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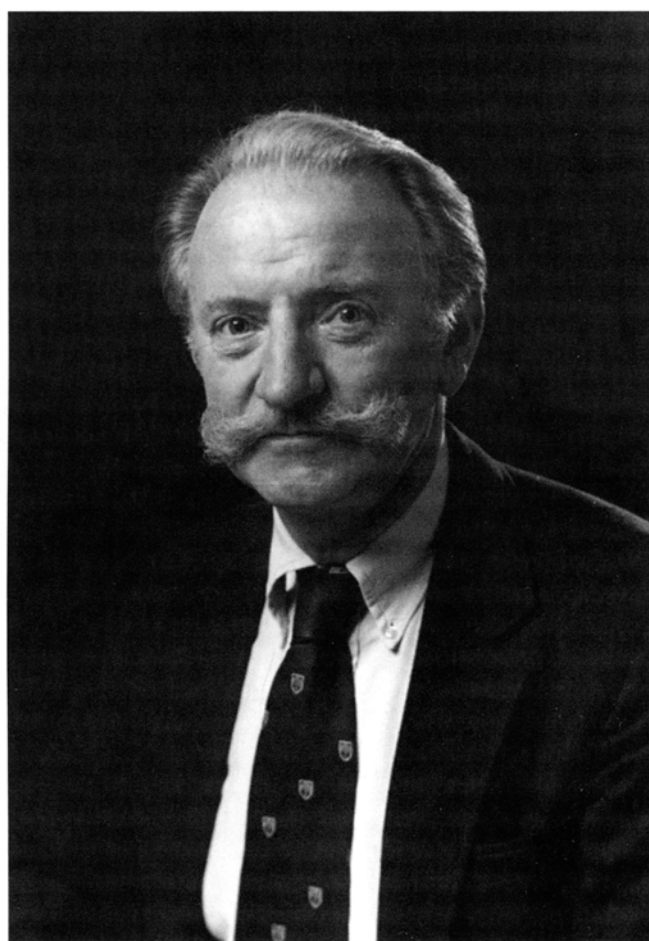
Alan Peters

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BORN:
Nottingham, England
December 6, 1929

EDUCATION:
Bristol University, B.Sc. (1951)
Bristol University, Ph.D. (1954)

APPOINTMENTS:
University of Edinburgh (1958)
Harvard University (1963)
Boston University (1966)

HONORS AND AWARDS (SELECTED):
Symington Prize in Anatomy, Anatomical Society of
Great Britain and Ireland (1962)
President, American Association of Anatomy Chairmen (1976)
Krieg Cortical Discoverer Award, Cajal Club (1990)
President, American Association of Anatomists (1992)
Henry Gray Award, American Association of Anatomists (1998)
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Alan Peters is one of the foremost anatomists of the mammalian nervous system. He was one of the first to determine the structure of myelin sheaths in the central nervous system and used light and electron microscopy to characterize the ultrastructure of neuroglia and neurons, as well as the microcircuitry and organization of neurons in cerebral cortex and the organization of thalamocortical projections. With S.L. Palay and H. deF. Webster, he co-authored three editions of the definitive text on the fine structure of the nervous system, and with E.G. Jones he created the authoritative series The Cerebral Cortex, which appeared in 14 volumes, 1984–1999. More recently, he has studied the anatomy of normal aging in the primate brain.

Alan Peters

When I received the invitation to contribute an autobiographical chapter to this volume I felt flattered; then I began to think what I might put into the chapter. Memories of events and people that I had not thought about for years came flooding into my mind, and I hope that most of the memories turn out to be accurate, so that I do not offend anyone I have omitted. Then in preparation for putting the chapter together I read some of the contributions to previous volumes. Most of the contributors cited their involvements with numerous collaborators, and I began to realize that although I know many people in science, basically I have been most content to either work alone or with one or two graduate students or postdoctoral fellows. I have never wanted to have a large laboratory with numerous collaborators. But I have been lucky in that I have collaborated with some very knowledgeable and pleasant people, and I have been especially lucky in having a series of excellent, bright and hard-working research assistants. Over the years they have made it possible for me to carry out a productive research program, despite the administrative duties I had as Chairman of a Department of Anatomy for 32 years. These research assistants, Charmian Proskauer, Julian Saldanha, Lauren Kimerer, Dan Kara, Kathy Harriman, Karen Josephson, and especially Claire Folger (Sethares), have made such major contributions to my research that I have always felt that their efforts should be recognized by including their names in the list of authors on articles.

If I omit someone that I have worked with, please forgive me. It is not that I do not appreciate the work we did together but only that there is no space to describe every research project in which I have been involved. The only way I can give a lucid account of my interests over the past 40 years is to confine myself to the major research themes I have followed.

Early Years

I was born in Nottingham, England, in 1929. My parents lived in a rented, semidetached house, but within a year of my arrival they moved into a row house in what was termed a working class district. The reason for the move was that my father, who was employed at a tobacco factory, was only on part-time work because of the depression, and the rent for the new house was more affordable. In those days, of course, it was not customary for a

wife to work, so there was only my father's income to support the family. Nottingham was basically an industrial town, and most of the neighbors worked in the lace trade, at Players tobacco factory, in the local coal mine, or at a factory that made bicycles. Nevertheless, it was a friendly neighborhood and not a bad place to grow up. Because the houses were so close to each other, the neighbors looked after one another and socialized; everyone knew everyone else's business, where the children were, and when anyone was ill. My mother and father often talked about moving into a larger house, but somehow they never got around to doing it. The house was nearby where my father worked, and my mother so liked the area that she lived in that house until she died at the age of 95.

When I was 4 years old I started my education at a nearby nursery school, and a year later I transferred to the local junior school, which was about half a mile away from home. I stayed there for the next 6 years, until the age of 10, when I took the entrance examination for the local grammar school. The system of public schooling in England in those days was such that all children up to age 10 went to one of the local junior schools, and then at the age of 10 or 11 the better students took the entrance examination for one of the grammar schools, which were the prime source of university applicants. In Nottingham there were three grammar schools for boys. If a student passed the entrance examination, he transferred to a grammar school where students stayed until at least the age of 16, and some stayed until the age of 18 if they were to go to university. Students who did not take or pass the grammar school entrance examination transferred at the age of 11 to the local middle school, and then advanced to senior school, where they stayed until the age of 15. Luckily, I passed the entrance examination and was given a place at High Pavement Grammar School.

The year I took the entrance examination for grammar school was an unusual one, because on September 3, 1939, the Second World War was declared. As a consequence, I remember that in my last year at junior school we attended school in shifts and had some classes in the local church hall, because the school had been taken over by the Home Guard and Civil Defence units. In addition, some of the teachers had been called to serve in the armed forces. Consequently, there was neither enough space nor teachers available to teach all of the pupils simultaneously.

Despite the beginning of the war, people went about their business very much as usual, and so in September 1940, I entered High Pavement Grammar School. It is interesting in retrospect to recall that the entering class of about 120 pupils was divided into four classes depending on their performance on the entrance examination. How they assessed abilities other than performance on that examination I have no idea, but nevertheless the brightest pupils were assigned to a "Classics" form in which the emphasis was on Greek, Latin, geography, history, and general subjects. The second group was assigned to an "A form," which was for pupils who were

mathematically oriented, and for them the emphasis was on mathematics, physics, chemistry, German, and Latin. The third group, the one to which I was assigned, was the "B form." We were considered not bright enough to understand advanced mathematics and physics, so our emphasis was on biology, chemistry, French (because it was less complicated than German), geology, and Latin. The fourth group was the "General" form, and their curriculum covered a wider range of topics, with emphasis on general science, geography, history, woodwork, and metal work.

On Monday through Friday, the school day lasted from 9 AM until 4:30 PM, and we had at least an hour of homework each evening. One afternoon per week and Saturday mornings, we played the sport appropriate for the season: rugby or cross-country running in winter and cricket or rowing in summer. Because I had a strabismus, I did not "have an eye" for a cricket ball, and I elected to row in the summer.

In retrospect it is clear that the education I received at grammar school was an excellent one, and being assigned a biology class was the first step in defining what I would do in my later life.

Although the education was excellent, it was somewhat disrupted by the war, because almost every night we were awoken by the air raid sirens and had to take shelter in the reinforced cellar under the house. It was not that many bombs were dropped on Nottingham, but the German airplanes followed the river Trent to fly to other locations, and as they passed over Nottingham, antiaircraft guns bombarded them. The result was that a great deal of shrapnel for the antiaircraft gun shells dropped from the sky, and unless one had a steel helmet it was dangerous to be outside. In addition, there was a general shortage of food, and most of the able-bodied men, as well as a number of women, were called up into one of the branches of the armed forces. One of those called up was my father, who was recruited into the Air Force, leaving my mother and I to fend for ourselves for the last couple of years of the war.

Otherwise, I progressed uneventfully through the first 5 years of grammar school, and in 1945 I sat the School Certificate Examination. This was a national examination given to pupils in the fifth form of grammar school, and the results of this examination largely determined how you would spend the rest of your life. Most pupils left school after taking the examination, but about 20% of them were selected to go on to enter the sixth form for a further 2 years of education to prepare for entry into university at the age of 18. I did sufficiently well in the School Certificate Examination to be selected to enter the sixth form, and I continued in the biology stream. That entailed a concentration in science and we took classes in chemistry, physics, zoology, and botany. Because there were only about 10 of us in the class, we received a great deal of individual attention, and in addition to lectures we were guided to carry out experiments in well-equipped laboratories. We also had a large amount

of free time in which we were expected to work by ourselves. But other responsibilities also came with being in the sixth form. Most of the sixth formers were made prefects, and essentially it was the prefects who oversaw the discipline in the school, because they took care of classes in the teacher's absence and could impose detentions on students who were misbehaving.

At the end of the 2 years in the sixth form I took the Higher School Certificate Examination, which was essentially the entrance examination for university. I recall that I obtained two honors and two passes in the four subjects that I submitted for the examination. This was good enough to gain entry into a university but not good enough to obtain a scholarship. This meant that if I were to go to university my parents would need to help pay for my education, which they could not afford to do. Because my parents knew nothing about higher education, both of them having left school at the age of 14, this obviously presented a dilemma. Mr. Crossland, who was Assistant Head Master at High Pavement School, offered a solution. Mr. Crossland was much feared by us boys, because he was in charge of the discipline in the school, and being taken before him was one of the things to very much avoid. However, under his tough exterior he was a very caring man, and after the results of the examination came out he asked me what I intended doing in the future. I told him that I would like to go to university but that my parents could not afford to support me. After a conference with Mr. Crossland, my parents were persuaded to let me stay on in the sixth form for 1 more year, after which I would take the Higher School Certificate Examination one more time to try to qualify for a state scholarship that would pay my tuition at university and a stipend. So I stayed on at school for 1 more year, and on taking the examination at the end of that year I gained honors in all of the four subjects tested and was awarded a state scholarship to Bristol University to read zoology. This was the university I had chosen because of the good reputation of its zoology department.

However, during this third year in the sixth form it was also recommended that I take the Open Cambridge University Entrance Examination to try for a place at Cambridge University. As a result of that examination I was awarded a scholarship to study medicine at Downing College, because my real ambition at that time was to become a medical doctor. However, when I went for the interview at Downing College, I was told that they could offer me a place, but I would not be able to activate it for at least 2 years, because they were giving entry preference to those who either had been called up into the armed forces at the end of the war or had completed 2 years National Service, which all males had to do at that time. Being stubborn and seeing my goal of going to university fading into the distance, I decided to give up the idea of medicine and to postpone doing my 2 years National Service until I had finished university. Consequently,

I went to Bristol University to read zoology, a decision that I have never had cause to regret, even though I never really became a card-carrying zoologist.

University

I started at Bristol University in September of 1948, and again because preference was being given to those who had been in the Armed Forces, I could not get accommodation in one of the halls of residence of the university. Instead, I was allocated lodging with a family. To save train fare, my father managed to get me a ride to Bristol on one of the lorries that transported goods from the tobacco factory where he worked to one in Bristol. Thus, I was delivered to my lodgings. I have forgotten the name of the family with whom I lodged for a couple of years, but the household consisted of a mother and her adult daughter, and quite soon after my arrival they told me that they belonged to a temperance society, which meant that they did not approve of drinking and the problems it caused. In any case, at that time I did not have money enough to spend much on drink and so it did not bother me. I suppose that I must have been a rather annoying lodger, but they were very kind to me: My room was comfortable and the breakfast and dinners they provided were good.

   Each morning I would either walk or get a bus to the university, where I took courses in my two major subjects, chemistry and zoology. Usually there were two lectures each morning and every afternoon, except Wednesdays when there were laboratory classes.

   On Wednesday afternoons, Saturday afternoons, and Sunday mornings I went out to the University Boat Club, which was located several miles away on the road to Bath. Rowing was not one of the major sports at Bristol University, so the membership of the Boat Club was not very large. Because I had spent some 5 years rowing while at school, I was soon given a place in the first eight. I continued to row for the entire time I was at Bristol, and in addition to getting my University Colors, I became Captain of Boats. Rowing was a very pleasant way to spend my spare time, but early on I was given a wake-up call, because I had spent so much time rowing in my first year that I almost did not pass the end of term examination.

   At the end of the second year at university, during which I learned all about the classification of animals and some ecology, I was selected to enter the honors class in zoology, and at the same time I moved to lodgings on the other side of town. There were only nine of us in the honors class, and being such a small group we received a great deal of personal attention from the faculty, especially in terms of the laboratory portion of the course. I do not think that any of the faculty had worldwide reputations, but they were very good teachers. Among the best was Jack Kitchen who was a protozoologist interested in contractile vacuoles, and one of the highlights of the year was

a course of lectures offered by the ethologist Kondrad Lorenz, who was visiting and gave a month-long course in animal behavior and imprinting. Among the students in the honors class were a few with whom I retained contact after graduation. One was Mike Mortimer who went off to Rhodesia after graduation to take up fish farming and then, after one of the more troubling times in Africa, came back to the United Kingdom as a forest ranger. Unfortunately Mike died at quite a young age. Another member of the class was Clive Edwards who after graduation joined the Ministry of Agriculture, went off to Purdue University to complete his Ph.D., and after retiring from the civil service in Britain came to America as Chairman of the Department of Entomology at Ohio State University in Columbus, Ohio, where he is one of the world's experts on soil ecology and earthworms. He and I shared an apartment during my three graduate years at Bristol.

At the end of the honors year I was awarded an upper second class honors degree, the only first class honors degree being given that year, and Professor John E. Harris F.R.S., who was the head of the zoology department, invited me to stay on to pursue a Ph.D., which would take another 3 years. I accepted his offer, and he put me on to a graduate support grant he had available. Because I had a strong background in chemistry, he suggested that a good project, and one that interested him, would be to investigate the mechanism of staining nervous tissue with reduced methylene blue and with silver salts. At that time, these were two of the main methods for the general staining of nervous tissue, but no one understood how these staining methods worked and how they could be controlled. Prof. Harris wanted to use the methods to stain the nervous systems of developing fishes, which was one of the research topics that interested him. But as far as I knew he never actually used the methods himself.

Graduate Study

When I started my graduate studies in 1951, I was allocated a laboratory in a small concrete building where all of the graduate students in the zoology department were housed. Following the traditions of the day, I was very much left to my own devices, conferring with my advisor, Prof. Harris, only about once every 3 months when I informed him what I had been doing, and he made suggestions about how I might proceed. Because there were six graduate students in the building, the atmosphere was congenial, and we frequently conferred with each other about our research, and in retrospect this was the main way that we expanded our education. Among the graduate students at that time was John Treherne, who was an entomologist and was later to play an indirect but important role in deciding my future. John was 1 year ahead of me, and after completing his Ph.D. he went into the army and later went to Cambridge University as an entomological physiologist. He eventually became president of Downing College. Another graduate

student was Lionel Carter, who was a biochemist; after graduation he went to work for Imperial Chemical Industries in Brixham, Devon, where he was in charge of their anti-fouling marine division and could indulge in his passion for sailing.

As I indicated previously, my first research project was concerned with the mechanism and conditions for the vital staining of nervous tissue with reduced methylene blue. The methylene blue was reduced so that it became colorless, and after tissue or whole fish embryos were put into the solution under anaerobic conditions the tissue or embryo could be removed and the preparation oxygenated. The nervous tissue, which had taken up the dye, eventually oxidized and became intensely blue, so that the nervous system could be examined (Harris and Peters, 1953). At that time I knew almost nothing about the nervous system, other than the rather superficial survey course I had during undergraduate studies, but looking at the stained cells and their processes I was fascinated by the complexity of it all.

After completing that study, I went on to examine the mechanism of silver staining of nervous tissue. The reason for carrying out these studies was because silver staining was one of the few routine methods available for examining the nervous system in tissue sections. These studies resulted in my first individual paper, which showed that the specificity of silver staining is largely due to binding of silver salts with hisitidine groups on proteins. The article was published in *Nature* (Peters, 1953), and as I completed my thesis, a series of other articles on the mechanism of silver staining followed (Peters 1955, a–d).

The last of the articles in this series (Peters, 1955d) was an electron microscopic study of the disposition of silver in stained nervous tissue. I had read about the emerging field of electron microscopy, and this seemed an interesting way to determine where the silver particles are located in nerve cells and their processes. I was lucky to obtain the help of a Professor Hewer from the pathology department, who had had a Cambridge rocking microtome for light microscopy adapted and geared to cut sections as thin as 0.1 microns. I also obtained help from Dr. Tony Lee in the veterinary school. After tissue sections had been silver stained, they were embedded in methyl methacrylate, and my first thin sections were made using thin, steel razor blades that had been sharpened by rubbing them on the inside of a wet drinking glass, a technique we had used during the war to extend the lives of razor blades. But as I was doing this study, it came to our attention that Harrison Latta had published an article on how to make glass knives, which made thin sectioning much easier. However, obtaining thin sections was only the first of the challenges: The second was getting the Phillips electron microscope in the physics department to remain stable long enough to get pictures of the thin sections on its 35-mm roll of film. The results were by no means spectacular, but they did show the metallic particles of silver formed during staining to be aggregated along the fibrous

components within the cytoplasm of the nerve fibers, not in the interspaces between the neurofibrils and not in the myelin sheaths.

In the autumn of 1954, I submitted my thesis for examination, and because my external examiner was out of the country, it was not until the beginning of 1955 that I heard I had been awarded a Ph.D. degree. I had no idea about what I would do with the degree in zoology. However, I did assure my fiancée, Verona, that she would not be marrying a zoo keeper, but naively I had not given my future a great deal of thought. I knew that at the beginning of 1955 I would be called up into the army to do my 2 years of compulsory National Service, and I more or less decided to defer making any decisions about my future until the end of that time.

Military Service

I need to say something about my military service, because effectively it was during this time that my professional scientific future became decided.

I was called up to serve in the Royal Army Signal Corps, presumably being posted to that unit because of my scientific background. I entered the army in January 1955, and, after completing my basic training, I was selected to go to Officer Training School at Eaton Hall in Chester. I spent 6 months there learning drill, military law and tactics, and physical education, which seemed to largely consist of running for miles with full packs. This latter experience convinced me never to take up jogging for pleasure.

At the end of the training I was commissioned as a 2nd lieutenant in the Royal Army Medical Corps (RAMC). The reason for that posting was as follows. John Treherne, who was a fellow graduate student at Bristol and who graduated a year ahead of me, was in the Medical Corps and was completing his service at a place called Porton, which was an army research station. His commanding officer at Porton had asked John if he knew of anyone who could replace him when he completed his 2 years of National Service. John recommended me as his successor, and this was the reason for my commission into the RAMC. After being commissioned, I was posted to the RAMC Depot, where it was intended that I should stay for a month or so until John Treherne was demobilized, when I would be posted to Porton as his replacement.

However, our well-laid plans went awry. We had not anticipated the regulations that governed postings from the RAMC Depot. The rule, strictly adhered to, was that officers were posted out of the depot in the same sequence in which they arrived, and while I was at the depot, a vacancy occurred for an adjutant at a field ambulance in Germany. Because I was the next officer in line for a posting, I was sent to Germany to take the position. As an aside the next officer in line after me was someone with a bachelor's degree in biology and no research experience, and he got the

posting to Porton. However, in retrospect, things worked out very well, because I gained a great deal of experience and confidence as a result of my experiences in the army in Germany.

I was allowed 10 days embarkation leave before departing for Germany, and so I called my fiancée with this news. We had already decided to get married some time in the future, and we concluded that it would be best if we got married before I left for Germany. In the space of a week, my wife to be, Verona, in her typical unflustered fashion, managed to get everything set for the wedding, including getting the wedding license and arranging for the reception. So we got married and had a honeymoon, after which I departed for Germany, leaving her behind. I had known my wife since I was in my teens, and I had always thought that she was unattainable. But when I was home for a week during my graduate student years, I had phoned her to ask if she would come to the Nottingham Goose Fair with me. To my surprise and delight she agreed to accompany me to the fair, which had its origin in medieval times, and that was the beginning of our courtship and eventual marriage. We have now been married for almost 50 years, for which I consider myself very fortunate.

As adjutant to a field ambulance, I was more or less in charge of the management and discipline of the unit, which included making sure that the soldiers did not get into too much trouble. Essentially, I was the only trained officer in the unit. All of the other officers had medical degrees and were concerned with medical and not military problems. We had a very sociable life, and one of the perks was that the officer's mess had been previously occupied by the German army, who had laid down a good stock of wines in the cellars, so that on mess nights we were able to sample good wines and brandy.

When I departed for Germany, there seemed to be little chance of my wife and I being together until I finished my military service at the end of 1956, but being an adjutant to a field ambulance, I had to be thoroughly familiar with a book called *Queen's Regulations*, which govern all aspects of military life. I found that in *Queen's Regulations* it was stated that National Service RAMC officers were allowed to have their wives with them. In reality, this referred to officers with medical degrees, who were either 1st lieutenants or captains. When the regulations were written there were no 2nd lieutenants in the RAMC, and in fact there were only about six of us when I was in the army. However, regulations being regulations, my colonel agreed that the regulations should be followed, and my wife gave up her job and was able to join me for the last 8 months of my military service.

At the end of 1956, as my demobilization from the army became closer, I began to think seriously about my future, but being in Germany made it difficult to make contact with people who might offer me a job. However, one day one of the medical officers, who was reading the *British*

Medical Journal and looking for a job for himself, saw that postdoctoral fellowships in basic science were being offered by Edinburgh University. In my graduate student days, I had been in contact with Prof. George Romanes, who was the professor of anatomy at Edinburgh, and so I knew that he had some interest in silver staining. Consequently I wrote to Prof. Romanes and asked him if I could come to work with him as a postdoctoral fellow. To my delight and relief, he offered me a postdoctoral fellowship, and we agreed that I should start working with him at the beginning of 1957, after I had been demobilized. So in January 1957 Rona and I caught the train to Edinburgh, arriving on a rather typical winter's day with gray skies and the gray stone of Edinburgh welcoming us.

Edinburgh

My first impressions of the Department of Anatomy at Edinburgh University were rather mixed. I found that it was basically a teaching department and that the faculty largely consisted of retired doctors and others with medical degrees who did not like working with patients. However, there were some exceptions and among them were George Romanes and Alan Muir, who I will say more about later. The general lack of interest in research is perhaps best illustrated by the fact that when I asked the chief technician for some glassware to resume my silver staining studies he went into the basement to find some for me. The only trouble was that the glassware was graduated in drachms and ounces, and glassware graduated in metric units had to be ordered for me. I was also struck by the fact that the professor, George Romanes, was the only one who had the authority to order anything, so every request for supplies or equipment had to go through him. He also oversaw the allocation of typists and of technical staff, each of whom had their own territories and would generally do nothing until requested to do so by either him or by the chief technician. However, I soon got used to the system, which was typical of departments of anatomy in Britain at that time, and it did not really interfere with my research.

I might add that George Romanes, who was a generous man with a very wide background and knowledge of anatomy, had largely given up research by the time I arrived in Edinburgh. In addition to running the department, George Romanes gave the bulk of the anatomy lectures, which were presented in an old-fashioned, tiered lecture theatre where dissections, including the public dissection of the body snatcher Burke, used to be carried out. Much of the rest of George Romanes' time was spent in rewriting the series of textbooks on anatomy that had been initially written by the Cunninghams, who were among the first professors of anatomy at Edinburgh. But despite these commitments, George Romanes was very

supportive, and because my laboratory was next to his office, most days we met to discuss my research as we puffed away on our pipes.

During this first year, I continued working on silver staining of nervous tissue and developed methods of staining that used silver proteinates, similar to the Protargol silver proteinate that was used in Bodian's silver stain (Peters, 1959). I also became very interested in physical developers as a means of intensifying the silver stains and undertook the staining of a series of rat embryos in which I intended to study the development of the spinal cord. However, as I began to learn more about the nervous system and become more interested in what was being stained by silver, I began to realize that silver stains are not all that useful for studying the central nervous system. Virtually all nerve cells and their processes are stained by silver, but only short lengths of any of the processes are visible in any one section. Consequently, processes of neurons cannot be followed for any distance, making connections between neurons almost impossible to determine. Another difficulty is that only the largest of the axonal boutons are stained by silver. I suppose that I had been too obsessed with the mechanism of the staining process and, at that time, had such a poor knowledge of the nervous system that it took me several years to realize the real inadequacies of silver staining.

A Faculty Member

After I had been a postdoctoral fellow for a year, George Romanes offered me a lectureship in anatomy, and I took the job. Apart from doing research, my responsibility was to help teach the dissection and histology laboratories on the nervous system. This meant learning the material, because my background in zoology had not covered any nervous system in detail. I also set about learning some general histology, but I could not become enthused by gross anatomy, which I have never learned.

At about the same time that I became a faculty member, Alan Muir in the department acquired an electron microscope, one of the new AEI 6As. Alan had been to America on sabbatical leave and had spent the year with Ed Dempsey in St. Louis, learning techniques of electron microscopy, techniques that he now offered to teach me. However, in 1958 the techniques for making thin sections were still somewhat primitive. The tissue had to be embedded in methyl methacrylate, which became warm as it polymerized, often causing the tissue to explode and fragment. Also, at that time, we had early model, MT-1, Porter-Blum ultramicrotomes that heated up when a light was turned on, causing the plastic embedded blocks of tissue to expand. This meant that the thickness of the thin sections was not very consistent. In addition, to make glass knives, we had to score sheets of glass and break it with pliers. Finding the best glass was always a challenge, and the consensus was that old and brittle glass made the

best knives. Consequently, it was with great glee that Alan Muir and I heard that Glasgow was scrapping its old tramcars to replace them with trolley cars. We knew that the windows of the old tramcars were made of plate glass, so Alan and I borrowed a truck and went over to Glasgow to get the plate glass from the breaker's yard. We brought it back and stored it in the basement of the anatomy department. That pile of glass kept us with knives for the remainder of the time I was in Edinburgh.

Among other things, Alan Muir was interested in peripheral nerves, and so the first project that we undertook together was on the development of nerve sheaths in phrenic nerves of rats (Peters and Muir, 1959). The instigation for the study was that some years previously, in 1954, Betty Geren had shown the myelin sheaths of peripheral nerves to have a "jelly roll" structure with the membranes of the Schwann cells being wrapped around the axons in a spiral fashion. It had also been shown that in mature animals several unmyelinated axons occupied the same Schwann cell, whereas Arthur Hess (1956) had shown that in developing sciatic nerves single, unmyelinated axons occupied Schwann cells, and such unmyelinated axons became less common as development proceeded and myelinated nerve fibers appeared. Our study was undertaken to examine the mechanism whereby the axons of developing nerves become partitioned by Schwann cells to achieve the mature state. In effect we found that early in development Schwann cells surround bundles of small axons and that as more Schwann cells are generated the axons gradually become segregated. Axons invested individually by Schwann cells become myelinated, whereas others, which share the same Schwann cell, remain unmyelinated. The diagram we produced to illustrate these developmental changes is often reproduced in modified form, even though the source tends to be neglected.

Later, Alan Muir and I went on to examine the membrane junctions at the terminal bars between endothelial cells (Muir and Peters, 1962).

Central Myelin Sheaths

At about the same time that Alan Muir and I were examining developing peripheral nerves, Mike Gaze, who was a lecturer in the Department of Physiology, asked me to do some silver staining of the optic nerves of *Xenopus* tadpoles, in which he was studying visual system development. I was amazed at the transparency of these tadpoles, in which the optic nerves were readily visible. So I decided to prepare some of the optic nerves for electron microscopy. At that time no one had managed to interpret the structure of the myelin sheaths in the central nervous system, although the "jelly roll" form of the myelin sheaths on peripheral nerves had been worked out a few years earlier as a result of the studies of Betty Geren (1954) and David Robertson (1957). However, it was assumed by

Sarah Luse (1956) and by De Robertis et al. (1958) that the structure of central myelin, and the mechanism of its formation, were different from peripheral myelin.

I began by examining myelin sheaths in the optic nerves of *Xenopus* tadpoles that had been fixed by immersion in osmium acid, but the fixation was not good. Optic nerves that had been fixed by immersion in potassium permanganate proved to be much better, because permanganate preserves membranes. John Luft had introduced potassium permanganate as an alternative to osmium tetroxide in 1956, and although it preserves membranes, it has the disadvantage that it does not preserve other organelles, such as ribosomes and neurofilaments. But by examining optic nerves fixed with permanganate, I was able to show that central myelin also has a spiraled structure, and the difference from peripheral nerve sheaths is that the cytoplasm on the outer turn of the sheath is confined to a small external tongue process that is a ridge of cytoplasm connecting to the parent oligodendrocyte (Fig. 1). Interpretation of the structure of central myelin sheaths had also been confounded by the absence of a basal lamina around the myelin sheaths, which allows adjacent sheaths to come into contact with each other and produce an intraperiod line (Peters, 1960a).

While I was preparing the paper on the structure of central myelin sheaths for publication, Colin Wendell-Smith, who is now in Tasmania, was spending some time in the laboratory learning the techniques of electron microscopy, and he and I talked about how I could prepare a diagram showing the spiraling of myelin lamellae. The problem was how to draw a spiral. After much debate we solved the problem by hammering a 6-inch nail into a board and tying a pen to the nail with a piece of string. Winding the string onto the nail allowed a very nice spiral to be drawn with the pen. It needs to be added that in the same issue of the *Journal of Biophysical and Biochemical Cytology* (vol 7, 1960) in which my results were published was an article by Maturana (1960), describing the structure of central myelin sheaths in the optic nerves of various anurans. Fortunately, our findings were in complete agreement.

Interpreting the structure of central myelin sheaths gave my career a significant boost. I later extended these studies to the optic nerves of rats and was able to show that the formation of central myelin is basically similar to that of peripheral myelin (Peters, 1960b). I was also able to demonstrate that there is a radial component in central myelin (1961,1964a), a component that was later shown to be due to narrow, tight junctions between myelin lamellae. Largely on the basis of these studies, in 1962, the Anatomical Society of Great Britain and Ireland awarded me the Symington Memorial Prize in Anatomy. Subsequently I was able to demonstrate the connections between oligodendrocytes and central myelin sheaths (Peters, 1964b), as well as the form of the nodes of Ranvier in the central nervous system (Peters, 1966).
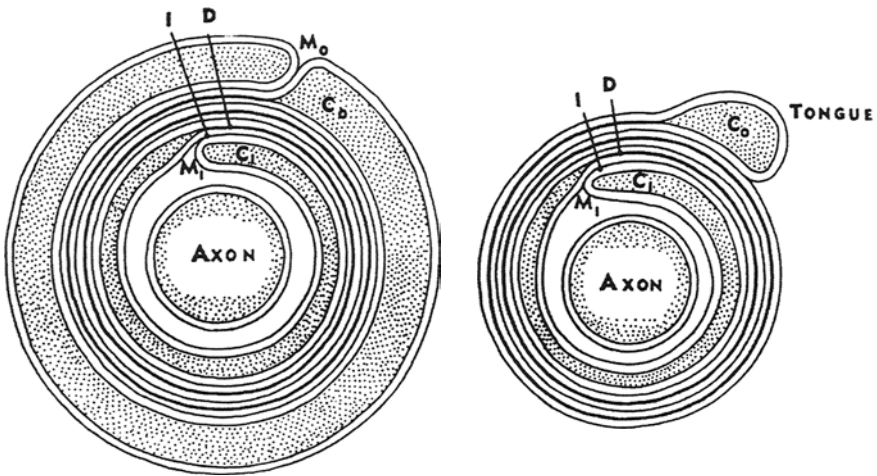
Fig. 1. Diagrams to show the structure of peripheral myelin (1A) and central myelin (1B). The cytoplasm of the axon, the Schwann cell, and the myelin forming glial cell are stippled. On both, the cytoplasm (C_1) lies internal to the first turn of the spiral, which begins at the internal mesaxon (M_1). The intraperiod line (I) arises within the internal mesaxon, which is formed by apposition between the external surfaces of the myelin-forming cell membrane. The major dense line (D) results from contact between the cytoplasmic surfaces of the same membrane. Both lines continue in a spiral and alternate throughout the thickness of the myelin sheath. In peripheral nerves, a continuous layer of cytoplasm (C_0) surrounds the myelin spiral, which terminates at the external mesaxon (M_0). In the central nervous system, the outer cytoplasm is confined to a tongue process so that the major dense line in the outermost line of the sheath, expect in the region covered by the tongue. (Reproduced from the *Journal of Cell Biology* 1960;7:121-126, by copyright permission of The Rockefeller University Press).

At that time, there were few electron microscopists in Britain looking at nervous tissue, but on trips to meetings of the Anatomical Society, I was able to hold discussions with George Gray, who was at University College in London and had described two types of synapses in cerebral cortex (Gray, 1959), and with David Robertson, who had been recruited by J.Z. Young to University College in London. David had a state of the art electron microscope laboratory that was the envy of us all, and he was working on peripheral nerves and his unit membrane theory.

Comparative Anatomy of Nerve Sheaths

Having a background in zoology, I decided that it would be interesting to look at the peripheral nerve sheaths in lower vertebrates, namely

Amphioxus and *Myxine*, by electron microscopy. As expected, I found that no myelin was present in *Amphioxus* and that the structure of the dorsal roots was such that small axons are aggregated into bundles and larger axons are segregated from the smaller ones. In fact, the nerves have features intermediate between those of developing nerves and of mature unmyelinated nerve fibers in mammals (Peters, 1963a).

The next step was to examine the fine structure of the peripheral nerves of *Myxine*, in which Nansen, the Norwegian polar explorer, had recognized in 1886 that the peripheral nerves were also unmyelinated. Because hagfish were not readily available in Britain, but were abundant in Norway, I wrote to Professor Jan Jansen at the Department of Anatomy in Oslo to ask if I could come to collect some *Myxine*. He readily agreed, and in 1962 I had the pleasure of spending 2 weeks in Oslo, where I was very impressed by the high standard of research going on in the department. At that time, Alf Brodal, Per Andersen, Ted Blackstad, and Jan Jansen Jr. were in the department so that it had a galaxy of scientific talent. Each of these people, as well as myself and others, made contributions to a book called *The Biology of Myxine*, which was dedicated to Prof. Jan Jansen and published in 1963. Electron microscopy showed that each axon in the peripheral nerves is surrounded by its own individual Schwann cell sheath (Peters, 1963b).

Boston 1963–1964

In 1960, I had met Sanford Palay at an international meeting in Delft, Holland, and we met again at the Anatomical Association meeting in London in 1961. Several months later, Sandy Palay came to Edinburgh to visit me, and we discussed the possibility of my spending a year in Boston. It was a possibility that I very much relished because Sandy Palay was in the forefront of neurocytology, and he had recently developed a method of perfusing brains with solutions of osmic acid (Palay et al., 1962). This produced a quality of fine structural preservation superior to that routinely obtained by immersing excised pieces of tissue in buffered solutions of osmic acid. Also, at that time, it was almost imperative for British anatomists to get their "BA," or "Been to America."

So I applied for a Carnegie Trust travel fellowship, which I was awarded, and in 1963 I took my wife and two daughters we had at that time on a transatlantic liner to Montreal, from where we embarked to Boston. There I spent a year at Harvard Medical School, where I was given the position of Visiting Lecturer in Anatomy.

Sandy and I discussed what we should do during my year's sabbatical. We concluded that it would be interesting to look at the lateral geniculate nucleus of the cat, because at that time little was known about the synaptology in the thalamus, and Torsten Wiesel and David Hubel in the physiology

department at Harvard had some interesting data on the responses of neurons in the lateral geniculate body to visual stimulation. It was hoped that it would be possible to produce anatomical and physiological correlations that would be enlightening.

Although Sandy Palay and his colleagues had been perfusing rats' brains with buffered osmic acid, they had never tried to perfuse the brain of anything as large as a cat. We calculated that it would take about a liter of 1% osmic acid to perfuse the brain of a cat, and as can be imagined, with that amount of osmic acid, the fumes given off during perfusion were strong, even though we were using a perfusion table with a down draft. The fumes became even stronger when we opened the skull to expose the coal black and brittle brain so that the lateral geniculate body could be removed for electron microscopic processing. We perfused two or three cats using this technique, and the osmic-acid fumes usually managed to fix my corneas and olfactory epithelium so that I had misted vision and could not smell anything for several days after each perfusion. Nevertheless we obtained some well-fixed tissue, and eventually Sandy Palay and I published a paper on the structure and synaptology of the lateral geniculate nucleus of the cat (Peters and Palay, 1966). We recognized the existence of what we called a synaptic glomerulus, which consisted of neuronal processes surrounded by astrocytic sheaths, and in the glomerulus we found that the axon from the retina, the central axon, forms asymmetric synapses with two other kinds of processes. One of these processes was obviously a dendrite, and the second kind of process, which also synapsed with the dendrite, contained synaptic vesicles. At that time, it was not known that dendrites can be presynaptic, so we assumed the second vesicle-containing process was an axon. It was not until several years later that Ted Famiglietti, who was then a graduate student in my laboratory, did a more thorough investigation to show that the second type of vesicle-containing processes, the one postsynaptic to the central axon of the glomerulus, and presynaptic to the other dendrite, is itself a dendrite. Consequently, the thalamic glomerulus contains dendro-dendritic synapses (Famiglietti and Peters, 1972).

In the 1960s the anatomy department at Harvard was arguably the foremost department for cell biology in the country. The head of the department was Don Fawcett, and he had recruited a galaxy of talent, which included such people as Sandy Palay, Betty Hay, Jean-Paul Revel, Sus Ito, and Dick Coggeshall. All of these faculty were doing electron microscopy, and the standards they set were very high, so I learned a great deal about cytology from them. In addition, in Sandy Palay's laboratory at that time were two other visitors. One of them was Facundo Valverde from the Cajal Institute in Madrid, who was producing exquisite Golgi preparations of visual cortex, and the other was Paulo Hashimoto from Japan.

In 1964, toward the end of my time in Boston, Sandy Palay and I discussed the possibility of writing a book on the fine structure of the nervous

system, which would explain what electron microscopy had contributed to our understanding of the nervous system and its components, and how electron microscopic (EM) images of the nervous system could be interpreted. However, after much discussion, we decided to postpone such an endeavor, because there were problems still to be solved and parts of the neuron that had not yet been recognized. One such part was the axon initial segment.

I was fortunate to be asked to give a number of seminars during my stay in Boston. One visit was to Los Angeles, where I met a number of people with whom I remained in contact, including Larry Kruger, Carmen Clemente, and Dan Pease. Another trip was to St. Louis, where I met Ed Dempsey, Bryce Munger, and Sarah Luse. I had not been warned that Sarah Luse thought the pale neuroglial cells, which I considered astrocytes, were oligodendrocytes, and so my descriptions of neuroglial cells caused somewhat of a stir. But at the barbeque following the talk, Sarah Luse and I amicably agreed to differ on which neuroglial cells were which.

Edinburgh 1964–1966

On returning to Edinburgh I continued to examine central myelin sheaths and started to examine the fine structure of the cerebral cortex, about which little was known at that time. I think that my choice of the cerebral cortex as an interesting part of the nervous system to study was influenced by the very insightful opening chapter on cerebral cortex largely written by Rafael Lorente de Nó in Fulton's *Nervous System*, in which there are interesting comments on the organization and connections of the neurons of cerebral cortex and descriptions of some of the cell types as they were known in 1938. One problem was getting good preservation of cerebral cortex, because we could not afford to use osmic acid as a perfusate and did not have the right equipment to cope with the fumes. Our problems were largely solved by perfusing brains using mixtures of formaldehyde and glutaraldehyde. Glutaraldehyde had been introduced in 1963 by Sabatini, Bensch, and Barrnett, and Russ Barrnett had told me about glutaraldehyde and its virtues during a taxi ride we took together in New York in 1964. The introduction of glutaraldehyde started the present era of greatly improved tissue preservation. Glutaraldehyde is a dialdehyde that has excellent cross-linking properties, but in the early days it was a very tedious process to produce pure glutaraldehyde from the commercially available product.

Soon after my return to Edinburgh I received a letter from James Vaughn, who was a graduate student of Dan Pease, whom I had met in California in 1964, asking me if he could come to Edinburgh as a post-doctoral fellow. I agreed, and in 1965 Jim Vaughn joined me in Edinburgh. Together we worked on the development of astrocytes in the optic nerves of rats in material fixed by perfusion with fixatives containing mixed aldehyde

solutions. The use of these glutaraldehyde/formaldehyde solutions made the preservation of the material much better than that obtained earlier, and we were able to show how astrocytes form the glial limiting membrane, how they segregate the initially unmyelinated nerve fibers, and how the frequency of intermediate filaments within astrocytes increases as they mature (Vaughn and Peters, 1967).

Soon after Jim Vaughn's arrival, I received a phone call from Boston University asking me if I would agree to be considered as a candidate for the chair of anatomy at the medical school. Of course I was flattered that they would even consider offering such a position to someone as young as me, because I was only 36 at the time. To satiate my curiosity I agreed to go to Boston for an interview. Before I left, I remember telling my wife that this was a waste of time, but I thought I may as well go to Boston to meet up with old friends I had made there during my sabbatical year. After several interviews I was offered the chairmanship of the Department of Anatomy, and it became clear that they wanted someone who would generate a research program in the department, in which they were prepared to create several new positions. During a subsequent visit it was arranged for me to go to Bethesda to make a visit to Natural Institute of Health (NIH) so that I could assess the possibility of obtaining funding to set up an electron microscope unit in the department. The officials at NIH were very encouraging and told me that such funding was indeed possible and so I returned to Edinburgh wondering what I should do.

At about this same time I had begun to talk to George Romanes about being promoted to senior lecturer, but he did not seem very keen on the idea because it would mean my jumping several steps of the promotions ladder, which was rarely done. One had to progress one step at a time, regardless of merit. I think that he was also reluctant because, unlike the large majority of faculty members in departments of anatomy in Great Britain, I did not have a medical degree. There were few doctoral programs in anatomical sciences at that time, and if I recall correctly every professor of anatomy in Great Britain had a medical degree, the one exception being J.Z. Young at University College in London.

At about the same time that I received an official offer of the chair in anatomy at Boston University, I did get a letter of promotion from Edinburgh University. But weighing my prospects of a career in anatomy in Great Britain, with the lack of medical degree, against the prospect of a career in the United States, I decided to take the offer from Boston University, where I have now worked for almost 40 years.

As an aside, Alan Muir and I left the anatomy department at Edinburgh at the same time. I, who had a Ph.D., came to Boston University School of Medicine, while Alan Muir, who had a medical degree, took the chair in anatomy at the Royal Dick Veterinary School in Edinburgh. Unfortunately Alan Muir died at the age of 50 in 1974.

Boston 1966–Onward

Because the Department of Anatomy at Boston University was essentially a teaching department at that time, I had negotiated with the dean for additional positions so a research program could be developed. My first appointment was Jim Vaughn, who had agreed to come to Boston with me from Edinburgh. He arrived in Boston ahead of us because the immigration authorities were holding up my family and me until Boston University had permission to fill their position with a foreigner. But in the end everything was settled, and I remember writing an NIH grant application while we were still in Edinburgh. I wrote it as we were packing our belongings, and my wife typed it for me, producing the necessary six carbon copies. Fortunately, despite my lack of experience with grant writing, the application was funded for 5 years and supported the setting up of an electron microscope facility. That grant was funded by the National Institute of Neurological Disorders and Stroke in 1966 and was continuously funded until the year 2000, when I decided not to renew the grant so that I could spend my time studying the effects of normal aging on the brain.

One of my main problems on leaving Edinburgh was what to do with the large number of $3\frac{1}{4}$-inch square glass electron microscopic negatives that I had accumulated over the years. I decided that the solution was to select 200 of them, the ones that I knew I could not live without. Consequently, I had a wooden box made specially, and I carried this heavy chunk of glass with me as hand baggage on our trip to the States, being concerned that the plates might be damaged by the baggage handlers. However, some 5 years later, I still had not taken the lid off the box, and eventually I ended up throwing most of those negatives away.

When I took over the department in 1966, we only had some 80 medical students and some 5 faculty members who taught. But the department had some reputation for studies of the nervous system, because my predecessor was Arthur Lassek, who worked on the pyramidal tract, and prior to him was LeRoy Conel, who had produced several volumes, which are still quoted, on the development of the cerebral cortex in humans.

In 1966 it was difficult to find faculty who were qualified in anatomical sciences, and I was lucky to know that Jay Angevine at Harvard had a graduate student named Jim Hinds, who was working on histogenesis in the olfactory bulb. So I offered Jim a faculty position, even before he had completed his doctoral degree, and fortunately he accepted the position and came to join us. Jim stayed in the department for several years, doing ground-breaking work on the development and the effects of aging on the olfactory bulb. I think that it was in 1984 that Jim told me he intended to give up science and was going to Maine to work as a chartered accountant. He is still working as a charted accountant, but he and his wife spend their spare time studying lichens.

Another early recruit was Ita Kaiserman-Abramof, who was working in Sandy Palay's laboratory. Consequently, by the end of 1968 the department had a nucleus of faculty who were doing research.

From my point of view, those early years were very productive. Jim Vaughn and I continued to work on neuroglial cells and made a series of studies on the development and structure of myelin sheaths. The studies on neuroglial cells led Jim and I to write a paper on a third type of neuroglial cell in the central nervous system (Vaughn and Peters, 1969). At that time no one had recognized microglial cells in the normal nervous system, and we were somewhat unsure whether this third cell type represented microglia. But in retrospect, I think our conclusion that the cell type we were describing was a stem cell is correct, and that our third cell type is now recognized as being an oligodendroglial precursor. Later, Jim and I summarized our work on the neuroglial cells in a chapter in which we described the morphology and development of neuroglia (Vaughn and Peters, 1971). Looking back at the illustrations in that chapter, it is interesting to see how much the preservation of nervous tissue had improved between 1960 and 1970.

The other set of studies that Jim Vaughn and I carried out were those on the formation and structure of myelin sheaths. These studies culminated in Jim and I collaborating to write a chapter on the morphology of myelin (Peters and Vaughn, 1970) in a short book called *Myelination,* which I edited with Alan Davison (Davison and Peters, 1970). I believe this chapter is a good summary of what was known about myelin at that time. Shortly afterward, in 1970, Jim, who was now recognized as being an excellent electron microscopist, was recruited by Eugene Roberts to join the Division of Neurosciences at the City of Hope in Duarte, California, where they were working on glutamic acid decarboxylase (GAD) and γ-amino butyric acid (GABA). Jim eventually became the chair of the department and has done seminal studies focused on the development of spinal cord.

In addition to these studies, I was still working on the neurons of the cerebral cortex, and one of the first articles we published from Boston was on the features of the initial axon segment in cortical neurons. As mentioned earlier, one of the reasons Sandy Palay and I had for deciding not to write the book on fine structure of the nervous system at the end of 1964 was that we had not yet recognized the initial axon segment. I had recognized this part of the axon of neurons in the cerebral cortex while still in Edinburgh, and on getting back to Boston in 1966, I told Sandy about my observations that in the cerebral cortex this part of neurons is characterized by fascicles of microtubules and by an electron dense undercoating of the axolemma. Sandy told me that he, along with Constantino Sotelo and Paula Orkand, had now also seen this portion of the axon in the lateral vestibular nucleus and in the cerebellum, and so we agreed to pool our data. Consequently, in 1968 we published two articles describing the

axon initial segment, one general article (Palay et al., 1968) and another one describing the axon initial segment and axon hillock in pyramidal cells of the cerebral cortex (Peters, Proskauer, and Kaiserman-Abramof, 1968). With the characteristics of all portions of neurons established, the way was clear for us to think about proceeding with a book on the fine structure of the nervous system, but before describing how we went about writing that book, I would like to make a few comments about Boston in the 1970s.

One of the nice things about being in Boston at that time was that there were many active groups working on the nervous system, and at Boston University one of my more interesting colleagues was Norman Geschwind, who had been appointed to the chair of neurology at the same time I took over anatomy. In addition to those working in the anatomy department at Harvard, there was the neuroscience group that was being developed by Frank Schmitt, and over at MIT was the group working with Walle Nauta. At least once a week I would go over to either Harvard or MIT to attend seminars, because in those days there was no trouble with parking, which made life easy.

One of the people I met at MIT was Lennart Heimer, who was working in Walle Nauta's group. Lennart, along with Robert Fink, had developed two methods for the selective staining of degenerating axonal boutons. These methods were essentially modifications of the Nauta method for staining degenerating axons but had the advantage that degenerating nerve fibers could be traced to their terminations. However, doubts had been expressed by a number of investigators about whether the Fink-Heimer method actually stained degenerating axonal boutons, and Lennart came to me to ask if it would be possible to do an electron microscopic study of tissue labeled by the Fink-Heimer method to really determine whether the silver was being deposited in degenerating axonal boutons. So we examined some tissue in which lesions had been placed in either hippocampus or olfactory bulb and were able to show that in material stained by the Fink-Heimer method the silver is indeed deposited in degenerating axonal boutons (Heimer and Peters, 1968). This proved the validity of the Fink-Heimer method, which for many years provided an important tool for tracing central nervous pathways.

The Fine Structure of the Nervous System

First Edition

As I have said, when I returned to Boston, Sandy Palay and I started talking once again about writing a book devoted to the fine structure of the nervous system. By that time, a number of excellent atlases showing electron micrographs of cells had appeared, the most notable ones being those of Porter and Bonneville (1963) and Fawcett (1966), but these atlases

had little on the central nervous system. We felt that an atlas devoted to the nervous system would be useful, but in addition to showing electron micrographs, we concluded that the book should have some descriptive text. Because Sandy Palay and I were then involved with studying the central nervous system, we decided that it would be a good idea to recruit someone with expertise in the peripheral nervous system, and an obvious choice was Henry deF. Webster, who was in the neuropathology department at Massachusetts General Hospital. Harry readily agreed to join us in the endeavor and in 1968 we started to select pictures and to write the text.

We had already thought about a suitable publisher for our book, and the Paul Hoeber Company seemed to be a good choice. However, in the 2 years it took us to complete the book, the Paul Hoeber Company had been bought out by Harper and Row, who seemed unprepared to receive our manuscript and plates and did not know who would do the half-tone printing. A detailed account of our problems has been given by Sandy Palay (1995), and despite our best efforts to ensure that the illustrations would be of good quality, we were very disappointed with the rather faint and lightly printed illustrations that finally emerged in the 1970 edition of the *Fine Structure of the Nervous System*. Nevertheless, despite little advertising, the entire run of 5000 copies had been sold by 1972. Looking back, is interesting to note that this edition listed only 550 references, practically all of the articles on neurocytology that had been published up until that time. Also there were obvious gaps in our knowledge. Microglial cells had not been characterized, almost nothing was known about the morphological correlates of inhibition, and except in the olfactory bulb, it was not recognized that dendrites could be presynaptic.

I later discovered that the Russians had published a pirated edition of our book. It appeared in 1972, and I met the translator, whose name I have forgotten, at a dinner party at Paul Yakovlev's house when he gave me a copy of his book. When I asked him if there were any royalties connected with this Russian edition, he told me that of course there were royalties and that all I had to do was to go to Moscow and collect them, although I would not be able to take the money out of Russia!

We received enough positive comments from colleagues that we began to think about a second, enlarged edition that would cover the many advances recently made in neuroscience. We approached Harper and Row and asked them if they would consider publishing a second and expanded edition of our book. They told us they were not interested in books with small-volume sales, but offered to sell the rights of the book to another publisher. Fortunately, when we signed the contract for the first edition, our attorney had insisted that one of us (A.P.) should hold the copyright. Harper and Row were very surprised to discover this fact, which allowed us to seek another publisher of our own choosing. This time we selected Saunders, who was represented by John Dusseau. He assured us that he personally

would ensure good-quality reproduction of our electron micrographs: a promise he kept.

Second Edition and Cambridge

We began preparations for the second edition in 1973, when I went to Cambridge on sabbatical leave as a visiting professor in the Department of Anatomy. The reasons for going on sabbatical leave were two-fold. The official reason was so that I could spend time in the university libraries to read and begin writing for this new edition. However, another reason was that Rona and I were still not sure whether we wanted to live permanently in the United States. By this time we had added a third daughter to our family, and we thought that exposing them and ourselves to living in Britain for an extended period would enable us to make up our minds about our future. We soon realized that there is no perfect place to live and that we were happy with our lot in the United States.

It is interesting to look back on facts that influence one's decisions. The trigger in this case was that I had a money order in dollars that I wanted to exchange into pounds sterling, but the bank in Cambridge was reluctant to make the exchange because, at that time, the dollar was fluctuating against the pound. This was aggravating because we had bills to pay and things we wanted to buy. Eventually I persuaded the bank to give me some pounds, and at the end of the day, I took the bus home and decided it would be nice to buy a bottle of wine for dinner. It was 6 o'clock in the evening when I got off the bus, and as I walked over to the local wine shop, the shopkeeper pulled down the blind to indicate that he was closing. I knocked on the door, but he would not open the shop for me, and so I went home angry, without the wine, and told my wife that if that was the way people behaved here, we were going back to the States, which we did.

Through the kind offices of Gabriel Horn, I was fortunate enough to be made a Visiting Fellow at King's College and enjoyed the break from administrative duties as chairman of the department at Boston University. Professor R.J. Harrison, who was the professor of the Department of Anatomy in Cambridge, was also good enough to allow me to learn something about scanning electron microscopy while I was there, and this resulted in a publication on the ependyma and chroroid plexus in the lateral ventricles of the rat (Peters, 1974), as well as some scanning electron microscope pictures that were included in the second edition of our book. This second edition appeared in 1976, and it had 118 plates, only 30 of which had been included in the first edition of our book. This new edition had an expanded text, and the illustrations covered such new items as freeze-fracture, scanning electron microscopy, and tract tracing. This edition contained some 1700 references, three times more than in the first edition, and even they did not cover the neurocytology literature completely.

By the early 1980s this edition was also out of print, and again, the success of the book led us to consider a third edition. However, as with our publishers for the first edition, Saunders decided it was not interested in small press runs. So we looked around for yet another publisher and finally decided on Oxford University Press, largely because Jeffrey House, who was the editor for Oxford, impressed us as someone we could trust and work with—an evaluation that turned out to be true.

Third Edition

We spent 2 years putting together the third edition, and to keep up momentum Sandy Palay and I met at his house every Wednesday to go over the text we had produced during the previous week and to select the plates. This time I took off to Hawaii to do some of the writing, a place that I had longed to see since growing up and reading books about the South Sea Islands. The third edition had a publishing date of 1991. The text was again expanded and the number of plates increased to 137, of which 51 were new. Oxford University Press did an excellent job of reproducing the plates, and this may be one reason why the Science Book Club selected it as one of their offers. It is interesting that even after 14 years, copies of the book are still being sold, largely because of the quality of the plates, because the text is now somewhat dated.

I like to think that this book set standards for the quality of electron micrographs of the nervous system and that it has served as a source of reference for those beginning to study the cytology of the nervous system.

A more detailed account of our tribulations with publishing our book on *The Fine Structure of the Nervous System* can be found in a memoir that Sandy Palay wrote for an issue of the *Journal of Comparative Neurology* published in 1995 in honor of my 65th birthday (Palay, 1995). This issue was put together by Jim Connor and Bob Skoff without my knowledge and contains articles by a number of former graduate students and postdoctoral fellows who had been in the Department of Anatomy at Boston University during my tenure as chairman. Of course, I was delighted to be honored in this special way and touched that these students, now colleagues, would plan this journal issue in my honor.

Sandy Palay retired from Harvard in 1990, and he spent the next 3 years continuing to edit the *Journal of Comparative Neurology* from his home in Concord. I would often go to his home and enjoyed our discussions of science and life in general. Unfortunately, at about the time that he gave up editing the *Journal of Comparative Neurology*, Sandy's health began to fail, and he died in 2002. The last venture we undertook together was a paper in 1996 on "The Morphology of Synapses" that was published in the 25th anniversary issue of *Journal of Neurocytology*, dedicated to "The Synapse" (Peters and Palay, 1996). This article was doubly appropriate,

because it will be remembered that in 1956 Sandy Palay published the first account of the fine structure of the synapse in mammals. When he examined thin sections of the abducens nucleus, he had encountered club-like profiles that were filled with mitochondria and vesicles aggregated against the presynaptic membranes abutting the surfaces of dendrites and cell bodies. Furthermore, this presynaptic membrane was separated from that of the postsynaptic one by a thin extracellular space, thus confirming Cajal's inference that neurons do not form a syncytium (Palay, 1956).

I felt very honored and pleased when, in 2004, I was selected by the editorial board of the *Journal of Comparative Neurology* and by the Cajal Club to be the first recipient of the Sanford L. Palay Award for contributions on the structure of the nervous system, an award funded by John Wiley and Sons to acknowledge Sandy's contributions as editor of the *Journal of Comparative Neurology*.

Cerebral Cortex: The Book Series

Toward the end of the 1970s, I realized that very few comprehensive accounts of the cerebral cortex existed, and I began to think that the gap might be filled through publication of a short series of books dealing with the kinds of neurons that are present in the cerebral cortex, how they are organized, the types of neurotransmitters they use, and their physiological properties. However, I felt that I needed someone to join me in this endeavor, and I approached Edward G. Jones to see if he would be interested. At that time Ted was the chair of anatomy at Irvine, California, and fortunately, he embraced the idea enthusiastically. After we had decided that the first volume should be devoted to the various cell types in cortex, we realized that it would be sensible to recruit an advisory board who would give the series more credibility, and we were fortunate that when we approached John Eccles, Ed Evarts, Norman Geschwind, Vernon Mountcastle, Walle Nauta, Sandy Palay, and Fred Plum they all readily agreed to serve on the advisory board for the project.

Our intent originally was to produce just three or four volumes, but ultimately, the series extended to 14 volumes. Plenum Press published the first volume in 1983, and the series continued through 1999. The compilation of the series was not difficult. In consultation with the advisory board, Ted and I decided which topics were suitable for a given volume and then in turn we would take on the task of writing to potential authors. After they had agreed to write a chapter, the one responsible for that volume would harass recalcitrant authors, make any necessary corrections to the manuscripts, and send them off to the publishers. Because the series was well received, it was rare that anyone who was asked to write a chapter turned us down. As different volumes continued to be produced, we recruited others to help edit given volumes, often members of the advisory

board, whose membership necessarily changed over the 15 years spanned by the series. With reluctance, Ted and I decided to terminate the series after Plenum Press, our original publishers, were bought out by Kluwer. The price that Kluwer wanted to charge for each volume was so high that we believed only few volumes would be sold. In our minds, this would necessarily reduce the availability of the books and make them less accessible to the readers we wanted to reach. The last volume in the series was meant to be one on *cat primary visual cortex,* which Bertram Payne and myself were to edit.

Fortunately, Kluwer/Plenum Press was not averse to releasing us from our contract, and Bert and I were able to work with Academic Press on the publication of a book called *Cat Primary Visual Cortex* (Payne and Peters, 2002). But even then the price was much higher than we would have wished, and the book was far from being a best seller. I think it is becoming increasingly obvious that most publishing houses no longer want to get involved with small-volume sales, even though one would think that with modern technology the cost of publishing books would have decreased.

Research Themes

As I began to write about the research I had done in Boston over the years, I realized that because of my various research interests a year-by-year account of the research I have done was not going to work. Consequently, I have decided to present the research with which I have been involved in terms of the themes I have pursued. That way, I hope that the following account has some logic and is not too disconnected.

General Studies of Cerebral Cortex

In the late 1960s there had been few studies of the characteristics of cells and their processes in cerebral cortex, and so every time one looked at a thin section of cortex with the electron microscope, something new turned up. In reality the only way to approach the problem of the identification of characteristics of the various components of the cerebral cortex was to sit down at the electron microscope for long hours and take pictures of anything that looked interesting. We would then go over the electron micrographs and use grease pencils to try to label each profile and assign it to a cell type. This approach led Ita Kaiserman-Abramof and me to discover that some of the profiles of asymmetric synapses, now known to be excitatory, on dendritic spines in cerebral cortex show the postsynaptic density at synaptic junctions to be in two parts, separated by a space. On reconstruction from serial thin sections, we found that this appearance is produced because the

disc-like postsynaptic densities of such synapses are perforated (Peters and Kaiserman-Abramof, 1969).

Following up on this observation, we found that in Golgi-impregnated preparations of cerebral cortex, the dendritic spines can be described as being of three types: stubby spines, which are rather short; mushroom-shaped spines, which have large bulbous heads; and thin spines, which are long (Peters and Kaiserman-Abramof, 1970). This classification of dendritic spines has stood the test of time and is still used as the basis for descriptions of changing frequency and morphology of dendritic spines in the cortices of animals that have been subjected to alterations in diets or behavioral environments, whereas increases in the frequency of perforations at axo-spinous synapses are regarded by some as one of the bases of learning.

For some reason that I am no longer certain about, Ita and I then went on to examine the Betz cells in cat motor cortex (Kaiserman-Abramof and Peters, 1972). I assume that we chose to examine these cells because at that time there were few neurons in the cerebral cortex that could be specifically identified, and the Betz cells can be readily recognized by their large size, making it possible to carry out a Golgi study of these cells in correlation with the electron microscopic analysis. It was in this study that we also developed methods for estimating the numbers of synapses on the surfaces of neuronal cell bodies in an attempt to try to provide data that would be useful to neurophysiologists examining the properties of these large neurons.

In 1971 Deborah Vaughan came to work with me as a postdoctoral fellow, and because Debbie had a great deal of experience in electron microscopy during her graduate work, we decided that it would be interesting to examine serial thin sections to reconstruct the three-dimensional shapes of dendrites and to find out how dendrites ended. Although it is not too uncommon to use serial thin sections for electron microscopy nowadays, at that time there had been only one or two studies in which serial thin sections had been used to make reconstructions. The first problem was to cut series of thin sections with the ultramicrotomes that were then available, and the second problem was how to mount the series of thin sections onto copper grids with single slots and no bars. We eventually developed a technique for doing this, and, using sections of layer 1 of cerebral cortex, which has few cell bodies and many dendrites running through it, we produced some of the first three-dimensional reconstructions of dendrites and their endings. After making the reconstructions by hand, we found that dendrites can have very long mitochondria and that both symmetric and asymmetric synapses are intermixed along the shafts of dendrites. We also discovered that although all dendritic spines appear to have an asymmetric synapse, some of them also have a symmetric one. However, we were disappointed to find that dendrites just end bluntly and that there is no specialization at their ends (Vaughan and Peters, 1973).

Quite soon after completing that study, Debbie Vaughan and I got involved with looking at the effects of aging on the central nervous system. I will have more to say about that later, but in 1974 we published one of the first studies of the effects of age on neuroglial cells, showing that all three classical types of neuroglia accumulate inclusions in their cell bodies with age (Vaughan and Peters, 1974).

Even in the 1980s, although we had looked at a variety of individual neurons in the cerebral cortex with Golgi-EM techniques that were developed in the 1970s, very little was known about the composition of the cortex in terms of the proportions of pyramidal and nonpyramidal cells present in the various layers, how they were arranged, and what criteria could be used to identify the various neuronal types in EM preparations. To get more information about the composition of the cerebral cortex, a technician that I had at that time, Dan Kara, persuaded me that we ought to undertake a marathon study of cell types in cerebral cortex. The approach was to take adjacent thin and 1-micron thick sections through rat visual cortex. We would then draw and number each cell that we saw in the 1-micron thick section, after which we would examine the adjacent thin section and take corresponding electron micrographs of each cell. We ended up by taking more than 2000 electron micrographs of cells in the various layers of rat visual cortex, but we did develop criteria for characterizing the pyramidal (Peters and Kara, 1985a) and nonpyramidal (Peters and Kara, 1985b) neurons in rat visual cortex, as well as for the first time determining how many profiles in each layer of cortex can be attributed to pyramidal or nonpyramidal cells and how many neurons there are present in this particular cortical area (Peters, Kara, and Harriman, 1985). For example, we found that there are some 120,000 neurons beneath 1 mm^2 of cortical surface and more than a million neurons throughout area 17 of the rat.

Golgi—Electron Microscopy

A problem with normal electron microscopy, in which individual thin sections are examined, is that one only gets a two-dimensional picture. The various profiles of neurons can only be related to each other through an analysis of serial sections. However, numerous serial thin sections are required to determine any relationships, and even with a herculean effort only portions of neurons can be reconstructed. On the other hand, successfully impregnated Golgi-stained material can show the cell bodies, dendrites, and sometimes a portion of the axons of individual neurons in three dimensions. In the 1960s this led a number of investigators such as Ted Blackstad (1970) and Bill Stell (1965) to attempt to blend these two techniques by making thin sections of Golgi-impregnated neurons, so that the synaptic relationships of impregnated neurons could be revealed.However, this approach met with limited success, because the electron-dense deposit

of silver chromate produced by the Golgi method either fell out of the thin sections containing portions of the impregnated neuron, tore the section, or heated up in the electron beam, causing the surrounding embedding plastic to expand. Various attempts were made to solve this problem, but with limited success, and in 1975–1977 Alfonso Fairén from the Cajal Institute in Madrid came to the laboratory to learn electron microscopy and to try to determine if the Golgi-EM procedure could be improved.

After several unsuccessful attempts at doing Golgi-EM, I was riding home on the turnpike one evening thinking about the problem, when it occurred to me that one solution was to try to gold tone the material impregnated by the rapid Golgi method. I had used gold toning many years earlier while working on the mechanism of silver staining. In gold toning of silver-stained sections, gold is used to replace the silver deposit, and the idea was to determine if the silver-chromate deposit generated by Golgi impregnation could be replaced by gold particles. I arrived at the laboratory the next day and suggested to Alfonso that he ought to try gold toning. To our delight, Alfonso achieved a successful gold toning of Golgi-impregnated neurons on the first attempt. The Golgi-impregnated sections were immersed in gold chloride, which was reduced by oxalic acid, and the silver chromate was then removed by immersion in sodium thiosulphate. The impregnated neurons were still visible but semitransparent, and in the electron microscope the gold could be seen as small, dense particles in a neuron with intact cytology. We perfected the technique and designed a microtome chuck that could be removed from the ultramicrotome to allow the progress of sectioning to be examined under the light microscope. We published this new Golgi-EM procedure (Fairén, Peters, and Saldanha, 1977), which was used for several years by ourselves and other laboratories, to examine the synaptology and axonal connections of neurons. Pushing this new technique to its limit, Alfonso and I also worked with Edward White, who had recently joined the department to show that it was also possible to use the gold-toning technique on sections of Golgi-impregnated tissue containing degenerating thalamocortical axons terminals, which had been caused to degenerate as a result of experimental lesions. Thereby both the presynaptic and postsynaptic components of thalamocortical synapses could be identified (Peters, White, and Fairén, 1977). This new technique allowed us to examine numerous types of neurons, especially the nonpyramidal cells of cortex, about which little was known, and most importantly to determine the types and locations of synapses formed by their axon terminals.

Nonpyramidal Neurons

One of the first nonpyramidal cell types we examined using our new Golgi-EM procedure was the smooth and sparsely spined stellate cell in cerebral

cortex. Alfonso Fairén and I were able to show that the axons of these neurons form symmetric synapses with a variety of postsynaptic partners (Peters and Fairén, 1978). Charmian Proskauer, who was one of my excellent research technicians, and I then went on to examine some of the cortical interneurons with myelinated axons. In addition to showing that their cytology and synaptic inputs are similar to those of the stellate cells with unmyelinated axons, we were also able to show that in those neurons with myelinated axons the Golgi impregnation stops at the initial axon segment (Peters and Proskauer, 1980a). By a stroke of good luck, as Charmian and I examined our many Golgi-impregnated sections, we were fortunate to come across two impregnated cells in the same section: One cell was a pyramidal cell and the other a multipolar stellate cell with an axon that appeared, on the basis of our light microscopic examination, to form synapses with the pyramidal cell. We indeed found that the axon of the stellate cell formed nine synapses with the pyramidal cell: five synapses with the cell body, one with the apical shaft, and three with basal dendrites (Peters and Proskauer, 1980b). I believe this was the first time that synaptic connections had been positively identified between two identified neurons in cerebral cortex, and the first demonstration that an inhibitory neuron can synapse with different parts of a pyramidal cell surface.

Bipolar Cells

We next used the Golgi-EM technique to examine the bipolar cells in rat visual cortex. Martin Feldman and I were among the first ones to describe these interesting cells in our light microscopic account of the forms of non-pyramidal cells in Golgi-impregnated rat visual cortex (Feldman and Peters, 1978). Lauren Kimerer, another research assistant, and I were able to show that our Golgi-impregnated bipolar cells have axons that form asymmetric synapses with the dendritic spines of pyramidal cells and that consequently they are excitatory neurons (Peters and Kimerer, 1981). This result was puzzling, because it had been assumed that all nonpyramidal cells are inhibitory neurons, and our result was at odds with the observations made earlier by John Parnavelas and his colleagues in London (Parnavelas et al., 1977). In their EM examination of Golgi-impregnated bipolar cells in rat visual cortex, they had found the axons of bipolar cells to form symmetric synapses. The situation became even more puzzling when in 1984 Alfonso Fairén and his colleagues in Spain described a Golgi-impregnated bipolar cell in cat visual cortex that formed asymmetric synapses (Fairén et al., 1984). Bipolar cells became more prominent when it was discovered that large numbers of them label with antibodies to vasoactive intestinal polypeptide (VIP), and, to further muddy the waters, it was found that such bipolar cells have axons that form symmetric synapses, as Jim Connor, then

a postdoctoral fellow in the laboratory, and I were also able to demonstrate (Connor and Peters, 1984).

A resolution to the problem emerged when Kathy Harriman, another research assistant, and I carried out further study of the bipolar cells (Peters and Harriman, 1988). We confirmed that VIP-positive bipolar cells have axons forming symmetric synapses, but examination of another 10 bipolar cells by the Golgi-EM technique showed that although eight of them formed asymmetric synapses, as we had described earlier, two of them formed symmetric synapses. So in effect there are two populations of bipolar cells. However, little attention has been paid to the excitatory bipolar cells, and their transmitters appear not to have been identified.

Chandelier Cells

One of the most interesting cells in cerebral cortex is the chandelier cell, which Peter Somogyi at Oxford University first showed to form synapses with the axon initial segments of pyramidal cells in cerebral cortex (Somogyi, 1977). Because of the location of these axo-axonal synapses, it was suspected that they were inhibitory in function. We were able to confirm this in a study we carried out with Charles Ribak, who had been my graduate student and had gone to do postdoctoral work with Jim Vaughn at the City of Hope. Using the Golgi-EM technique in combination with labeling with a GAD antibody, we were able to show that the symmetric synapses, which the axon terminals of chandelier cells form with the axon initial segments of pyramidal cells, use GABA as their neurotransmitter (Peters, Proskauer, and Ribak, 1982).

Other Golgi-EM Studies

If I recall correctly, the only other studies we carried out using the Golgi-EM technique were on cortical development and on stellate cells in cat visual cortex. The study of cortical development was carried out with Michael Miller, who came to the laboratory as a postdoctoral fellow. We were able to follow the maturation of pyramidal cells in rat cortex and show that dendrites acquire asymmetric synapses before they acquire symmetric ones. We also found that the spines of dendrites begin as low, broad protrusions, which acquire synapses and gradually become longer as they mature into the common lollipop type spines of the adult (Miller and Peters, 1981). These and other studies that Michael carried out on normal development formed the basis for his very interesting subsequent observations on the effects of alcohol on development.

Although it was not difficult to generate Golgi-impregnated pyramidal cells, to impregnate stellate cells in layer IV of monkey visual cortex was a much more serious challenge. But Richard Saint Marie, who came to the laboratory as a postdoctoral fellow, persisted and was eventually able

to impregnate a few stellate cells with visible axons. Using the Golgi-EM technique, he was able to show that like pyramidal cells, only symmetric synapses occur on the cell bodies of the layer IV stellate cells and that most of the synapses received by stellate cells are on their dendritic spines. Dick was also able to show that the spiny stellate cells have axons that form asymmetric synapses and the synapses they make with dendritic spines outnumber those made with dendritic shafts by two to one. This research clearly showed that spiny stellate cells and pyramidal cells have many features in common, and basically they differ only in the distribution of their dendritic trees (Saint-Marie and Peters, 1985).

The era of EM studies of Golgi-impregnated neurons essentially came to an end with the development of the technique of intracellular filling by neurophysiologists. This technique allows the physiological properties of neurons to be evaluated before they are filled to study their morphology, and early on some of the most insightful descriptions of cortical cells were those given by Kevan Martin and David Whitteridge, who at that time were at Oxford (see Martin, 1988). Interestingly, intracellular filling showed the axonal plexuses of cortical neurons to be much more wide-spread and complex than Golgi impregnations had revealed. As a consequence, we now know much more about the projections of cortical neurons and where their axons synapse. However, even now, although we have extensive information about axonal terminations, as far as the cerebral cortex is concerned, there is a dearth of information about the origins of the synaptic inputs that specific types of cortical neurons receive. However, it can be estimated that a pyramidal cell in cortex probably receives input from as many as 1000 other pyramidal cells and as many as 75 inhibitory neurons (Peters, 2002). Such complex interactions between cortical neurons are difficult for me to comprehend.

Thalamic Inputs to Cerebral Cortex

One of my long-term interests has been the nature of the thalamic input to cerebral cortex. This interest began with a study that Charles Ribak, now a professor at Irvine, California, did as a graduate student in my laboratory. He carried out an autoradiographic study of the projections from the lateral geniculate nucleus, which in the rat had received little attention at that time, even though a number of studies had been carried out on the geniculate input to cat and monkey visual cortex. Chuck showed that there is a major peak of labeling in layer IV of rat visual cortex, with minor peaks in layers I and VI, and some extension of the labeling into areas 18 and 18a. He was also able to demonstrate very extensive projections from the ventral geniculate nucleus to the brainstem (Ribak and Peters, 1975). Soon after completing this study, Chuck left to do a postdoctoral fellowship with

Jim Vaughn at the City of Hope, where they had developed an antibody to GAD, and, as I mentioned earlier, Chuck and I later collaborated on showing that the input from the chandelier cells to the axon-initial segments of pyramidal cells in cortex is inhibitory.

The autoradiographic study sparked our interest in trying to determine which types of cortical neurons are postsynaptic to the thalamic axon terminals. Consequently, using the only appropriate method available at that time, Martin Feldman and I made lesions in the lateral geniculate nucleus of the rat, and after a suitable time had elapsed, we carried out a series of EM studies to determine where the thalamic terminals, now degenerating, form their synapses. We found the axon terminals form asymmetric synapses, most of which are with dendritic spines, although a small percentage of thalamic terminals synapse with dendritic shafts and with neuronal perikarya (Peters and Feldman, 1976). By reconstructing the postsynaptic dendrites using serial thin sections, we were able to show that the latter two postsynaptic sites belong to nonpyramidal cells (Peters et al., 1976), and when we subsequently reconstructed the dendrites that receive thalamic inputs on their spines, it became apparent that various kinds of neurons with spinous dendrites can receive thalamic afferents (Peters and Feldman, 1977). This led us to postulate that the geniculate afferents to layer IV and lower layer III of visual cortex terminate on any neuronal entity capable of forming asymmetric synapses. This postulate was strengthened by the results we obtained using our newly developed Golgi-EM gold toning technique to examine Golgi-impregnated neurons in the visual cortices of rats whose lateral geniculate nuclei had been lesioned (Peters et al., 1979).

Consequently, we came to the conclusion that the thalamic afferents to cortex do not synapse with specific types of neurons but synapse with any neuronal component in layer IV and lower layer III capable of forming asymmetric synapses. More information about this postulate can be found in the book *Cortical Circuits*, written by Ed White (1989), who found similar results in his combined degeneration–Golgi-EM studies on the thalamic projections to the barrel field of the mouse. Ed wrote this very useful book after leaving Boston University to take the chair of anatomy position at Ben Gurion University of the Negev at Beer Sheva, Israel. After he left, I acquired Ed White's electron microscope, and I am still using it. However, I have used only once the 70-mm camera that Ed had installed to take the numerous electron micrographs of serial thin sections he generated to produce the balsa wood models of dendrites that hung from the ceiling of his laboratory and were constructed to examine the thalamic input to mouse barrel field.

Essentially, these studies on the thalamic input to cerebral cortex had come to an end by the 1980s, but my interest was revived as a result of

conversations I had with Bertram Payne. These conversations led us to analyze what we know about the geniculate input to cat primary visual cortex. Bert had a life-long interest in cat visual cortex and was interested in the results that Engin Yilmaz, who was a postdoctoral fellow from Turkey, had recently obtained on the organization of neurons in cat visual cortex. Engin and I had shown that the neurons in this cortex are organized into modules that are centered on pyramidal cells. On average, these pyramidal modules are 56 microns in diameter (Peters and Yilmaz, 1993), and Bert Payne and I began to consider how many geniculate afferents each module might receive. We soon realized a great deal of quantitative data had been published on these geniculocortical afferents, so we set about to correlate and analyze this data (Peters and Payne, 1993). The analysis turned out to be very interesting. I will not give the results in detail, but it emerged that there is an enormous convergence of thalamic input onto individual pyramidal cell modules, and that individual spiny stellate cells in layer IV each receive 100–125 synapses from the geniculocortical afferents. This accounts for only about 5% of the total excitatory inputs these cells receive. Correlated with this conclusion are two other interesting facts. One is that Rodney Douglas and Kevan Martin (1991) had previously calculated that at least 100 active synapses are necessary to generate an output discharge from spiny stellate cells. The other is that most of the earlier studies using degeneration had shown that 3 to 4 days after making lesions of the lateral geniculate body only about 5% of the terminals in visual cortex degenerate. Because the thalamus dominates the functioning of the cortex, it was generally thought that this number must be far too low. It was thought that the small percentage must be due to the fact that all of the thalamic axon terminals do not degenerate simultaneously, so that at any one time only a small percentage of them show signs of degeneration. This explanation was generally accepted even though the peak of degeneration is at about 3 to 4 days postlesion, and most of the terminals have disappeared by 5 days.

Subsequently, Bert Payne and I, along with Julian Budd from the University of Sussex, also analyzed the published data on the geniculocortical input to layer IVC of monkey striate cortex (Peters, Payne, and Budd, 1994). The data showed that the input to a given layer IVG neuron is derived from about 24 overlapping axonal plexuses and that only 1.3–1.9% of the excitatory input to layer IV cells is provided by the magnocellular layers of the lateral geniculate nucleus (LGN), and 3.7–8.7% from the parvicellular layers. Again, there is the question about whether these low values are correct. They seem to be consistent with most findings, although Ed White and his colleagues have reported that in the barrel field of the mouse cortex as many as 20% of the asymmetric synapses are from the thalamus.

Other Work Carried Out with Bertram Payne

I looked forward to the many discussions that Bert and I had about the structure and functioning of cerebral cortex, because he had a very good analytical mind and a tremendous background knowledge of visual cortex and its connections. However, despite much talk, we only really worked together on two experimental projects. One project we did together was on the transcallosal projections from cat visual cortex (Peters, Payne, and Josephson, 1990). A graduate student in my laboratory, Candice Hughes, had recently shown that after injection of horseradish peroxidase (HRP) into the 17/18a border in rat cerebral cortex, some of the neurons labeled in the contralateral cortex were nonpyramidal cells (Hughes and Peters, 1990), and Bert wondered if some nonpyramidal cells in cat visual cortex had similar callosal projections, because it had been shown that in cat, callosal projections can either excite or inhibit neurons in the opposite hemisphere. Consequently, we did a similar experiment in cats, making injections of HRP at the area 17/18 border. As in the rat, we did find labeled, callosally projecting nonpyramidal cells in the cat and concluded that as many as 10–32% of the nonpyramidal cell population at the area 17/18 border may have callosal projections. We sought to pursue this result by planning further experiments, which we presented in a grant application. Unfortunately, the reviewers knew that only pyramidal cells have axons that project across the corpus callosum, so we were not able to continue these studies, although others have since confirmed that some nonpyramidal cells do project callosally.

The other experimental project Bert and I undertook was on the distribution of cytochrome oxidase patches and Meynert cells in monkey visual cortex (Payne and Peters, 1989). We were able to show that the Meynert cells predominantly lie beneath the spaces between the cytochrome oxidase patches in layer II/III. On the basis of the functional connections of neurons in area 17, we suggested that this arrangement indicates that the Meynert cells are involved in the detection of movement in the visual field.

The last combined endeavor that Bert and I undertook was to edit a book on cat primary visual cortex (Payne and Peters, 2002), which was supposed to be one of the books in the cerebral cortex series. Bert and I wrote an extensive introductory chapter for the book, making the case that primary visual cortex in the cat consists of both area 17 and area 18. Bert did most of the writing for the chapter, and I think that both the chapter and the book are useful references, but, unfortunately, the book did not sell many copies because the publishers made the price of the volume prohibitive.

Unfortunately, shortly after the book on cat visual cortex was published, Bert became ill and died prematurely in May of 2004, at a time when his work on the reorganization of cortex after lesioning was reaching its peak.

Pyramidal Cell Modules

Another long-term interest of mine has been in the organization of pyramidal cells in cerebral cortex into modules. I had been searching for some columnar organization in the cerebral cortex since reading the early account of the cortical neurons and their connections by Lorente de Nó (1938), who suggested that cortical neurons are interconnected to form vertical columns. This concept was reinforced by Vernon Mountcastle's (1957) demonstration that in cat S1 cortex, neurons are organized into microcolumns, such that if an electrode is passed vertically through the cortex, successive neurons along the path are excited by the same stimulus to the body surface.

After searching for some vertical organization in cerebral cortex, in 1972 Terry Walsh, who had recently joined the department as a faculty member, and I noticed that in semithick tangential sections through layer 4 in area 3 of plastic embedded rat cerebral cortex, the ascending apical dendrites of layer 5 pyramidal cells are arranged into groups, or clusters, spaced about 50 microns apart, and that as these apical dendrites ascend through layer 3, the apical dendrites of the layer 3 pyramidal cells are added to the clusters (Peters and Walsh, 1972). We suggested that the clusters of apical dendrites are the centers of modules of pyramidal cells and that these modules might be the morphological equivalents of the microcolumns that Vernon Mountcastle (1957) had proposed to be the basic functional neuronal units of cerebral cortex. Independently, and almost simultaneously, Fleischhauer and colleagues had found similar clustering of apical dendrites in the rabbit and cat (Fleischhauer et al., 1972), and so it appeared likely that organization of pyramidal cells into modules might be a common feature of neocortex.

To investigate how these pyramidal cell modules might form, Martin Feldman and I undertook a study of the development of rat cerebral cortex using both Golgi-impregnated material and electron microscopy. We found that in tangential sections the radial glial cells of the developing cortex are surrounded by clusters of apical processes belonging to migrating neurons, and we concluded that the pyramidal cell modules in the mature brain are derived from the neuroblasts that migrate along individual radial glial fibers (Peters and Feldman, 1973). This is in line with the results of the studies made by Pasco Rakic on the migration of neuroblasts along radial glial fibers in monkey cerebral cortex (e.g., Rakic, 1990) and would imply that the radial glial fibers should be at the center, or form the axis of, the pyramidal cell module. However, over the years, the mechanism of how the pyramidal cell modules are formed has continued to nag at me. Because the cortex is formed inside out, the layer 5 pyramids are formed before the layer 3 pyramids, and if these neurons use the same radial glial fibers to migrate through the cortex, it would seem that migrating and ascending processes of the layer 3 pyramidal cells would push the processes

of the layer 5 neurons away from the radial glial fibers so they could have access to them. Consequently, in the pyramidal-cell modules of the mature cortex, the apical dendrites of the layer 3 pyramids would be in the middle of a cluster, and those of the layer 5 pyramids would be on the outside of a cluster. But the reverse is true (Peters and Kara, 1987), and in a later study of development using tangential sections through the developing cortex of rats, I was able to show that the profiles of the radial glial fibers are not at the centers but at the peripheries of the clusters. I presented this proposition at a symposium held in Edinburgh in 1991 to mark the retirement of Mike Gaze, who first introduced me to *Xenopus* tadpoles and to their optic nerves (Peters, 1993).

Marty Feldman and I continued our studies on the organization of pyramidal cells by showing that dendritic clusters are present in the cortices of rats, cats, monkeys, and humans, suggesting that they are a general feature of mammalian neocortex (Feldman and Peters, 1974). We also looked ardently for structures resembling "barrels" in cortices other than rat and mouse somatosensory cortex, but after we had hallucinated many times, we had to conclude that barrels are not a common feature of neocortex.

In the early studies, the only way to examine how pyramidal cells are organized into modules was through reconstruction using serial, 1-or 2-micron thick sections of plastic embedded tissue, as we had done in rat visual cortex (Peters and Kara, 1987). This is obviously a very tedious process and did not encourage us to extend our studies of pyramidal cell modules into the cortices of other animals. However, toward the end of 1980, a number of investigators had shown that antibodies to microtubule associated protein 2 (MAP2) label the cell bodies and dendrites of neurons. Ken Kosik in the Department of Neurology at Harvard Medical School was kind enough to supply us with antibody to MAP2, and so Claire Folger, and I set about to examine the organization of pyramidal cells in primary visual cortex of the monkey (Peters and Sethares, 1991). This antibody clearly shows the cell bodies of neurons and the clustering of the apical dendrites of the pyramidal cells in monkey visual cortex, so that we were able to generate a three-dimensional model of the pyramidal-cell modules in this cortex, for which Claire used her artistic talents to produce a nice diagram. These also showed that the apical dendrites of the pyramidal cells of layer 6A do not contribute to the clusters but form bundles that ascend only as far as layer 4C. The other feature of this cortex that became obvious through use of the MAP2 antibody is that the cell bodies of the neurons of layer 4A are arranged into cones, which fit into the pockets, or holes, in the honeycomb pattern of cytochrome oxidase staining in layer 4A. I will not give more details, because they can be found in the publication (Peters and Sethares, 1992). We were very pleased by the results we obtained with the MAP2 antibody, and this encouraged us to examine the pyramidal-cell modules in cat visual cortex (Peters and Yilmaz, 1993), the mouse barrel

field (White and Peters, 1993), and in area 18 of rhesus monkey cortex (Peters et al., 1997). It became evident that although there are variations in the patterns, the organization of the pyramidal cells into modules is a common feature of the neocortices of a variety of mammals (Peters, 1997).

I was able to present what was known about the organization of neurons in cerebral cortex into modules as part of a symposium that I arranged at the 106th meeting of the American Association of Anatomists (AAA) in San Diego when I was President of the organization in 1993. I was fortunate enough to persuade Vivian Casagrande, Ted Jones, and Francis Crick to make presentations at the symposium, which was well-received by the members of the association and led to a long discussion that went on well beyond the time allotted to it. Unfortunately, the presentations made at that symposium were never published. The symposium was toward the end of my term as president of the AAA, and for me the symposium was one of the highlights of my tenure. But looking back at that year, it seemed that I was kept quite busy because there were many decisions to make. However, there seemed to be little time to complete anything satisfactorily, and my frustration with that situation led me to propose that the term of president of the AAA should be for 2 years and not 1 year. But to achieve this, it was necessary to rewrite the constitution of the AAA. Luckily, there was little opposition to the changes I proposed, and, in retrospect, there is no doubt in my mind that changing the constitution was my most significant accomplishment during my tenure as president of the AAA.

Getting back to cerebral cortex—another question that arose as we investigated the cortex is whether any of the nonpyramidal cells contribute to the modular pattern of organization. By using various stains and antibodies to label different types of nonpyramidal cells, it became evident that in monkey primary visual cortex, at least, the only nonpyramidal cells that repeat regularly are the double-bouquet cells, whose axons are aptly described as resembling horse tails. Antibodies to calbindin show these cells and their horse-tailed axons rather nicely, and so we were able to show that there is one horse-tailed axon per module (Peters and Sethares, 1997). However, the role that these double-bouquet cells play is still not clear, and it can only be supposed that they provide some form of vertical inhibition. It is interesting that these double-bouquet cells rarely appear in Golgi preparations and little was known about their morphology until antibodies became available.

If one looks at myelin-stained sections of most cortical areas, especially those of the primary visual cortex, well-defined, vertical bundles of myelinated nerve fibers can be seen passing through the cortex, and, in tangential sections, it is evident that, like the clusters of apical dendrites of pyramidal cells, these vertical bundles of nerve fibers are arranged in a regularly spaced pattern. Moreover the center-to-center spacing of the vertical bundles of myelinated axons is similar to that of the clusters of

apical dendrites that define the axes of the modules, and the obvious question to ask is: How are they related? It turns out, of course, that many of the nerve fibers in the vertical bundles arise from the pyramidal cells and, consequently, we proposed that each pyramidal cell module has its own output bundle of nerve fibers (Peters and Sethares, 1996). The basic concept that we proposed is that each pyramidal cell module has a unique set of inputs and response properties, which are slightly different from those of the modules surrounding it. This being so, nerve fibers originating in that module carry a unique set of efferent information, which is passed on to other cortical areas.

Unfortunately, although it is generally recognized that there are pyramidal cell modules in cerebral cortex, the level of interest in these morphological modules is relatively low. I hope that more neuroscientists will eventually pay attention to them, because I believe that they are the fundamental functional units of cerebral cortex and that the pyramidal cell modules are synonymous with the microcolumns defined by Vernon Mountcastle.

Studies on Normal Aging

In 1972 I became involved in studies of changes that the normal brain undergoes as it ages, and in recent years these studies have been the focus of my research effort. I initially became involved in this research through my contact with Marott Sinex, who at that time was the chairman of the Department of Biochemistry at Boston University.

Marott had been very influential in setting up the National Institute on Aging, and he had been encouraged by the new institute to submit a program project grant to them, which he did. The outcome was that this was the first program project funded by the institute. At that time there were few scientists interested in the effects of normal aging, and so the program lacked focus. For example, it covered such topics as the effects of aging on the olfactory and auditory systems and cerebral cortex, effects on connective tissue, and the effects of age on chromatin and RNA. Then in 1979, when Marott stepped aside, I became the Principal Investigator and decided to focus the program more on the effects of aging on the nervous system. At that time we were using rats, and it became increasingly apparent that under laboratory conditions rats became grossly overweight and died at a younger age than normal. Also there are few tests that can be done to assess the effects of age on the cognitive status of rats, and so it seemed to us that rats did not provide the best model for normal aging.

Serendipitously, at about the time we were becoming concerned with the value of the rat model for normal aging, I met an old acquaintance, Dr. Johannes Tigges, at a scientific meeting and discovered that Yerkes Regional Primate Research Center at Emory University had a number of

aging rhesus monkeys. The primate center was willing to cooperate with us, and so in 1984 we changed our animal model of aging to the rhesus monkey, which closely resembles the human and is capable of responding to a variety of behavioral tests that can be used to establish cognitive status. Moreover, although rhesus monkeys can develop senile plaques in their brains, they do not acquire neurofibrillary tangles and do not get Alzheimer's disease, making them an excellent model for normal aging studies. In the realm of human aging, much of the emphasis is on Alzheimer's disease, which is certainly devastating for both the patient and their families, but it should be borne in mind that the majority of humans age normally, and yet we know almost nothing about what changes are taking place in their brains. For me, this is what makes the study of the effects of normal aging on the primate brain so exciting, because almost every study of normal primate brain aging enters virgin territory. It is one of the few fields of neuroscience research where one can still make fundamental and new discoveries. The effects of aging on the brain are so intriguing that for the past several years, essentially since I resigned from the chair of anatomy in 1998, I have devoted most of my time to this area of research and hope that I can continue to do so for several more years.

When we began our studies of the effects of aging on the brains of rhesus monkeys, very little was known about their longevity and so the first task was to determine the life span. This was done by examining the animal records kept at Yerkes, and it turned out that the maximum life span is about 35 years (Tigges et al., 1988), so that each monkey year is equivalent to about 3 human years.

With this change in species, Drs. Douglas Rosene and Mark Moss, who had worked with monkeys previously, undertook the behavioral testing to assess the cognitive status of the monkeys we were using, and Tom Kemper, who has a profound knowledge of neuropathology, also joined the program so that we could relate our findings on the monkey to what happens in the aging human brain. In these initial studies of normal aging in rhesus monkeys I, along with Johannes Tigges, started to examine cerebral cortex; Dr. Deborah Vaughan began to study the spinal cord; and Drs. Marty Feldman and Mike Harrison focused on the auditory system.

When this program started, it was assumed that normal cognitive decline was due to a loss of cortical neurons. Hence, the expression "He, or she, is losing his/her neurons" when someone is forgetful, but as we learned more about the effects of aging on the monkey cortex, it became apparent that there is not a significant overall loss of cortical neurons with age, and I think that the concept that cognitive decline is not due to extensive neuronal loss from cerebral cortex is now generally accepted (Peters et al., 1998; Peters and Rosene, 2003). However, it must be stated that few cortical areas other than motor cortex (Tigges, Herndon, and Peters, 1990), primary visual cortex (Vincent, Peters, and Tigges, 1989; Peters and

Sethares, 1993), and area 46 of prefrontal cortex (Peters et al., 1994) of primates have been thoroughly examined, and so some surprises might yet be in store as other cortical areas are scrutinized.

Morphologically, the cells that show the greatest effects of aging are the neuroglial cells, and if one wants to determine whether a given section of cortex is from an old monkey, it is best to look at the neuroglial cells and not the neurons, because, with age, neuroglial cells become filled with inclusions (Peters et al., 1991). The origins of most of phagocytosed material in the astrocytes and the microglial cells is not yet known, but it is evident that some of the material is derived from myelin sheaths, which break down during aging and even degenerate, especially if the axon degenerates.

Our first indication of myelin degeneration was when Marty Feldman and I encountered large, round, fluid-filled profiles in gray and white matter of old monkeys. It turned out that these profiles were due to ballooning of some myelin sheaths (Feldman and Peters, 1998), and as we looked more closely at nerve fibers in the electron microscope, it became evident that other aging myelin sheaths accumulate dense cytoplasm in pockets between their lamellae (Peters et al., 2000; Peters, 2002c). However, despite these degenerative changes, myelin continues to be produced with age, so that there is an increase in the numbers of myelin sheaths that have redundant myelin, producing sheaths that are much too large for the enclosed axon, as well as an overall increase in the thickness of myelin sheaths, which is partly due to an increase in the frequency of unusually thick myelin·sheaths (Peters et al., 2001). But the situation becomes even more complicated, because looking at electron micrographs of myelinated nerve fibers in areas 17 and 46, Claire Folger and I noticed that there is an increase in the frequency of profiles of paranodes with age (Peters and Sethares, 2003). This occurs even though there is not a significant increase in the lengths of paranodes, which occur at the two ends of lengths of myelin and are the sites where the myelin lamellae terminate adjacent to the nodes of Ranvier. We concluded that the increase in the frequency of paranodes could only occur if the numbers of internodal lengths of myelin increase with age. This would happen if there were remyelination taking place, such that some of the original internodal lengths of myelin degenerate to be replaced by shorter lengths of myelin. We then went on to look for evidence of remyelination in old monkeys and found some short internodal lengths of myelin and inappropriate thin myelin sheaths, both of which are considered to be the hallmarks of remyelination.

Interestingly, this apparent increase in the number of myelin internodes is correlated with an increase in the number of oligodendroglial cells in cerebral cortex (Peters and Sethares, 2004), and the question is: Where do these new oligodendroglial cells come from? Others, for example, Dr. Joel Levine at Stony Brook, have proposed that these new oligodendroglial cells arise from oligodendroglial cell progenitors in the brain and that these

progenitors label with an NG2 chondroitin proteoglycan antibody (e.g., Levine et al., 2001). Joel Levine was kind enough to supply us with some of this antibody, and when we examined the labeled cells in the electron microscope, it turned out that they had some features in common with astrocytes. In fact these cells had been described several years ago by Reyners et al. (1982), who called them β astrocytes. However, it turns out that these cells are not astrocytes but a fourth type of neuroglial cell that has been largely overlooked by electron microscopists because of their similarity to astrocytes (Peters, 2004). How wonderful after all of these years of looking at thin sections to encounter a cell that has been overlooked.

We now know that the galaxy of myelin changes that occur in the cortex are also present in white matter. Moreover, Julie Sandell and I have found that not only is myelin disrupted but that there is also a significant loss of nerve fibers with age from white matter. We have shown such a loss of nerve fibers from the optic nerve (Sandell and Peters, 2001), anterior commissure, (Sandell and Peters, 2003), and the corpus callosum. As is well known, the nerve fibers in the anterior commissure and the corpus callosum belong to neurons in cerebral cortex, and yet there is no significant overall loss of these neurons. This leads to the conclusion that some of the cortical neurons lose their long projecting axonal branches, although their cell bodies and dendrites remain intact. Their profuse local axonal plexuses are probably sustaining these neurons. This may not be too surprising, because the local plexuses have more axon terminals than the projection branches, because some 95% of the axon terminals forming synapses in the cortex originate from these local axonal plexuses (Peters, 2002b).

So what effects do these age changes in nerve fibers have on the aging brain? The proposition is that loss of nerve fibers from white matter leads to decrease in connectivity between parts of the brain, resulting in what clinicians refer to as a disconnection syndrome. Concomitantly, degenerative changes in myelin, as well as the increase in the frequency of internodal lengths of myelin brought about by remyelination, lead to a decrease in the speed of conduction along nerve fibers, which in turn results in alterations in the timing in neuronal circuits. The consequence of this may be the phenomenon experienced by almost every old person, the inability to instantly remember names. However, old people do not forget; it just takes longer to recall, which may be because the timing in a neuronal circuit must be just right, to mimic the timing when the memory was consolidated.

Finis

This seems like an appropriate theme upon which to end this account. It has been good to recall events in my scientific life, some events that I had not thought about for years, with memories gradually coming back to me as I have undertaken the writing. I am sure that there are events

that I have not been able to recall, but hopefully, nothing of great import. I have certainly enjoyed my scientific life, and as I have often said to students, we are very fortunate that we are paid to do what we enjoy most—scientific research. In my case I have also been very fortunate in other ways, especially in my marriage of almost 50 years to my wife Verona. She has provided a very stable and loving environment for myself and our three daughters, has always supported me in my career moves, taken care of the social side of our lives, and has always been uncomplainingly willing to move from place to place as my career progressed.

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