—comments, crossings, underlinings and marks of exclamation and interrogation in the margin and "Please see me immediately!" In his office he asked me to tell him plainly what I had done, how I thought when I planned the experiments, what the results were and what I thought they meant. After I told him he said, "Why don't you write what you just told me, instead of filling your report with a meaningless series of words!" A very sensible lesson for a pretentious young student!

Hillarp had a disgust for large international meetings. He was incurably shy and hated to dress up. Therefore, he was very happy to send his younger collaborators to international meetings, which of course made us all well known internationally at a very early stage in our careers.

without being deeply moved. I clearly see Nils-Åke's funeral and my dear, late, father before my eyes.

Later in the spring of 1965 the dissertations of Kjell Fuxe (1965), Karl-Axel Norberg (1965) and Torbjörn Malmfors (1965) took place. My own thesis was defended in the following year (Dahlström, 1966) and the year after that, Gösta Jonsson (1967) and Tomas Hökfelt (1967) presented their theses. Bertil Hamberger's dissertation took place in 1968 and Lars Olson, Charlotte Sachs and Urban Ungerstedt defended their theses in 1970. All these dissertations were awarded Cum Laude.

To Work with Hillarp

As indicated above, Hillarp was extraordinarily generous. He was also very unconventional. I remember the first summer when Nils-Åke had installed himself in the department. His office was on the second floor and in the corridor near the door to his office he had placed a large freezer filled with scientific items but also with ice cream. Outside was a box where anyone who felt in need of an ice cream could pay for what he/she fished up from the freezer. My own little office was in the floor below. Since the new professor was going to be in the house, I was neatly dressed, in stockings, long cotton skirt, and a long-sleeved blouse buttoned with cufflinks and buttoned up under the chin. It was a hot summer and in Sweden air conditioning is not included in the average building. I went upstairs for an ice cream and bent down into the freezer to pick my choice. Then Hillarp's door opened. He had the same need for an ice cream. We stared at each other, both mildly shocked. He was in a pair of minimal shorts, nothing else, with bare-feet. I was 'dressed up' as described above. He said, with notable surprise in his voice, "For Christ's sake, how are you supposed to be able to carry out creative work dressed like that in this heat! Dress in a swimsuit and sit outside in the shade with your typewriter!" We all soon followed his example and felt quite relaxed.

He had always been unconventional, even a bit challenging. In an old photograph from his early days in Lund he is seen entertaining a couple of important international visitors in the backyard of the Histology Department (Fig. 14). It was warm and consequently Hillarp is very lightly dressed, in contrast to his conventional visitors, who sit in the sun sweating in suits, white shirt and tie.

Hillarp was one of the last scientists to construct and build technical and electronic equipment himself, rather than have it built by engineers in the machine shop. During his very early years as apprentice (amanuensis), he had studied by mail in order to acquire enough knowledge of electronics and technology to build his own research equipment (Fig. 10). Today, when all instruments are bought prefabricated, this may seem strange. However, Hillarp felt that only by designing and constructing

fluorescence method. He simply had no place in his mind for anything trivial which only concerned his own person! One week after the operation he gathered us together to inform us that he had only a few months left to live and strongly suggested that we all worked hard, even harder, to accomplish as much as possible during his last months alive. He was troubled, but very matter of fact. His own person did not matter so much to him as the future of the group and the fluorescence method. (In the 1960s a malignant melanoma was a fatal diagnosis. The 1-year survival was less than 5%. Today this disease can be managed much more successfully.)

We were shocked but soon realized that there was only one thing to do: work, work, work! We worked frenetically the whole summer. Nobody took any vacation, weekends or holidays. Hillarp's creative mind was more productive than ever and he flooded us with notes and letters about experiments that must be done, that could be done and which probably would be ingenious if they were carried out. The atmosphere he created stimulated us to come up with our own more or less smart ways to solve problems. So many brilliant hypotheses were born during discussions around Hillarp during those days. He cared about and took interest in every member of the group. Even the newcomers had their thesis work planned out on paper by Hillarp. It was without doubt the most productive time in our lives. During these months he outlined the thesis content for many of us. In October 1964 he was admitted to hospital for periods mixed with periods at home or at work. The last time he visited home was on New Year's Eve in 1964 and he returned permanently to his hospital ward on 1 January 1965.

He continued to see us, one or two at a time, in his hospital room to discuss science and our theses. He was suffering severe pains but postponed the morphine injection until the pains were unbearable, in order to have a clear scientific mind as long as we were with him. When he could no longer write his messages to us himself, his wife Ulla took dictation and his ideas continued to reach each of us. Unfortunately he did not live long enough to see any of his Stockholm students defend a thesis.

He died on 17 March 1965. I was at that time working at the Pharmacology Department in Göteborg and I can remember the numb grief that blanketed my mind when I received the news of his death. This remarkable mind would no longer be with us. He had left us, his 'scientific children', to carry on without him.

Hillarp had decided upon a non-clerical burial and this took place in the chapel near the Karolinska Hospital. My father, a professional organist and teacher, had been asked to be responsible for the music. Nils-Åke had decided that his favourite piece of music be played, the prisoners choir from the opera *Aida* by G. Verdi. Even today I cannot hear this music

humidity and during hot summers, when the temperature rose for a few days, the PF powder contained more water than was anticipated from the sulphuric acid concentration. In the end a controlled climate room was installed where the PF desiccators were stored and where all transfers of tissues from freeze-dryer to PF reaction vessels took place.

During the difficult years before all this was investigated we were often close to despair. Hillarp then reminded us that medical research was "95% struggle and failure and 5% success, if we were lucky!" This was a useful reminder for all of us and encouraged us to be patient and persistent in our experimental future.

'In the Dark'

At the end of the day, after freeze-driers had been opened and/or started, it was time for microscopy. Mrs Mirta Baidins, our skilled chief technician, never tired of sectioning the multitude of paraffin blocks and produced an endless number of trays of neatly cut and Entellane-mounted sections. These had to be inspected within a few days and this meant that several hours every day had to be devoted to the dark room where three or four fluorescence microscopes were lined up. Many evenings and nights were spent there and many happy exclamations (good reaction) or curses (bad reaction) were heard. A particular frustration was experienced when the fine, weakly yellow fluorescent 5-HT containing nerve terminals were seen at the moment when the sections were first inspected, only to have faded some seconds later, rendering photography (which took 30–60 s for catecholamine fluorescence) impossible. Nowadays the immunofluorescence technique, employing antibodies to 5-HT, has verified our early observations which could never be properly demonstrated, only described verbally. Only thick serotonin terminals, where enough fluorophore molecules were concentrated in a small spot and protected each other against photodecomposition, could be photographed.

Hillarp's Illness

In May 1964 Hillarp had noted a tumour in his right axilla. The late Professor Jack Adams-Ray, legendary surgery professor at the Karoliska Hospital and collaborator with the group on mast cells, extirpated the tumour and found immediately by macroscopic examination that it was a metastasis of a malignant melanoma. The mother lesion was a 'birth mark' on the chest, which had started to itch several months earlier. It had aroused the attention of Hillarp's wife, Ulla, who tried in vain to persuade him to see Jack Adams-Ray several months earlier. But Nils-Åke was too preoccupied with science, his students and the perfection of the histo-

role for monoamines in central monoaminergic transmission was thus suspected but since the precise cellular localization was so far unknown, this remained conjecture for a number of years.

In 1962 the freeze-drying procedure was used for brain tissue and the monoaminergic neurons were seen for the first time. Photographs of varicose nerve terminals in the hypothalamus and the caudate nucleus were published in a 1962 article by Carlsson *et al.* (1962) and nerve cell bodies with green fluorescence were described. Reserpine was demonstrated to lower the fluorescence and, thus, the first pharmacological experiments using the new histofluorescence method were conducted.

Many species of mammalian brains were dissected and freeze-dried during winter and spring 1962–1963. The fluorescence reactions varied and therefore all experimental tissues had to be freeze-dried, formaldehyde treated and embedded in the same experimental session. We all worked hard and got excellent and exciting results, and a large number of cell body groups were described. Since the locations of these fluorescent cell bodies did not always coincide with known anatomical structures, we introduced a new nomenclature. Green fluorescent cell bodies were called group A1, A2, etc., while yellow fluorescent cell bodies, containing 5-HT, were called B1, B2, etc. (Dahlström and Fuxe, 1964). This publication became one of the 100 most-cited references during the years 1964–1984 (Science Citation Index) and is still cited 30 years after publication.

The Formaldehyde Reaction and Water

The fluorescence reaction varied considerably and Kjell and Nils-Åke discussed the possibility that the water content in the PF powder could be of importance. The summer of 1963 was trying for all of us. The fluorescence became diffuse and whole freeze-dryings, one after another, had to be discarded as useless. Especially bad reactions were noted on rainy and warm days and gradually the importance of moisture and temperature was realized. By the end of 1963 we started to store the PF powder in desiccators with sulphuric acid of varying concentrations to control the water content. The relationship between water and temperature and the success of the reactions was described in detail by Hamberger *et al.* (1965). Hamberger, in his thesis (1967), clarified the complete theoretical basis of all our difficulties: (1) each new batch of PF powder contained some, varying, amount of water. Therefore the PF powder had to be dried completely over desiccant before any equilibration of water content could be made; (2) sulphuric acid of different concentrations created, in a closed vessel (desiccator), air humidities varying with the concentration of acid; (3) the PF powder adsorbed water according to the air humidity in the desiccator; and (4) the temperature in the room also determined the air

compare Bengt Falck's set up with that in Stockholm. They discussed the diffusion occasionally seen in the tissues without finding any clue to explain it.

Hillarp and his family moved to Stockholm in late August of 1962. Very soon he informed us, in informal gatherings, about the autonomic nervous system. He described the multitude of anatomical, pharmacological and physiological questions that could be investigated and solved with this new epoch-creating histochemical method that could render monoamines visible at the cellular level in the microscope. His enormous enthusiasm and curiosity was indeed contagious and we were all seriously infected by the 'science bug'.

To improve efficiency and avoid in-fighting, Hillarp divided the vast field between us. Kjell chose the central nervous system (CNS) and suggested that Dahlström should join him in that area since they already cooperated well. Their work was extremely fruitful, as judged by the many publications appearing between 1962 and 1968, when Dahlström moved to Göteborg. Karl-Axel Norberg, later joined by Bertil Hamberger and Urban Ungerstedt, investigated the peripheral nervous system and studied the pathways between different sympathetic ganglia and innervated structures. Torbjörn Malmfors, Charlotte Sachs and Lars Olson studied the physiology and pharmacology of the adrenergic system using spread preparations of the iris as a model organ. The electron microscopy of CA and 5-HT systems was explored by Tomas Hökfelt. Gösta Jonsson became involved in the chemistry of the formaldehyde reaction together with Martin Ritzén and the late Hans Corrodi from Arvid Carlsson's group in Gîteborg. Martin Ritzén was working at the Institute for Medical Cell Research, which was headed by Professor T. Caspersson.

In Lund, Bengt Falck with his students Berndt Ehinger and Christer Owman, later joined by Anders Björklund (who, to his regret, never met Hillarp in person), commenced work on the endocrine system. This was Falck's main interest since his thesis work under Hillarp. The adrenergic innervation of orbital structures became Ehinger's main field of interest and he is now Professor of Ophthalmology in Lund.

Monoamines in CNS Neurons

CA as well as 5-HT had been demonstrated by biochemical means to be present in the brain (von Euler, 1946; Twarog and Page, 1953; Amin *et al.*, 1954) but the monoamines were considered to be affiliated with blood vessels. However, Vogt (1954) remarked on the uneven distribution of CA and suggested that the amines had functions other than innervation of the vessels. DA was also demonstrated in brain (Montague, 1957). The highest concentrations were present in the caudate nucleus (Bertler and Rosengren, 1959), clearly pointing to involvement in motor regulation. A

paraffin, was carried out during the autumn of 1961 and published in 1962 (Falck, 1962). It is interesting to note that a similar procedure was independently but concurrently developed in the USA, where Lagunoff *et al.* (1961) developed a method for studying histamine in mast cells. After treatment with formaldehyde gas at 60°C a strong yellow fluorescence developed. Lagunoff and his collaborators attributed this fluorescence to histamine but in retrospect it seems more likely that it was due to 5-HT, which was in fact also mentioned as a possibility by the authors. This observation was published in the September issue of the *Journal of Histochemistry and Cytochemistry*, which reached the library in Lund via surface mail in December 1961.

Starting the Group in Stockholm

In the spring of 1962 Hillarp received and accepted a call to the Chair of Histology at the Karolinska Institute. The author of this article was there at the time, working on her first project. This involved tissue culture of mouse uterine epithelium and was carried out together with Kjell Fuxe and Ove Nilsson, now Professor of Anatomy in Lund. This project was not really interesting and the old Professor, Häggqvist, was far from inspiring. All microscopes were kept locked away under plastic hoods. Somebody wanting to use a microscope had to ask the professor for the key and then relate in detail why the use of a microscope was necessary. In fact, most of the plastic hoods were very dusty. I was seriously considering leaving the department when the new professor came to inspect his new territory in April of 1962. It was like a hurricane sweeping through the old department, blowing away dust and stiff authoritarian reign and introducing a strange new feeling of enthusiasm into the building. The students working there at that time were Kjell Fuxe (aged 24), Torbjörn Malmfors (23), Karl-Axel Norberg (29), Tomas Hökfelt (22) and Annica Dahlström (21). Later in the same year came Bertil Hamberger (20), in the spring of the following, year Gîsta Jonsson (24), and in the autumn of 1963, Lars Olson (20), Urban Ungerstedt (20) and Charlotte Sachs (23).*

Fuxe had already done some work involving fluorescence microscopy with Assistant Professor Ove Nilsson and was introduced to Hillarp by Lars Gyllensten, who was at that time Associate Professor. Lars Gyllensten later became an author and resigned from the Karolinska Institute, for some years serving as secretary of the Swedish Academy. Hillarp introduced Kjell to the new method. Kjell immediately caught on and started to build a histofluorescence laboratory in August of 1962 with the invaluable help of Georg Thieme. He also spent a few days in Lund to

*For pictures illustrating the group in Stockholm, please see Dahlström and Carlsson (1986).

and vapour treatment. This was instead of, as in the later modifications of the method, being treated in vapour before embedding. During this work, Eränkö visited Lund and was shown the heating procedure. He had also observed this effect of heating on the development of fluorescence but had not published his observations. He communicated his results to Hillarp and Falck, who acknowledged this personal communication in the 1961 papers which were issued from Lund and Göteborg and submitted for publication in August and September 1961. But, dear readers, we are not yet there! Still some developments had to take place.

Fluorescent Nerve Terminals, at Last!

One day in August (25th or 26th) 1961, when Hillarp spent a weekend in Lund visiting his old department, he proposed to Falck that they should try air-dried stretch preparations of tissue specimens such as iris or mesentery. These tissues had been much used by Hillarp in his thesis work but this time the tissues were to be treated with formaldehyde vapour generated from paraformaldehyde (PF) powder during heating. The outcome was dramatic. The same nerve plexus pattern as had been seen with the methylene-blue staining method was now revealed, brilliantly fluorescent, beneath the microscope! The NA had been converted into a strongly green fluorescent product, as in the model experiments. In mesentery specimens, cells with a yellow fluorescence were noted. This yellow fluorescence was 5-HT.*

Sometimes in science all conditions are optimal, as on this occasion. The momentous outcome of this spontaneous experiment was duly celebrated with champagne. However, the experiment could not be repeated until several months later, in October–November, and for this reason this brilliant observation was not mentioned in the 1961 papers.

The Freeze-drying Procedure

A big step had been taken but there was still work to be done. How could this procedure be applied to larger tissues, such as the brain? The method would have to be applied to embedded tissue specimens. This essential development was carried out by Bengt Falck in the Histology Department at Lund. A freeze-drier was built by Falck, based on the Pearse principle. This tedious work, to develop the freeze-drying, then the step of formaldehyde vapour treatment, followed by embedding in

*For illustrations of the early microscopic and fluorescence microscopic photographs please see the publication by Dahlström and Carlsson (1986), which dealt specifically with the development of the histofluorescence method.

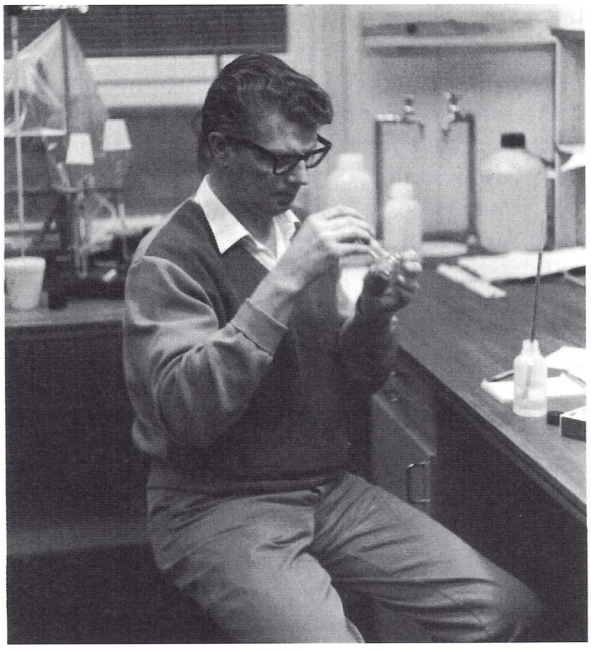

Fig. 13. Nils-Åke Hillarp in the laboratory of the Pharmacology Department in Göteborg, dissolving substances to be used in the testing of the fluorescence method. (Photograph by G. Thieme, 1961.)

spotted on to glass slides and air-dried. Upon treatment with formalin vapour, generated from a 35% solution of formaldehyde, the spots that contained NA, dopamine (DA), A or methoxy-tyramine developed a strong fluorescence. However, this only occurred if the amines were present in a dried protein layer. Proteins thus catalysed the reaction and it was found that an increased temperature enhanced the fluorescence. It was also discovered that the fluorescent products which developed upon the formaldehyde treatment were tetra-hydro-isoquinolines, later verified by the work of the late Hans Corrodi and Hillarp (1964). These fundamental model experiments were published in a famous article by Falck *et al.* (1962), submitted for publication in late August in 1961.

Together with Bengt Falck, who had participated in Hillarp's earlier attempts to use the THI method, Hillarp and Carlsson now proceeded testing the formaldehyde method on tissue sections cut on a Pearse–Slee cryostat. This was especially purchased for this purpose by the Pharmacology Department in Göteborg. The cryostat sections were dried *in vacuo* and exposed to formaldehyde vapour at 50–75°C for various times. At 75°C a strong fluorescence developed within 30 s. Adrenal medulla was tested but the specimens were embedded in paraffin before sectioning

The Histofluorescence Method for Catecholamines and Serotonin

In the following year (1960), Nils-Åke Hillarp and Arvid Carlsson moved to the Department of Pharmacology at the University in Göteborg (Fig. 12). Arvid Carlsson had been appointed to the Chair in Pharmacology and Hillarp's move with him was made possible by a grant from the Medical Research Council, enabling him to devote himself full time to research. In order to receive this grant, Hillarp and Carlsson had proposed devoting the time to two projects: (1) to characterize further the mechanism of amine storage; and (2) to study the function and cellular localization of monoamines in the brain. Both projects were successful, largely due to Hillarp's remarkable technical skill and the presence in the department of equally skilled technical assistants and engineers. An optimal atmosphere and a 'critical mass', as it is called today, were present.

Shortly after the move to Göteborg, Hillarp and Carlsson read the article by Hess and Udenfriend (1959) describing the successful use of formaldehyde for inducing fluorescence in tryptamine and detecting small amounts of amines in their spectrophotofluorimeter. Together with Georg Thieme, a research engineer, Hillarp started a systematic investigation of the formaldehyde reaction in model experiments (Fig. 13). Various amines were dissolved in serum albumin, sucrose, gelatine or gliadin, were

Fig. 12. Arvid Carlsson, holding the binoculars, Margit Lindquist (a very skilled collaborator of Arvid's, deceased) and Nils-Åke, inspecting the final construction of the Pharmacology Department in Göteborg. The preclinical institutions were constructed in 1959–1960 on the 'Medical Mountain'. The body of the histology building, present location of the author, can be seen at the rear. At the far distance, the main building of the Sahlgren hospital.

fluorimeter and could measure very small concentrations of serotonin. Back home in Lund, Carlsson and Hillarp discussed whether fluorescence, in contrast to light absorption, might be sensitive enough to be used not only for the detection of CA in biochemical assays but also for histological detection. The question was, how could CA be made fluorescent?

The first method tried was the trihydroxyindole (THI) method, used for the biochemical assay of CA (Ehrlén, 1948). Tissue sections were first exposed to iodine to oxidize the CA to adrenochromes. Then, in a second step, ammonia was added to arrange the adrenochromes to fluorescent adrenolutines, all according to the THI method. Carlsson and Hillarp were successful concerning the adrenal medulla. A very intensive fluorescence was developed and this was markedly decreased after reserpine treatment. Thus, the fluorescence could indeed be due to the presence of CA. Adrenergic cell bodies or terminals, however, were never observed.

Another way to make CA fluoresce was using formaldehyde. In 1932 Erös had observed a yellow fluorescence in certain gut mucosal cells in tissue specimens fixed in routine formalin solution. Twenty years later, in 1952, this fluorescence was demonstrated to be due to the presence of 5-hydroxytryptamine (5-HT or serotonin, Barter and Pearse, 1953) in gut enterochromaffin cells. In the same year, Eränkö (1952) described that formalin-fixed adrenals contained cells with a green fluorescence. He later demonstrated (Eränkö, 1955) that this fluorescence was due to the presence of NA in the cells. However, Hillarp compared this fluorescence with that of THI-treated adrenals from many species and found that the formalin-induced fluorescence was much weaker than the THI-induced fluorescence. So, for a while he gave up the idea that this formaline method could be useful for developing a histofluorescence method for adrenergic nerves.

Four years later, in 1959, Hillarp and his new pupil Bengt Falck, visited the Finnish Endocrinological Society where Hillarp was invited to give a talk entitled "Storage and Release Mechanisms of Catecholamines". After the meeting, Eränkö, who was the chairman of the session, brought Hillarp and Falck to his office where they discussed the observations on adrenal glands for several hours. As related by Olavi Eränkö in a telephone interview the author made with him in 1985, he had at that time started to experiment with vapour from liquid formalin to induce fluorescence in hamster adrenals at room temperature. Eränkö could not remember if he ever demonstrated these fluorescent adrenals or not. Hillarp never mentioned such a demonstration. He no doubt would have done had it occurred, since his sole goal for many years had been to make CA fluorescent. Bengt Falck, when I interviewed him in the same year, did not remember any demonstration of fluorescent tissue sections but could not rule out that vapour treatment of tissues may have been discussed.

Acknowledgements

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A Decade of Collaboration with Nils-Åke Hillarp: Recollections from 1956 to 1965

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Nils-Åke Hillarp and I received our basic education in biomedical research in the Departments of Histology and Pharmacology, respectively, of the University of Lund, Sweden. Hillarp was born in 1916, that is, 7 years before me. Our collaboration started in 1956. The background was the following.

In 1955 I spent a post-doctoral 6 months working in the Laboratory of Chemical Pharmacology of the National Heart Institute, headed by the late Dr Bernard B. Brodie. In the spring of 1955, only a few months before my arrival there, Brodie, Shore and their colleagues had made one of the major discoveries in the history of neuroscience, that is the depletion of serotonin stores in the brain and other tissues by reserpine, a drug recently introduced in the treatment of hypertension and schizophrenia (see Pletscher *et al.*, 1955). Brodie and his colleagues generously introduced me into the field of chemical pharmacology. His laboratory was at that time one of the most important research centres in this discipline and can, in fact, be said to be the cradle of modern biochemical pharmacology. An intense research was on-going not only in psychopharmacology, but also in drug metabolism. The prototype of the spectrophotofluorimeter had just been constructed in this laboratory by Robert Bowman, who was an M.D. but above all an ingenious inventor. This instrument, later manufactured and marketed as Aminco-Bowman spectrophotofluorimeter, is in my opinion one of this century's major breakthroughs in the analysis of drugs and endogenous chemical substances. It became widely used and was to become my main research tool for more than two decades but was thereafter largely superseded by even more powerful methodology.

Brodie was a remarkable person, who managed to stir up emotions wherever he went. He gained much sympathy but also made many enemies. He met with mixed feelings even among his collaborators. He was always full of new ideas and was impatient to have them experimentally tested, which was often disturbing for the continuity of the work in his laboratory. But most of his students became strongly devoted to him and looked upon him as a father. He seemed to have no ambition to be a true mentor but was mainly driven by his enormous scientific curiosity and ambition. Nonetheless he managed to transmit to his collaborators something apparently very important, because an amazing number of these people later became prominent scientists in the USA and in several other countries. To describe what he actually transmitted is not easy. He had an intensely searching mind and little respect for textbook knowledge. He courageously entered research fields that he did not know much about. Sometimes he managed to formulate basic questions that had previously been ignored. Perhaps this was the secret behind his remarkably successful career.

In Brodie's laboratory I worked on the effect of reserpine on the storage of serotonin in platelets, studied *in vitro*. I suggested to Brodie that we should also examine the action of reserpine on some closely related amines, especially the catecholamines. But Brodie considered this waste of time. He was convinced that serotonin was the important agonist in this context. While still in Brodie's laboratory I read an article by Hillarp *et al.* (1955), that had just appeared in *Nature* and described the storage of adrenaline together with adenosine-5'-triphosphate (ATP) in particular intracellular organelles in the adrenal medulla. In preliminary work I had found fairly large amounts of ATP in platelets, and this, in conjunction with Hillarp's report, strengthened my suspicion of a common storage mechanism for serotonin and catecholamines. I then wrote to Hillarp and suggested that we collaborate on the action of reserpine on the catecholamines, and he agreed.

Monoamines and Brain Function: A Controversial Issue

Hillarp was, at that time, Associate Professor of Histology at the University of Lund. He had already made a number of remarkable contributions in neuroscience and endocrinology. Especially important for our collaboration was his above-mentioned recent discovery of special organelles in the adrenal medulla, responsible for the storage of catecholamines. As we later found (Carlsson *et al.*, 1963), it was specifically these organelles and their counterpart in monoaminergic nerves, i.e. the so-called synaptic vesicles or granules, that reserpine acted on. Hillarp and I started to work together in 1956, and our collaboration lasted until his untimely death in 1965. We (Carlsson and Hillarp, 1956) soon found that

reserpine caused depletion of the adrenal medullary hormones, and this was soon followed by the discovery that similar depletion took place in other tissues, including brain. These findings offered a possible explanation of the hypotensive action of reserpine, and we could confirm this by experiments where stimulation of sympathetic nerves no longer caused release of their neurotransmitter noradrenaline following reserpine treatment (for review and references, see Carlsson 1987a).

These discoveries made us very excited but placed me in an awkward position in relation to my highly esteemed mentors, Drs Brodie and Shore. Our results challenged their interpretations in two respects. First, they indicated that the action of reserpine should not necessarily be interpreted as due solely to its effect on serotonin, and second, they argued against our mentors' proposal that continuous release of the putative neurotransmitter serotonin onto its receptors is responsible for the action of the drug. Rather, our results suggested that at least the hypotensive action was due to an effect on catecholamines and that this effect was caused by depletion rather than release. Unfortunately, this divergence of opinion was to place my mentors and myself in different 'camps' for many years to come and led to a large number of sometimes vivid debates in writing as well as at various meetings. This was unfortunate, because we, despite these divergences, were much more on common ground than a great number of other workers in this field, as will be apparent from the following.

To resolve the issue concerning the mode of action of reserpine my colleagues and I administered DOPA to reserpine-treated rabbits and mice and discovered the central stimulant action of this amino acid as well as its ability to reverse the akinetic and sedative action of reserpine. Since the serotonin precursor 5-hydroxytryptophan was not capable of reversing the action of reserpine we suggested that depletion of catecholamines rather than serotonin was responsible for some important behavioural effects of reserpine (Carlsson *et al.*, 1957).

However, when we analysed the brains of the animals treated with reserpine and DOPA, we found them still fully depleted of noradrenaline. Further analysis revealed that the behavioural action of DOPA could be explained by the accumulation of dopamine in the brain. Moreover, our studies disclosed that dopamine is a normal brain constituent and is released by reserpine, like noradrenaline and serotonin. The data suggested to us that dopamine is not just a precursor to noradrenaline, as previously assumed, but is an endogenous agonist in its own right (Carlsson *et al.*, 1958). This received further support when my students Bertler and Rosengren (1959) shortly afterwards discovered the marked difference in regional distribution between dopamine and noradrenaline, the former being largely accumulated in the basal ganglia. We could thus suggest that the parkinsonism induced by reserpine is due to dopamine depletion and that dopamine is involved in the control of extrapyramidal

motor functions. This suggestion was further supported by the fact that the motor disturbances in Huntington's chorea can be alleviated by reserpine and similar drugs (Carlsson, 1959).

Thus, for the first time evidence was forthcoming for a role of endogenous agonists, present in brain tissue, in animal behaviour. At first serotonin had come into focus, but the subsequent experiments pointed to a role of the catecholamines, and especially dopamine, for the sedative and akinetic actions of reserpine, and the reversal of these actions by L-DOPA. We were very excited by these findings but were disappointed to meet with considerable resistance by some prominent investigators. In particular, a meeting in London in the spring of 1960 on Adrenergic Mechanisms (Vane *et al.*, 1960) was a rather surprising experience to me. At this meeting practically all prominent workers and pioneers in the catecholamine field were present. It was dominated by the strong group of British pharmacologists, headed by Sir Henry Dale. I was impressed to see how the British pharmacologists, as well as many other former Dale associates, behaved towards Sir Henry, like school children to their teacher, although some of them had indeed reached a mature age. It was also remarkable to find how little disagreement was expressed among these people, who behaved more or less like a football team. At this meeting I reported on our data indicating a role of the catecholamines in motor functions and alertness. No doubts were expressed about our observations as such. In fact Drs Blaschko and Chrusciel presented observations that confirmed our findings on some essential points.

I have re-read the discussions recorded in the Symposium volume, and I am still puzzled by them. To start with Sir John Gaddum's Summary of the session on Central Adrenergic Mechanisms, he concluded (p. 584): "The meeting was in a critical mood, and no-one ventured to speculate on the relation between catecholamines and the function of the brain". As mentioned, my paper, which was entitled "On the Biochemistry and Possible Functions of Dopamine and Noradrenaline in Brain", as well as a considerable number of remarks that I made during the discussion sessions, dealt precisely with this issue. Obviously, in Gaddum's mind I was nobody! Why did he and the other British pharmacologists so completely ignore us? At first there was some concern about L-DOPA being a 'poison'. This appeared to be mainly based on the observation by Weil-Malherbe, that large doses of L-DOPA, given together with a monoamine oxidase inhibitor, could be lethal. This discussion ended by a concluding remark by Sir Henry Dale (p. 551) that L-DOPA is, in fact, a poison, which he found very remarkable for an amino acid. Then Paton referred to unpublished data by Edith Bülbring, suggesting the presence of catecholamines in glia rather than nerve cells. Responding to a question of Dale, Marthe Vogt concluded (p. 551) that there was absolutely no evidence that the catecholamines in the brain act as synaptic transmitters

or serve a general hormonal function. The proposal that this may be the case was said to depend on the particular pharmacological agents used. A critical survey of all the available evidence led, according to Marthe Vogt, to the conclusion that any of the theories on a relation between catecholamines or serotonin and behaviour is "a construction which some day will be amended" (p. 579).

Today this reluctance to accept a role for the monoamines in brain function may seem strange, especially since the doubts were expressed by some important pioneers in the theory of chemical transmission. It must be recalled, however, that at this time the predominating mechanism of neurotransmission in the brain, in contrast to the peripheral nervous system, was believed to be electrical. Moreover, the idea that the loss of a nerve function could be replaced by a drug and, thus, Parkinsonian symptoms be alleviated by L-DOPA, was hard to reconcile with the concepts of classical neurophysiology. It should be recollected, on the other hand, that several years earlier Gaddum (1953) had proposed that serotonin may serve to keep us sane, and that Marthe Vogt (1954) had pointed out that the 'sympathin' of the hypothalamus was probably not entirely derived from the peripheral sympathetic nervous system. It would seem that their original thinking had been followed by some 'pale cast of thought'.

Hillarp also attended this meeting, and we had good reasons to be grateful for this scepticism because it prompted us to increase our efforts to strengthen our views. I had just been appointed Professor and Chairman at the Department of Pharmacology, University of Gothenburg. Immediately following this meeting Hillarp and I agreed that he should join me to work on catecholamines in my new department, provided that he could be set free from his associate professorship in histology in Lund. We applied for the necessary funds at the Swedish Medical Research Council, and our grant was approved. We decided to focus on two problems: (1) to investigate a possible active amine-uptake mechanism by the adrenal medullary granules and its inhibition by reserpine; and (2) to try to develop a histochemical fluorescence method to visualize the catecholamines in tissues. Both these projects turned out to be successful. (Concerning the first point, see Carlsson *et al.*, 1963 and below.) Since detailed accounts of the histochemical fluorescence method and the subsequent mapping of monoaminergic pathways have been given elsewhere (Carlsson, 1987a; Dahlström and Carlsson, 1986; Dahlström, this volume), they will not be repeated here.

A Paradigm Shift—Emerging Synaptology

During the early part of the 1960s a large number of observations were made in Sweden by Hillarp, myself and our respective collaborators,

based on the combination of histochemical, biochemical and functional studies and using a number of pharmacological tools, which had an impact on the scientific community's view concerning the role of biogenic amines as neurotransmitters, not least in the central nervous system. That we can speak here of a true paradigm shift is evident from the proceedings of an international symposium held in Stockholm in February, 1965 and entitled "Mechanisms of Release of Biogenic Amines" (editors, von Euler *et al.*, 1966). In his introductory remarks Uvnäs stated that "these amines play an important role as chemical mediators in the peripheral and central system". None of the distinguished participants in this symposium expressed any doubts on this point.

While the scepticism had thus faded, it was followed by an intensive debate on the function of various synaptic structures and mechanisms. A few early recollections of this debate will be reviewed below.

A major issue dealt with the role of the synaptic vesicles in the transmission mechanism. In the mid-1960s opinions still differed concerning the subcellular distribution of the monoaminergic transmitters. In the fluorescence microscope the accumulation of monoamines in the so-called varicosities of nerve terminals was obvious. This corresponded to the distribution of synaptic vesicles, as observed in the electron microscope. In fact, Hökfelt (1968) was able to demonstrate the localization of central as well as peripheral monoamines to synaptic vesicles in the electron microscope. However, there was controversy about the nature and size of the extravesicular (or extragranular) pool of neurotransmitter. This is evident from the recorded discussions of the above-mentioned symposium "Mechanisms of Release of Biogenic Amines". For example, Drs Axelrod and von Euler (p. 471) maintained that a considerable part of the transmitter was located outside the granules, mainly in a bound form. This fraction was proposed to be more important than the granular fraction, since it was thought to be more readily available for release. Indeed, the granules were facetiously referred to as 'garbage cans'. Our group had arrived at a different model of the synapse, based on combined biochemical, histochemical and pharmacological data (Carlsson, 1966). We were convinced that the granules were essential in transmission, and that the transmitter had to be taken up by them in order to become available for release by the nerve impulse. In favour of this contention was our finding that reserpine's site of action is the amine uptake mechanism of the granules. The failure of adrenergic transmission as well as the behavioural actions of reserpine were correlated to the blockade of granular uptake induced by the drug, rather than to the size of the transmitter stores (Lundborg, 1963). Moreover, extragranular noradrenaline [accumulated in adrenergic nerves by pre-treatment with reserpine, followed by an inhibitor of monoamine oxidase (MAO) and systemically administered noradrenaline], was unavailable for release by the nerve

impulse, as observed histochemically (Malmfors, 1965). We proposed that under normal conditions the extragranular fraction of monoaminergic transmitters was very small, owing to the presence of MAO intracellularly, and that the evidence presented to the contrary was, in fact, an artifact. Subsequent work in numerous laboratories has lent support to these views. Already at the Symposium, Douglas presented evidence suggesting a Ca^{2+}-triggered fusion between the granule and cell membranes, preceding the release. The release is now generally assumed to take place as 'exocytosis', even though the complete extrusion of the granule content may still be debatable.

An important issue in the early debate dealt with the site of action of major psychotropic drugs. In their first studies on reserpine Brodie and his colleagues had proposed that this agent was capable of releasing serotonin on to receptors, which would suggest the cell membrane to be its site of action. However, our observations, quoted above, demonstrated that reserpine acted on the storage mechanism of the synaptic vesicles. As to the tricyclic antidepressants, Brodie *et al.* suggested their site of action to be on the synaptic vesicles. In their original studies reported in 1960 Axelrod *et al.* (see Axelrod, 1964) observed that the uptake of circulating catecholamines by adrenergic nerves could be blocked by a variety of drugs, for example, reserpine, chlorpromazine, cocaine and imipramine. These studies obviously did not distinguish between a number of different pharmacological mechanisms. In our own combined biochemical (Carlsson *et al.*, 1963; see also the independent, simultaneous work of Kirshner, 1962) and histochemical studies (Malmfors, 1965) two different amine-concentrating mechanisms could be distinguished, i.e. uptake at the level of the cell membrane, sensitive, for example, to cocaine and imipramine, and uptake by the storage granules or synaptic vesicles, sensitive, for example, to reserpine. These two mechanisms have of course different, essentially opposite functional consequences, implying enhancement and inhibition, respectively, of monoaminergic neurotransmission.

Receptor Research

In the early 1960s we were puzzled by the fact that the major antipsychotic agents, such as chlorpromazine and haloperidol, have a reserpine-like pharmacological and clinical profile and yet lack the monoamine-depleting properties of the latter drug. We found that chlorpromazine and haloperidol accelerated the formation of the dopamine metabolite 3-methoxytyramine and of the noradrenaline metabolite normetanephrine, while leaving the neurotransmitter levels unchanged. In support of the specificity, promethazine, a sedative phenothiazine lacking antipsychotic and neuroleptic properties, did not change the

turnover of the catecholamines (Carlsson and Lindqvist, 1963). It did not seem far-fetched, then, to propose that rather than reducing the availability of monoamines, as does reserpine, the major antipsychotic drugs block the receptors involved in dopamine and noradrenaline neurotransmission. This would explain their reserpine-like pharmacological profile. To account for the enhanced catecholamine turnover we proposed that neurons can increase their physiological activity in response to receptor blockade. This, I believe, was the first time that a receptor-mediated feedback control of neuronal activity was proposed. These findings and interpretations have been amply confirmed and extended by numerous workers, using a variety of techniques. In the following year our research group discovered the neuroleptic-induced increase in the concentrations of deaminated dopamine metabolites (Andén *et al.*, 1964). Later papers by Andén *et al.* (1970) from our own laboratory and by Nybäck and Sedvall (1970), emphasized the effect of neuroleptics on dopamine, and the work of Aghajanian and Bunney (1974) described the effect of dopaminergic agonists and antagonists on the firing of dopaminergic neurons. Other important, subsequent discoveries were the dopamine-sensitive adenylate cyclase by Greengard and his colleagues (Kebabian and Greengard, 1971) and the binding of dopamine to specific cell-membrane sites, from which it could be displaced by neuroleptics (Seeman *et al.*, 1976; Creese *et al.*, 1976).

The further analysis of receptor-mediated feedback control of neuronal activity revealed that this control was largely, if not entirely, mediated by a special population of receptors, apparently located on the monoaminergic neuron itself. These receptors have been called presynaptic receptors or, perhaps preferably, autoreceptors, since they have various locations on the neuron but share the property of being sensitive to the neuron's own neurotransmitter (Carlsson 1975a, b, 1987b). The first suggestion of the existence of such receptors came from studies by Hillarp's pupils Farnebo and Hamberger (1971) on brain tissue slices, demonstrating inhibition and stimulation of nerve-impulse induced dopamine release by dopamine agonists and antagonists, respectively. Subsequent *in vivo* studies in our laboratory demonstrated inhibition of striatal dopamine synthesis by the dopamine-receptor agonist apomorphine and blockade of this action by the neuroleptic agent haloperidol; moreover, this effect persisted after cutting the dopaminergic axons, thus demonstrating that this feedback control was not loop mediated but was restricted to the nerve-terminal area (Kehr *et al.*, 1972). Aghajanian and Bunney (1974) demonstrated a similar control in the somatodendritic part of dopamine neurons, leading to a decreased firing by dopamine-receptor agonists and a blockade of this action by dopamine-receptor antagonists. Further work along this line has led to the discovery of selective dopamine-autoreceptor agonists and antagonists with interest-

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ing pharmacological properties and potential clinical utility (see Clark *et al.*, 1985a, b; Svensson *et al.*, 1986).

The Ups and Downs of Serotonin

While dopamine, like Cinderella, had to dwell in obscurity for a long time until it came into glory, serotonin took another path. Very soon after the discovery of serotonin as a normal brain constituent it started to attract a great deal of interest, as soon as a link between serotonin and LSD was discovered and brain serotonin was proposed to serve a role to keep us sane (see Gaddum, 1953). This culminated with the discovery of reserpine's serotonin-depleting action already referred to. However, when subsequent work disclosed the catecholamine-depleting action of reserpine, noradrenaline and later dopamine came into focus, and serotonin lost its dominating place. But serotonin made a splendid comeback in a different context.

The tricyclic antidepressants were first shown to block the re-uptake of noradrenaline, and thus this neurotransmitter was proposed to play a major role in the control of mood and drive. Later it was discovered, however, that the tricyclic antidepressants also have powerful actions on the re-uptake of serotonin and that this applied especially to some of the most widely used antidepressants (Carlsson *et al.*, 1968; for further references, see Carlsson, 1976, 1982, 1986). Together with the late Dr Hans Corrodi, a highly talented Swiss chemist, we then developed the first selective 5-HT uptake inhibitor zimelidine (Berndtsson *et al.*, 1972), which turned out to be an active antidepressant agent (see Carlsson *et al.*, 1981) but was withdrawn because of certain rare but serious side-effects (Bengtsson, 1992). Subsequently a number of other selective serotonin uptake inhibitors were developed and likewise found to be efficacious antidepressants. This in conjunction with the discovery of an antidepressant action of L-tryptophan (Coppen *et al.*, 1963) and of reduced concentrations of 5-hydroxyindoleacetic acid in the cerebrospinal fluid of depressed and suicidal patients (Träskman *et al.*, 1981) led to a marked increase in the visibility of serotonin, which is now generally recognized as an important neurotransmitter in the control of mood.

Recently serotonin has also started to attract a great deal of attention in the control of anxiety. Panic disorders appear to respond especially well to serotonin uptake inhibitors. Most remarkably, obsessive–compulsive conditions appear to respond specifically to serotonergic drugs (see Eriksson and Humble, 1990). The ability of these agents to influence personality aberrations, also within the range of normal variation, has attracted considerable interest, as evident from the book *Listening to Prozac* (Kramer, 1993).

Concluding Remarks

Hillarp made his scientific career in histology, and some of his most important scientific contributions fall within the boundaries of morphology. However, his work was always largely directed towards elucidating physiological problems. When morphology proved insufficient he spared no pains to acquire the knowledge and experimental skill necessary to solve his problems, and thus in the course of his career his theoretical and practical abilities and achievements expanded far into biochemistry and physiology. The uniquely broad background thus obtained, together with brilliant imagination, power of combination and boldness, seem to have formed the key to his many remarkable successes.

Nils-Åke Hillarp was a modest person with a strong dislike of pompous manners. In front of a large audience he was reticent, and he only attended a few international scientific meetings. In fact, the Symposium on Adrenergic Mechanisms referred to above, was the only meeting outside the Nordic countries that he attended. But in more intimate surroundings he was lively and outgoing. His many friends, co-workers and pupils will always remember him for his generosity, warmheartedness and enthusiasm.

The present review covers only a part of Hillarp's many contributions during the last decade of his life. Other aspects are dealt with in other papers of the present volume. For example, the important histochemical and morphological aspects are discussed in Annica Dahlström's and Gösta Johnsson's reviews. In the present review little or no distinction has been made between papers authored by the different groups separately or in collaboration. In fact, the different contributions may well be looked upon as one single collaborative effort carried out by a complex network that started out at the University of Lund in 1956 and then expanded to the University of Göteborg as well as the Karolinska Institute in 1960 and 1962, respectively. Various aspects of the work continued in the three sites after Hillarp's death in 1965 and are still on-going. Some examples of later activities have been included above in order to illustrate the profound impact of the initial discoveries.

In the present three-decade perspective it is fair to state that very few, if any, workers in Swedish biomedical research during this century have had an impact comparable with that of Nils-Åke Hillarp.

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