The History of Neuroscience in Autobiography
Volume 7

Edited by Larry R. Squire
Published by Society for Neuroscience
ISBN: 0-19-539613-8

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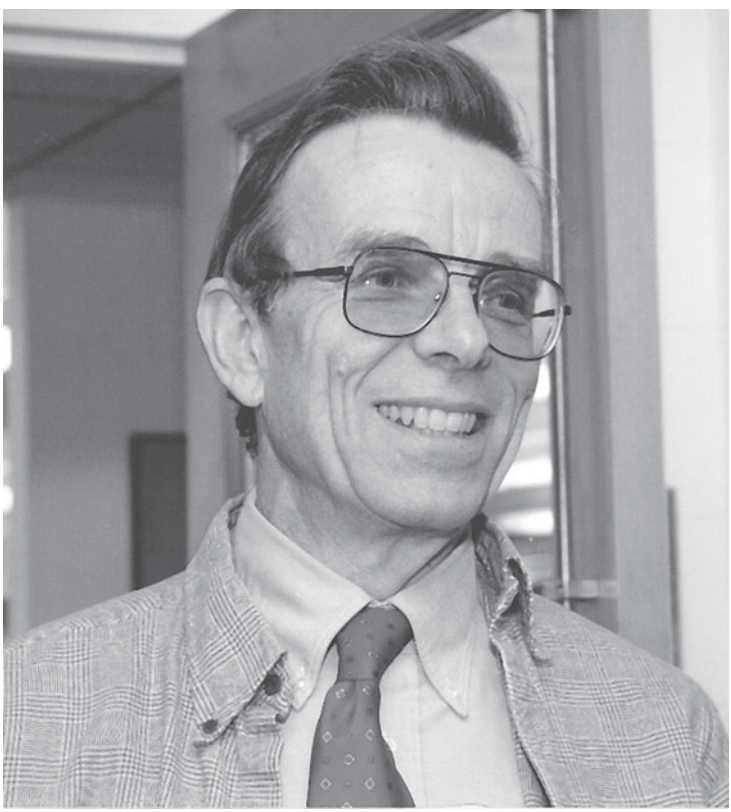
pp. 140–187

https://doi.org/10.1093/acprof:oso/9780195396133.003.0004



Bertil Hille

Born:

New Haven, Connecticut
October 10, 1940

Education:

Yale University, B.S. (1962)
The Rockefeller University, Ph.D. (1967)

Appointments:

Fellow, Cambridge University, Physiological Laboratory, Cambridge, England (1967–1968)
Assistant Professor, University of Washington, Department of Physiology and Biophysics (1968–1971); Associate Professor (1971–1974); Professor (1974–present)
Wayne E. Crill Endowed Professor, University of Washington, Department of Physiology and Biophysics (2005–present)

Honors and Awards (Selected):

Kenneth S. Cole Award of the Biophysical Society Membrane Biophysics Subgroup (1975)
Mathilde Solowey Award in Neurosciences from NIH Foundation for Advanced Education in the Sciences (1976)
Member of NIH NINDS Physiology Study Section (1981–1984)
National Academy of Sciences, U.S.A. (1986)
Research Award from the McKnight Endowment Fund for Neuroscience (1988–1994)
3rd Annual Bristol-Myers Squibb Award for Distinguished Achievement in Neuroscience Research with Erwin Neher and Jean-Pierre Changeux (1990)
Columbia University Louisa Gross Horwitz Prize for outstanding basic research in Biology or Biochemistry with Clay Armstrong (1996)
1999 Albert Lasker Award for Basic Medical Research, with Clay Armstrong and Roderick MacKinnon (1999)
The 2001 Gairdner Foundation International Award with Clay Armstrong and Roderick MacKinnon (2001)
Institute of Medicine (2002)
Wayne E. Crill Endowed Professorship, Department of Physiology & Biophysics, University of Washington, Seattle (2005–)
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Bertil Hille helped establish the concept of ion channels as membrane proteins forming gated aqueous pores. He showed that Na^+ and K^+ channels of axons can be distinguished by drugs such as tetrodotoxin and tetraethylammonium ion, and that their ionic selectivity can be understood by a limiting pore size, the selectivity filter, and by movements of ions through a series of saturable sites. He showed that local anesthetics enter Na^+ channels in a state-dependent manner. In later studies of modulation of ion channels by G protein–coupled receptors he distinguished two new signaling pathways. A fast, pertussis toxin-sensitive pathway turned on inward rectifier K^+ channels and turned off Ca^{2+} channels by G protein $G\beta\gamma$ subunits. A slow, pertussis toxin-insensitive pathway turned off some K^+ and Ca^{2+} channels by depleting the plasma membrane phosphoinositide PIP_2. Hille wrote the widely used textbook "Ion Channels of Excitable Membranes."

Bertil Hille

This essay assumes that less senior readers might be more interested in what scientific life was like one and two generations ago and how ideas arose. The more recent science is a matter of published record and does not need as full repetition. My own trajectory illustrates, like those of many others, that serendipity and opportunity are as significant as careful planning in determining a career. People are not born to fill a certain role. Much happens during early development. We are molded and redirected by interactions with mentors and unexpected opportunities.

Parents

I was born into an academic family in New Haven, CT. My parents were Scandinavian immigrants, so my brother Harald and I are first-generation Americans, born just before the United States entered World War II. My father, C. Einar Hille (1894–1980), was a Yale math professor and scholar (Ph.D., Stockholm University, 1918) with many honors, including being elected to the U.S. National Academy of Sciences and the Royal Swedish Academy of Sciences. My mother, Kirsti Ore Hille (1906–2001), was energetic, intellectually and artistically gifted, and an avid reader of books. Her honors included several dozen engraved silver cups for taking first place in downhill slalom ski races in Norway. Her hands were often busy with crafts, including weaving. She encouraged conversation, scientific thinking, causal explanations, and the academic community. My father was fully occupied with higher mathematics, so my mother undertook most of the tasks of parenting and the household. At that time, professors were not as well paid as today, and we lived with some New England frugality, although always in the larger classic houses that could be rented within walking distance of the university—my father didn't drive.

Early Education and Languages

During my first years, my father spoke Swedish and my mother Norwegian, the two languages being mutually understandable. Gradually our household shifted to English, but as is common among Europeans, my parents were polyglots who spoke and read German and French easily as well as fragments of other languages. Their English was interlaced with foreign phrases. It would be a rare dinner if we had not discussed the etymology and linguistics

of several foreign expressions, and we had not finished with an encyclopedia, a dictionary, or an atlas on the table. My father gave his mathematical lectures in several languages. Later on, my mother became an article abstracter for the *Quarterly Journal of Alcohol Studies*, summarizing papers from the French, German, and Scandinavian. While we were children, my father had sabbatical and other leaves, which gave us a half year in Stockholm, Sweden, a full year in Nancy, France, and a half year in Mainz, Germany. European cities still were deeply scarred by damage from World War II. We crossed the Atlantic by ocean liner each time. In Europe, my brother Harald and I went to the public grade school, lyc*é*e, or Gymnasium, so we too received some classical European education in several countries. For several months in advance, 78 RPM records of language practice would be playing during dinner. Each European visit included several months of touring in our automobile (mother driving) to visit the best art museums, cathedrals, castles, and mathematicians. Our cars were small and without radios or heaters. We read aloud from the Guides Michelin, Baedeker's, and Guides Bleus in several languages, and my parents, especially my father, explained which king was married to whom from where and who succeeded whom and intrigued or fought where. He had a deep fund of geography and history. On one of these trips when I was 12 years old, we were visiting German mathematicians and stopped at Wetzlar, where my parents secretly bought me a Leitz compound microscope including an oil immersion objective and a dissection kit. I got them on my next birthday. The same year, I was missing first-year Latin at home, so my brother (a year older) had to drill me in Latin verbs as we drove between castles of the Loire valley. Eventually, Harald also studied several Slavic languages and became a linguist and terminology expert in many languages. He still works with languages at the United Nations in New York.

We attended private schools—on scholarship. The Foote School was the grade school of choice for Yale professors' children; thus, many of my classmates had an academic family background. The education, environment, and student body were supportive and excellent. Mrs. Hitchcock, the wife of a Yale School of Medicine professor, drilled into us a powerful, concise, Anglo-Saxon style of writing. I also became fond of drawing and water coloring. I liked the intersection of art and geometry and remember holding forth to my sixth grade class for an hour on two-point and three-point perspective and how correctly to turn a house plan into a full perspective drawing. With my dissection kit I held a demonstration of the inner organs of the frog complete with its heart beating in situ for somewhat reluctant friends. I collected butterflies, spread their wings, and pinned them with tiny labels into cigar boxes. Entomologist Professor Charles Remington across the street from our home at the Connecticut Agricultural Experiment Station kindly discussed collecting and showed me their many drawers of pinned and labeled type specimens.

When I was 13 years old, my mother decided that, like my brother and most graduates of the Foote School, I should be sent away to boarding school for 4 years to be taught essential American ways. Westminster School offered a strong traditional education to white boys in coats and ties, with outdoor sports led by the Masters and discipline entrusted to the older boys, the Sixth Formers. It followed the English style of many New England prep schools then. The motto was "with grit and grace." I profited from Ashley Olmstead's writing discipline, enjoyed singing in the choir (first soprano), and studied Latin and French literature. The formal science teaching was classical, yet still new and fascinating for me. Electrons went around atomic nuclei in stately and precise planetary Bohr orbitals. Biology taught morphology and taxonomy but not yet mechanisms. When Thomas Hooker and I finished the school's math offerings and declared that we would try for the Advanced Placement Exam in mathematics in our final year, the teacher (for just the two of us) and we had to struggle with a quickly purchased calculus book since none of the three of us knew how derivatives or integrals worked. Collaboratively we made it. Today calculus is available in most high schools. Many of the boys at Westminster were from more moneyed classes and knew much more about machismo, girls, style, popular culture, alcohol, and tobacco. They were also physically more developed than me. I often felt shy, uncomfortable, and awkward in their company. In times of loneliness I retreated to the choir room and played the piano for a few hours. Our class was the first to have a black student, Booker T. Bradshaw, from Richmond, Virginia. He was strong in sports and excelled as a student. Although from an elite family, he felt out of place for other reasons. Booker and I were roommates and close friends for the last 3 years. He had a deep collection of jazz, country, and popular music. Later, Jack Partridge, my roommate at Yale, introduced me to bluegrass music.

Starting in a Lab

I have had the extraordinary good fortune to receive generous and supportive guidance at many stages in my scientific career. When I was 16 years old, I began working summers in the Yale Biology lab (1957). This was after my junior year at Westminster. Edgar J. Boell, the Chair of Yale Zoology and a close friend of our family, made good on a promise he had made years before. I was given a research project to study the respiratory function of the larval gills of the salamander *Ambystoma tigrinum*, an organism that Ross Harrison had pioneered for embryology decades before at Yale. Boell was the Ross G. Harrison Professor of Zoology. I worked with those larval salamanders until 1962 when I graduated from Yale. For three summers, I measured their respiration volumetrically using the classical Warburg apparatus, a sort of shaking water bath with perhaps 20 gasometric manometer chambers holding one larva apiece. Ed Boell was an extremely supportive mentor,

who introduced me personally to scientific culture and to each step of preparation and laboratory measurement. His warm enthusiasm for careful work and patient observation leading to reliable results was an excellent formative lesson and role model for a high-school apprentice.

Yale

In 1958 it seemed perfectly natural to go on to Yale (and become a zoologist), where my tuition would be free, and to apply to nowhere else. Extraordinary teachers included the memorable Vincent Scully in history of art, a real showman whose long wooden pointer slapped the two giant screens in an otherwise totally dark room as he paced on the elevated stage and orated loudly in dramatic sentences. He kept his audience of 800 (all boys) in rapt attention while expounding wonderful original ideas. I have often hoped to bring even just a fraction of his drama to my lecturing. I still enjoy art history. E. Robert Beringer teaching honors physics had a beautiful gift of explanation and wonderful homework problems. He made physics challenging and exciting to learn. I took most of the advanced undergraduate courses in zoology and enjoyed them all, from invertebrate zoology, comparative anatomy, up to biochemistry. Everything made sense through descent from a common ancestor and adaptation to the environment. Cell biology was a standout with R. Bruce Niklas, just one year after his Ph.D. on moving chromosomes. I also took all of the lecture courses for the Biophysics major and was strongly influenced by Harold J. Morowitz's many biophysical and theoretical insights. His thinking about free energy, entropy, science, and Eyring rate theory became very useful to me more than a decade later. We read Schrődinger, "What Is Life." Physical chemistry in the Chemistry Department was a beautiful combination of natural science with simple math. Could biology achieve that? All of those courses introduced exciting ideas that were new for me. One time, George von Békésy was invited to give a series of lectures in Zoology on the cochlea; perhaps it was the year before his Nobel Prize. A few of us undergraduates asked if he would have lunch with us. Naturally in our youth we would ask him what was the secret to success, and he told us, "I never decide that something is correct until I have shown it three ways!"

Although my zoology curriculum was mostly classical, in my senior year (1961–1962) we became aware of a turning point toward a new biology. I had been reading in past issues of my father's collection of the *Proceedings of the National Academy of Sciences* (*PNAS*), including Linus Pauling's proposals of the α-helix and the β-sheet, and started looking at the new-journal shelf in Zoology each week. In that year, François Jacob and Jacques Monod defined the lac operon and the lac repressor (*Journal of Molecular Biology*), Francis H. Crick and Sydney Brenner argued for a triplet code (*Nature*), and Marshal W. Nirenberg, Severo Ochoa, and competitors began uncovering

the triplet codons one-by-one (*PNAS*). I was excited to report on those advances in the weekly Biology Club that five of us seniors held. There we also discussed amber mutants of *Neurospora*, the territorial calls of frogs, respiration in the turtle cloaca, and pigmentation of butterfly wings.

Part of my senior honors thesis was to measure and analyze the time course of uptake and clearance of ^{137}Cs (as a K^+ tracer) in intact *Ambystoma* larvae. I developed a several-compartment model description much like those of pharmacokinetics. Timothy Goldsmith, then a new faculty recruit, became my honors advisor. He would challenge me with exotic readings such as on the liquid-junction potential, in German (Henderson, 1907), and on the voltage clamp and the squid action potential (Hodgkin and Huxley, 1952). That was the first time I saw this kind of work. The squid axon papers were less than a decade old and still not fully accepted, because they seemed to biologists to be like, "give me ten parameters and I can make an elephant wag its tail." I was hearing a distrust of modeling in biology from my zoology teachers and came away not sure whether that was a good way to go. I didn't realize at that time that this would be a core of my future work.

Woods Hole

Four summers in Woods Hole also had an enormous formative influence. After my sophomore year at Yale, I spent some weeks at the Oceanographic Institution, WHOI, being a free crew member on oceanographic cruises, with jobs like collecting deep water samples with Nansen bottles, measuring the thermocline, making plankton tows, or assisting in pressing water for analysis out of cores as they were collected from the glacial clay deposits off the continental shelf. We had day watches and night watches around the clock. I spent the following summer in the undergraduate research program at WHOI measuring photosynthesis and photodamage by ultraviolet light on samples of intertidal algae. Our supervisor John W. Kanwisher, with his wife Joan and little girls (including future cognitive neuroscientist, baby Nancy Kanwisher), also needed crew for their new boat on weekends, so I learned to sail a gaff-rigged schooner. Mario R. Capecchi (of homologous recombination in mouse) was an undergraduate in the same small program that summer. Another summer and a half I worked with Roger D. Milkman at the MBL on heat-shock perturbation of posterior-crossvein development in *Drosophila* pupae. These measurements could be made very quantitatively and begged for a quantitative model, which we made with multiple Q_{10}s and numerous kinetic steps. I got a lot of practice in developing multicompartment models on a portable analog computer by myself. Reinforcing the concept that modeling may not be good for biology, the resulting manuscripts were rejected by seven journals and finally were published in the *Biological Bulletin*, but little cited. I still like that work! A final Woods Hole summer while I was in graduate school (1965) was spent in Harry Grundfest's

lab at the MBL, voltage clamping what seemed to be a voltage-gated chloride conductance in the weak electric organ of the skate, *Raja erinacea*. Michael V. L. Bennett cosupervised my work. It fascinated me that this putative channel for chloride behaved kinetically so much like the well-known delayed rectifier K^+ channels. It is abundant in the electric organ and could be followed up today.

Quite beyond the actual summer lab work, the MBL was a place of vigorous biological foment, a place to fill a curious mind with wonderful new ideas, a renaissance education in a cutting-edge science cafeteria. Everybody who was anybody was there in the summer and they were open to casual discussions of all of life science. Without being registered, I attended all the lectures of the physiology course, maybe twice, and some of the embryology and the invertebrate zoology courses. There I heard Matthew S. Meselson tell about the Meselson and Stahl experiment on DNA and Edwin J. Furshpan and David D. Potter explain about gap junctions in the crayfish and the Hodgkin-Huxley work. I attended all the Friday night lectures by great scientists and some Tuesday night "electrobiology" presentations, where Ichiji Tasaki and Harry Grundfest often challenged each other stridently. Together Yale and Woods Hole evoked a passion in me for a very broad range of sciences, although with little special focus. I learned that success in science would be to trace all natural phenomena back to the laws of physics and chemistry. Woods Hole may have been the "school" that contributed most to my appreciation of modern life sciences and a life of science. During this time I grew up physically, became self-confident, brash, and an outspoken "young Turk."

The Rockefeller University: A Community of Scholars

Early in my senior year at Yale, Ed Boell told me he had written a letter to the Rockefeller Institute in New York to recommend me for graduate studies. I had not heard of it before (!), but as soon as I had my interview, there was no point in looking elsewhere. The interview began with the Dean, Frank Brink, Jr., who asked me what book I was carrying to read on the train. It was *Biophysical Chemistry* by John Edsall and Jeffries Wyman. I asked him if he knew of it. Fortunately, that book and its authors were well regarded at Rockefeller. I visited with Philip Siekevitz and Paul Weiss, brashly telling them my instant opinions on their specialties. Most of my day was spent shadowing Detlev Bronk, a biophysicist, the President of the University, and simultaneously the President of the U.S. National Academy of Sciences, ex-president of Johns Hopkins, and ex-Chairman of the National Research Council. He was fielding telephone calls from David Rockefeller, inspecting many parts of his campus, talking with his employees, and impressing his interviewee with his philosophy and scope. All of science has intellectual unity. Science is an international community of scholars.

Scientists should engage in science policy and public service. Bronk was proud that there was no need for or dependence on federal support for research at his elite institution. At the end he invited me to join their community of scholars. Irresistible! Dr. Bronk considered recommendation letters only from people he knew well. By this method in 1962 he selected 21 students for my class, six of whom eventually became members of the National Academy of Sciences: Wyatt W. Anderson, Anthony Cerami, Harvey F. Lodish, David D. Sabatini, Daniel W. Stroock, and me. It must be rare when such a collection of future scientists is so successfully chosen from undergraduate applicants!

Since 1901, the Rockefeller Institute for Medical Research had been at the forefront of what is now called biomedical research, conquering major diseases and greatly advancing the basic life sciences. In 1955, a graduate program was begun under the new President Bronk. The name was shortened first to The Rockefeller Institute and in 1965 to The Rockefeller University. In our time, there were many more faculty than students, the idea of formal teaching was unfamiliar to them, and our curriculum was self-determined. Everything was done with a style that eventually drew down the endowment. The students were pampered, made to feel like privileged scholars, and provided with an apparently unlimited budget for whatever we wanted to investigate. Faculty and students ate in the formal dining room, coats and ties required. At lunch the linear tables held 30 people, and you never knew whom you might sit down next to—sometimes even the President. I think our stipend was $2000 with an additional $1000 for intellectual and cultural enrichment.

I continued to read broadly from the weekly table of new journals, under the watchful eye of Antoine Lavoisier on the library wall. His stunning, life-sized seated portrait with Mme. Lavoisier standing by him painted by Jacques Louis David now hangs in the Metropolitan Museum of Art. Friday afternoon lectures provided a role model in how to present a scientific story. They were so grand and of such wide scope that it was hard to imagine ever achieving so much in a life's work. Eclectic course offerings I took were extraordinary: cell biology (George E. Palade, Siekevitz, David J. L. Luck), a tutorial in biochemistry (Alfred E. Mirsky), chemical kinetics (David Mauzerall), theoretical organic chemistry and enzymology (Daniel E. Koshland), topics in physical chemistry including hyperchromicity of DNA, helix-coil transitions, properties of water, the hydrophobic effect, and regular solution theory (Walter J. Kauzmann visiting from Princeton), osmosis theory, electrochemistry, and the Nernst-Planck diffusion regime (Alexander Mauro), electricity and magnetism and thermodynamics (George E. Uhlenbeck), neurophysiology (Victor J. Wilson), Egyptian hieroglyphics (Samuel A. Goudsmit), humanism (Ludwig Edelstein), and more.

Among my own class I became especially close to Harvey F. Lodish, David I. Hirsh, Daniel W. Stroock, Alan B. Steinbach, and Alan M. Kapuler

(who had initiated our Biology Club at Yale). Harvey had a B. S. in chemistry from Kenyon College. Within the first week, I declared that he needed to know some biology. "Fine," he said, "do you have something I can read?" I gave him the 900-page *Histology* by Ham and Leeson. The next day he came back with the book, saying, "That was OK, do you have another?" Thus, he began to prepare himself for writing *Molecular Cell Biology* 23 years later. In my first month at Rockefeller, I met Frederick A. Dodge, in the dining room. He was a very senior graduate student. For his thesis he had worked with Bernhard Frankenhaeuser in Stockholm to develop a voltage clamp for single nodes of Ranvier of frog myelinated nerve fibers (Dodge and Frankenhaeuser, 1958, 1959) and then set it up in New York for further experiments. This was the first full duplication of the Hodgkin-Huxley program of separation of ionic currents, model fitting, and simulation of the action potential for the vertebrate phylum—indeed for anything other than the original squid giant axon. I could understand what he was telling me at dinner about the nodal Na^+ and K^+ currents, but despite his invitation to come up and see the lab, I was reluctant to get involved because of the lack of confidence in such model-oriented approaches I had absorbed from my Yale mentors. Other senior students at the time included Alan Finkelstein, David L. Baltimore, and Charles F. Stevens. I remember an hour-long heated dispute between Siekevitz and Baltimore in our cell biology course on whether the newly proposed concept of polyribosomes was just an artifact (Siekevitz) or correct (Baltimore). Chuck Stevens kindly transferred custody of the students' analog computer to me and showed me how to use it. That was just the tool I needed to complete the modeling of *Drosophila* crossveins, so I took it to Woods Hole the next summer and could complete that project with Roger Milkman.

Technology

Technology plays a pivotal part in biophysical experiments. We used to say that if you could buy the apparatus commercially, the experiment already had been done. In 1960, while at Yale, I took a free course in how to use their digital computer, a whole building by itself full of the vacuum-tube 709 computer. The International Business Machines Corporation taught us for 3 hours each night, three nights in a row, a language they called FORTRAN. The 709 offered the first FORTRAN compiler. We each could enter a program on punch cards in a 5-minute time slot for practice. My program was on the order of: A = 1, B = 3, C = A + B, PRINT C, with appropriate formatting. After the 9 hours of instruction and debugging this program, I felt like an expert in computing! In 1963 at Rockefeller, I wandered into one of the lab rooms of H. Keffer Hartline (Nobel 1967 for lateral inhibition in the *Limulus* eye) where he had just installed the Rockefeller University's first digital computer, a transistorized Control Data CDC 160A. It boasted a

massive desk-like console with many associated racks of equipment, a 13-μs add time, 32K (total) magnetic core memory in an external box with noisy fans, and "fully solid state." Their idea was to make it into a laboratory computer for recording on-line and analyzing responses of the *Limulus* eye. The electronics department was busy designing interface modules for analog-to-digital (A-D) and digital-to-analog (D-A) conversion, which could not be purchased off the shelf as they can today. Fred Dodge was also there. By now he was Dr. Dodge and an employee of IBM, with an affiliation with the Hartline lab. He and Bruce W. Knight were beginning to construct software that would eventually determine the transfer functions for eye responses to sinusoidal inputs of light or current. However, although Fred had programmed the nodal Hodgkin-Huxley calculations of his thesis using SICOM, a machine language for an IBM 650 drum computer at Columbia University, neither of them knew how to use the FORTRAN II compiler that had come with the CDC machine. I sat down and wrote the cross-correlations for them on the spot after they had explained the math to me.

For over a year at Rockefeller I had looked around at possibilities to study membranes, including electron microscopy of membrane complement lysis and the kinetics of the then new Na^+-K^+ ATPase activity. Finally Alex Mauro invited me to come to his lab to study the effect of D_2O on action potential propagation in the lobster circumesophageal giant axons. This was formally the President's lab of biophysics that comprised Bronk, his Dean Brink, his Associate Dean Clarence M. Connelly (none of whom had research programs), Alex Mauro, and W. Paul Hurlbut. Alex had worked on a wide range of phenomena: muscle satellite cells (he discovered them), black widow spider venom, the *Limulus* ventral eye, theory of osmosis and filtration, Nernst-Planck theory, and cardiac pacemakers. Alan Steinbach joined the same lab and we were lab mates doing our theses in the same room. On the same floor was the lab of Hartline and Floyd Ratliff, studying lateral inhibition and photon responses of the *Limulus* lateral eye, as well as the University Electronics Department.

Alex Mauro was a very enthusiastic presence in the President's lab with strong outspoken liberal emotions about the escalating Vietnam conflict, the civil rights movement, and social injustice. But he was not so interested in deciding what the research questions would be or in the practical aspects of setting up a lobster experiment. The whole lab was low key, and little lab research was going on. I am forever indebted to Fred Dodge for helping me get going several times that year, as he did for a generation of biophysics Ph.D. students. With the Faraday cage and extracellular wires or intracellular microelectrodes, D_2O slowed the propagation of the action potential, and the nearly intact lobsters accumulating in the freezer made wonderful bouillabaisse parties. I had started recording from nerve, but it was not clear where this project could lead.

By 1964 I had to take my oral qualifying exam, one of the few formal requirements. I don't remember all the questions or even all the examiners,

but I do remember Zanvil A. Cohn asking me to explain the functions of the liver and David Mauzerall asking for a formulation of enzyme kinetics in terms of irreversible thermodynamics. All this went well enough, but fortunately the committee determined that if I was hoping to be a biophysicist I had better know more about electronics. Although I could handle Ohm's Law and the simplest cable equation, I had regarded electronics as in the realm of engineers and not for pure basic scientists like myself. Alex Mauro was assigned to rectify my deficit. He had worked at General Electric during World War II, and he took a book from his shelf to get me up to speed. It was a 500-page tome called *The Pentode* on the metal alloys, grid spacings, electron-cloud space-charge regions, optimal filling gases, and the ideal glass envelope for this type of high-gain vacuum tube, in short everything one might need to manufacture and design new ones. I did read this book from GE but still did not gain practical facility with electronics. Fortunately my real mentor and role model in this again was Fred Dodge. As I began to use them, the old oscilloscopes, stimulators, and amplifiers would break down, and Fred would pull them out, show me the circuit diagram, trace the waveforms until we found the problem, and then replace the part. I began to study circuit diagrams for my instruments and eventually was able to understand for example what every resistor, capacitor, and vacuum tube was doing in my Tektronix 565 dual-beam oscilloscope. I also became skilled in using the new transistorized operational amplifiers that began to appear on the market. By 1966 I had to use the soldering iron alone. Fred left for Bernard Katz's lab in London (Nobel, 1970 for neuromuscular transmission) for postdoctoral study of the effects of low calcium on the endplate potential with Rami Rahamimoff. Despite this valuable practical education, I regret that I never acquired any depth in AC-circuit analysis or in de novo design of transistor circuits in the way that I later admired in Wolfgang Nonner and Fred Sigworth. Although my father was a mathematician who later wrote textbooks on complex variables and differential equations, I never gained adequate facility with these tools to read typical electrical engineering books.

The year 1964 was pivotal for me in yet another way. I married Merrill Burr, who was a couple of classes ahead of me at Rockefeller finishing her thesis work on chymotrypsin as Dan Koshland's first graduate student. Ours has been a lasting and happy union. Shortly before we married I flew Icelandic Air to Luxemburg and hitchhiked alone to and around Greece visiting Classic Greek, Mycenaean, and Minoan archeological sites for a month—my last excursion as a bachelor.

After my oral exam, I asked Fred Dodge if I could learn to voltage clamp the node of Ranvier, the technique of Dodge and Frankenhaeuser. Maybe I could try modification of nerve functions with a more mechanistic readout. That was fun. A single myelinated fiber had to be teased from the others in a frog sciatic nerve trunk, sealed into a multicompartment chamber with

Vaseline across partitions, and, when addressed with a rack full of vacuum tubes and power supplies, produced beautiful ionic current traces on the oscilloscope. The dissection with fine needles was difficult, since the largest frog nerve fibers were only 20 μm in diameter and could not be touched directly or stretched. Although I used that frog preparation in sole-authored papers for 15 years, I never achieved the manual skill shown, for example, by Robert Stämpfli or Wolfgang Nonner, whom I visited later in Homburg (Saar), Germany. Retrospectively, my talents seemed rather to be in recognizing and interpreting new phenomena, developing logical arguments and unifying hypotheses, and synthesizing novel stories simply and transparently.

Analysis of voltage-clamp records turned out to be slow. The oscilloscope traces were photographed by a Grass camera on long rolls of 35 mm film. They were projected from a photographic enlarger onto graph paper and traced by pencil. Then points were read off, baselines subtracted, currents separated, values transformed, and replotted onto semi-logarithmic paper to fit time constants. I wasn't patient enough to spend a week analyzing an hour of recording and sought a faster way. By early 1965 Fred Dodge had the idea that Hartline's computer might help. He taught me to program data sampling with hand-coded machine language (there seemed to be no macroassembler!), and this code was linked with a FORTRAN section. The code could not control the experiment, but it sampled sweeps online, synchronized by pulses from a ganged bevy of vacuum-tube Tektronix pulse generators. Initially the raw data had to be saved on long rolls of punched paper tapes, but later in the year the arrival of a large digital magnetic tape drive (as big as a closet) made possible a more compact and secure format. Dr. Hartline very generously conceded Saturdays for this activity unrelated to his lab. I could wheel my full rack of equipment and the temperature bath down to the Hartline lab and plug in on weekends. For my thesis and the next 15 years, all my personal experiments were done on Saturdays, and I still am in my office on many Saturdays. I got used to the full day of quiet that allowed me to focus entirely on doing experiments without interruption. Off-line analysis of the current records using a FORTRAN program I wrote could be done at lunch times or in evenings of other days when the computer was not in use. This was the first time that any voltage-clamp experiment was recorded and analyzed by a digital computer.

Graduate Research and Ion Channels

It is hard to reconstruct your state of thinking 45 years ago, but some notion comes from looking at my lab notebook. Once I had the new relatively high-throughput online system operational, my very first Saturday experiments (June 1965) before going to the Grundfest lab for the summer involved lidocaine, tetrodotoxin, and chlorpromazine, and in September, immediately after returning from Woods Hole, it was tetraethylammonium ion (TEA).

With tetrodotoxin and TEA it was clear at once that one drug was selective against Na^+ currents and the other against K^+ currents. While getting ready to do experiments, I had taken Koshland's enzymology course, saw many attempts to identify residues in enzyme active sites, and began reading books about structure–activity relations for antagonists, partial agonists, and full agonists of various receptors. It seemed natural to suppose that the Na^+ and K^+ carrying systems (as Hodgkin and Huxley called them) of axons might also be investigated using the concepts that had been developed by enzymologists and pharmacologists. A question that no one at Rockefeller seemed to have any opinion on (or interest in?) was whether the ions were passing through pores and, if so, whether Na^+ and K^+ used the same pore or separate ones. Without any evidence, I was cockily sure that it was pores and that there were two kinds, which should be called Na^+ and K^+ channels. This would be the hypothesis of my work. Clay M. Armstrong, then a post-doc at the National Institutes of Health (NIH) with Kenneth S. Cole, already had been thinking along exactly the same lines; he began a beautiful series of papers on K^+ channel block by axoplasmic TEA (Armstrong, 1966, 1968, 1969, 1971). We thought the same way and fed on each others ideas. Many others thought differently. For example, American biophysicists had published articles proposing that ions pass through cracks in the membrane, that K^+-carrying inward rectifiers are carriers, or that Na^+ and K^+ pass through a single pore that goes through a graded change in diameter and selectivity during the action potential. In 1966 or 1967 at the Biophysical Society meeting, there was a "discussion-only" session on axon electrophysiology. Toshio Narahashi, the chair, announced that as a ground rule one could not use the word *channel* in this discussion. Clay and I objected (our abstracts even contained the word in the title). Finally, Narahashi conceded that *channel* could be used provided that no mechanistic connotation was implied! For at least 10 more years some people used the word *channel* even if they rejected the pore hypothesis.

For the thesis, I use voltage clamp to screen a very large number of the agents that had been reported to change the excitability or shape of action potentials and selected those that were best suited to argue for the separate-channel hypothesis (Hille, 1966, 1967, 1968a, 1968b). Tetrodotoxin and saxitoxin (obtained from Ft. Detrick) blocked Na^+ currents selectively. My 1966 *Nature* paper states, "The sodium channels of the nerve are closed or clogged individually by the binding of the anaesthetic molecule to a complementary structure of the channel (p. 1222)." TEA and analogs blocked K^+ currents selectively. TTX, STX, and TEA blocked as though entering the pore and binding to a single binding site that fully eliminated an aliquot of conductance rather than reducing current by gradual changes of gating. Raised extracellular Ca^{2+} shifted the gating of Na^+ channels without changing K^+ channel block by TEA, and adding the insecticide DDT seemed to keep Na^+ channels open after they had opened ("foot-in-the-door" hypothesis)

without changing K^+ channel sensitivity to TEA. Acidic solutions blocked Na^+ channels as if titrating a negatively charged acid group with a pK_a of 5.2 that was essential both for conduction of ions and for the binding of TTX. Such a negative charge might attract permeating Na^+ ions and would repel any anions from the pore. Twenty-five years later the amino acid sequences of voltage-gated Na^+ channels were shown to have several acidic residues in the putative selectivity filter region, whose neutralization decreased TTX binding and ionic selectivity (e.g., Terlau et al., 1991). In 2010, we still await structural work to show how they are arrayed in three dimensions.

These well-cited thesis papers were written with little input or oversight from others. In retrospect it is evident that they should have been critiqued locally first and that they were recklessly submitted without the normal number of replications and with no indication of reliability. Fortunately the phenomena seem to have been mostly correct. On reading my thesis (1967) David Mauzerall (physical chemist) said that it was "typical of biophysicists" to think that every experiment will be the same as the last one so that statistics were not needed. Even looking back at the Hodgkin-Huxley papers, one sees that although they present tables with the results of several axons, only sometimes is there a mean, and there are no "statistics." I have been fortunate in that once my lab got into experiments where we had to compare means, my students and postdocs brought enough basic statistical background to know how to do it.

Alex Mauro knew George Camougis and Bertil H. Takman of Astra Pharmaceuticals, the makers of lidocaine. One day he drove his two students, Alan Steinbach and me, to Worcester, Massachusetts, to visit Astra. This started a long useful relationship with Bertil Takman, who knew a vast array of compounds and analogs that had been explored in their studies of local anesthetic action and development. He had a fund of ideas that moved our work forward 10 years later. In 1966 he introduced us, for example, to membrane impermeant QX-314 that is now widely used to silence single neurons from the inside. It gave me respect for industrial research and medicinal chemistry.

My thesis papers and my thesis lab began my long happy association with the *Journal of General Physiology* (JGP). It was owned by the Rockefeller University, so President Bronk appointed the editor who usually sat in Bronk's lab where I worked. Earlier editors had included Bronk, Brink, and Mirsky. While I was there, it was Associate Dean Clarence Connelly (1961–1964), J. Woodland Hastings (1964–1966) at Harvard, followed by Paul F. Cranefield (1966–1995). Connelly was very sensitive to American usage. I still follow his preference that the present tense rather than the future tense be used to refer to a later part of a text (i.e., avoid the expression, this *will* be explained later) and his impression that "both" is greatly overused (it is meaningless to say, *both* A and B are identical).

In spring 1967 I defended my thesis and concluded with the following words, taken verbatim from my written lecture notes:

> The different permeability mechanisms can be called the Na channels, the K channels, and the leakage channels. The sodium and potassium channels of nerves must be special structures inserted in the matrix of the cell membrane that provide diffusion paths tailored for specific ions. Almost every example of molecular specificity in biology involves the interaction of a protein with something. We have come to know that proteins are more versatile than other macromolecules in the variety of conformations that they may assume and in the kind of chemical moieties they offer. Their production is intimately tied to the genetic material and therefore their structure is most directly subject to the actions of natural selection and their synthesis most directly responsive to the activity of genes. I feel that the changes of ionic permeability of nerve membranes reflect the conformation changes of proteins that form the diffusion channels through the membrane. Somewhere in these channels, various amino acids are gathered in a constriction that is able to distinguish between Na^+ and K^+ ions. Biochemists could expect to find these [protein] molecules in the axoplasm and surface membranes. . . . . It would probably be feasible to use TTX binding as an assay for the Na channel proteins.

This was the channel manifesto that Clay and I were advancing each in our own way. Eleven years later, the first Na^+ channel subunit was purified from electric organ on the basis of TTX binding (Agnew et al., 1978). In my thesis lecture I had used as one argument in favor of pores that the fluxes of Na^+ in Na^+ channels show no saturation and behave linearly, the independence principle of Hodgkin and Huxley (1952). Presciently, Fritz A. Lipmann (1953 Nobel for coenzyme A) asserted in the question period that, like enzymes, pores must also be able to saturate.

Finding a Job

During my last year, President Bronk arranged that I would be going to Cambridge, England, to work with Alan L. Hodgkin (1963 Nobel laureate for the basis of nerve action potentials). I was awarded a Helen Hay Whitney Fellowship for that. Hodgkin said he would tell me the research problem after I was there. The fellowship application asked how my proposed work would help in connective tissue diseases. I answered that connective tissues must have cell membranes and the squid giant axon was the best place to study them.

Because I would be out of the country for a year, I felt that it would be best to find a faculty job to return to at the end. My mentors arranged several visits. At Harvard Biology (George Wald and Keith R. Porter) I realized I would need more infrastructure (shops) than they had available. At Duke Zoology (Knut Schmidt-Nielsen) the dinner conversation with faculty and wives focused on whether any of them would be comfortable with inviting a black guest to dine in their house. I had a marathon West Coast tour visiting my mentors' friends: UCSD (Robert B. Livingston and Theodore H. Bullock), UCLA (Wilfried F. Mommaerts), Berkeley (Robert I. Macey), University of Washington Seattle (Chuck Stevens, J. Walter Woodbury, Harry D. Patton), and my brother-in-law's department of Biology at Simon Fraser University in Burnaby, Canada. UCSD was going to start its first medical class in the next year and seemed like a gamble since they had no idea what teaching responsibilities their faculty would have or even what departmental structure. I really did want to continue using an online computer connection to my experiments, and in 1967 a beginner could not imagine having one of his own. Mommaerts, Chair of Physiology at UCLA, was very charming and courted me then and several times afterwards; I asked if they had an online computer. They did, and he took me on a 30-minute hunt and finally located a dusty card-reader terminal in a dark corridor. Seattle's Department of Physiology and Biophysics seemed the best. There already were excellent membrane biophysicists like Woodbury and Stevens. They had a machine shop, an electronics shop, a vigorous graduate program, and above all—the trump card—an online computer facility connected to physiological laboratories, and lots of computer savvy, all within the department. Furthermore, the Pacific Northwest seemed great, and Merrill, who had been there years before for a summer, thought it ideal. She was right. I was 26 and still had not defended my thesis, but I had a job!

Most institutions on this visit had said that of course they didn't have jobs but I could visit anyway. Happily, jobs appeared. This was before job openings had to be advertised, and instead Chairs just would go to their dean after some interesting candidate had been identified—the old-boy network. Of all the places I visited, only Simon Fraser University in Canada offered to pay a portion of the airfare for this big trip. The University of Washington did not. When I was back in New York with job in hand, Harry Grundfest accosted me, "No! You are not going to that intellectual desert!" He was so wrong.

Postdoctoral Work in Plymouth

Merrill finished her postdoc with Severo Ochoa at NYU (determining the last codon for termination and purifying the first three bacterial initiation factors), I submitted the last two manuscripts of my thesis publications to the Editor of the *Journal of General Physiology* in the next room, and we

flew to England. It was only my third airplane trip. Alan Hodgkin told me that my task would be to go to Plymouth to check out Ichiji Tasaki's reports that squid axons could make action potentials in bathing media containing elevated Ca^{2+} and no Na^{+}. They would be calcium action potentials. He would visit Plymouth only infrequently when he could get time away from his Cambridge duties, but he would be working on another project. Remembering that Tasaki's axons had been internally perfused with an unusual medium containing 5 mM CsF and pronase dissolved in glycerol and water, I asked him what I would need for internal perfusion. He said I would not be perfusing. I asked then what kind of voltage clamp I should use to analyze the phenomenon. He said I would not be voltage clamping (indeed, they had no voltage clamp amplifiers) and that Mr. Cook, his very versatile assistant, would make a pair of cathode followers to measure potentials. When Mr. Cook laid out the parts for the followers, a full rack, several panels, four pentode vacuum tubes, four 45-V, four 9-V, and two 6-V batteries, and a collection of resistors, potentiometers, and cables, I commented that I knew how to make followers using the new field effect transistors (FETs) that would be no larger than your thumb. Mr. Cook responded that this circuit had been designed by Professor Bernhard Frankenhaeuser of Stockholm and could not be improved upon. I had much to learn about being an American postdoc in Professor Hodgkin's lab.

Some of the faculty I eventually interacted with at Cambridge included Richard Adrian (skeletal muscle excitation-contraction coupling, later to be Lord Adrian, son of Edgar Lord Adrian), Ian M. Glynn (Na^{+}/K^{+} ATPase), and Denis A. Haydon (lipid bilayers and gramicidin single channels). In the Hodgkin lab room in Cambridge where I was given a place to sit temporarily, there was a Faraday cage holding the three-microelectrode muscle fiber voltage clamp that had been used for the experiments of Adrian, Chandler, and Hodgkin (1970). W. Knox Chandler was at the next desk. He had been finishing the muscle clamp work that summer, and earlier (1962–1965) he had done perfused squid axon work with Hans Meves as Hodgkin postdocs. He was now projecting voltage-clamp data from films onto graph paper to take them back to New Haven for analysis on Yale's 7090 (solid state now) computer. He came back each summer for this, as the films could not leave Cambridge. Mordecai P. Blaustein was in Hodgkin's lab preparing for his second postdoctoral Plymouth season to finish characterizing the Na^{+}-Ca^{2+} exchange process of the giant axon. In that banner year for Ca^{2+} transport, the Na^{+}-Ca^{2+} exchanger was being discovered in squid in Plymouth (Baker et al., 1969) and in cardiac muscle in Berne (Reuter and Seitz, 1968), and the plasma membrane Ca^{2+} ATPase was being discovered in red blood cells in Berne (Schatzmann and Vincenzi, 1969). Note that in those days authors were listed alphabetically on articles in the *Journal of Physiology (London)*; men usually used their initials, whereas women were required to use their full names! Hodgkin's lab then was rounded out by Shigehiro Nakajima

(visiting research fellow) and Peter Stanfield (research student), both working with electrical properties of skeletal muscle fibers.

In October, the squid-axon contingent moved to the Plymouth Marine Laboratory in Devon. There, when the weather permitted, commercial fisherman would bring a few squid for the scientists to the dock around 5 PM. They had already been decapitated and stored in Dewar flasks filled with sea-water ice slush, so they had to be dissected at once. The scientists worked until the morning. The squid were few, and it was evident when Hodgkin allocated them that the Hodgkin-Blaustein Na^+-Ca^{2+} exchange project had very much higher priority than mine. When I did get one, it was also made clear that I should not bother them during their pressing work to learn what to do next. Fortunately in a few days, Trevor Shaw came from London and he kindly showed me the dissection. I was to use a plastic vertical chamber (made from the design of Huxley) and the double-spiral wire axial electrode. The axon hung vertically from a glass cannula tied into one end, and the long intracellular electrode was threaded through the cannula and down the fiber axis without touching the membrane. Making this delicate electrode could take days, winding two stiff wires in a spiral along a very thin glass rod and tacking them down all along with tiny dabs of shellac under a microscope. Fortunately a clever jig with a lathe-like screw held the parts and advanced the rod appropriately during construction. Despite all efforts, the axons made no action potentials in high-calcium solutions. That seemed understandable to me since they were not perfused with Tasaki's exotic intracellular medium, which would eliminate all K^+ currents by Cs^+ block, chelate all Ca^{2+} by F^-, enormously shift the voltage dependence of the Na^+ channels and any Ca^{2+} channels by low ionic strength to allow excitability, and, we know now, remove inactivation from Ca^{2+}-permeable Na^+ channels by proteolysis.

What to do? The only way I knew to analyze the state of the membrane would be to voltage clamp. The next time Hodgkin visited Plymouth, I asked him if I could use a little of the $1000 he got with my fellowship to buy three operational amplifiers to make a clamp. They would cost £20 each (about 100 times what they cost today). He responded that it would be a bad investment: Someone had given the lab an operational amplifier some years before, and they "had never found a use for it." I was bailed out again by Trevor Shaw when he came a few days later. Trevor was a very dear person. The Plymouth Marine Lab, of which he was a trustee, might be able to buy three amplifiers if I could convince him that someone else there could use them after I was done. It became apparent that almost no one in these circles, even their electronics technicians in Cambridge, knew what an operational amplifier was. As there were no squid, I gave Trevor a full evening tutorial, and he arranged for the purchase. I set out to construct the circuit. The Plymouth Lab had no electronics shop, but after World War II they were given a collection of obsolete radar sets that could be used to scavenge parts.

(Hodgkin and Huxley had worked in radar design during the war, as had many other future electrophysiologists of that generation.) The resistors were as large as my little finger and used a different version of color coding from the convention I knew, but I could identify some. For the lack of a chassis, I hammered two rows of nails into a wooden board, a miniature Parthenon, and soldered the enormous components to their heads. There were no power supplies, so power was from batteries. It was grotesque, but it worked.

We had a period of gales when the fishing boats could not go out. Hodgkin had come and, looking for something to do while we waited, he asked to read the copy of my thesis that I had brought. It was 175 pages with a lot of discussion of channel ideas and speculations on the mechanisms of action of the many drugs I had tried. It also included some theoretical sections, including a calculation of ion fluxes and conductance through pores of assumed geometry as in the appendix of Hille (1968a). After reading, he flattered me by proposing that I should write a review article. I said I thought that I should gain more scientific maturity first, but later I learned that he must have passed the idea along to Denis Noble at Oxford. Another time Hodgkin described how exasperating it was to train American postdocs who take his good research ideas back to the States. Although I was feeling blamed, what he meant was that the United Kingdom had no postdoctoral support system. Had there been one, he could have trained 20 British postdocs during his career, and his good ideas would have taken root in the British Isles, enriching British science, a very valid argument for a change in national policy. During gales, I also wrote my first NIH R01 research grant to support my future lab in Seattle. I had no examples to work from, and there were no experienced Americans in Plymouth to give advice. England was going through a period of austerity, so when I asked Hodgkin about requesting an oscilloscope on my grant, he said he had thought about asking for one himself 5 years before, but then had thought better of it since perhaps someone else would be more deserving. In the end, I sent in a request for 3 years of support at a modest $18,000, $4000, and $4000, respectively. In those days the State of Washington paid 100% of our salaries and it was considered inappropriate to ask for salary on our grants.

Another American postdoc using squid giant axons that season was Lawrence B. Cohen. He worked for Richard Darwin Keynes (then Director of Babraham Animal Research Institute, just outside Cambridge). Much of Keynes's life research had pursued the hypothesis that beyond the electricity that Hodgkin and Huxley had analyzed so successfully, one should be able to learn about mechanisms of excitability from other physical signals such as isotope fluxes, thermal changes, and light. He had obtained very important isotope flux results, including showing the quantity of Na^+ and K^+ ions passing the membrane during the action potential (Keynes, 1951), measuring single filing of K^+ ions in K^+ channels (Hodgkin and Keynes,

1955), and discovering basic properties of the Na^+-pump (e.g. Caldwell et al., 1960). In Babraham, Larry Cohen had detected various optical signals in excited nerve bundles, so Richard had sent him to Plymouth to see whether the signals correlated with the action potential in squid giant axons. As Larry had done his thesis measuring the nucleotide-dependent viscosity of actin solutions, he was not yet at ease with electrophysiology or electronics. Hearing that I had made a voltage clamp and that my project was not going well, Richard Keynes persuaded Hodgkin (his thesis supervisor many years before) to let me join Larry's optical project. We managed to voltage clamp the axon under crossed polarizers and found that the fast membrane birefringence signals tracked the changes of membrane potential within microseconds and almost linearly (Cohen et al., 1968). An added bonus was that Richard was very skilled at dissecting axons and threading the electrode. Whenever he was in Plymouth, we could save our energies for the midnight experiments. Richard had a facility for falling asleep (naps) at any time and in any place, and Larry and I often connected signal wires and passed instruments across his large seated frame after Richard fell asleep late at night with his feet up on the counter, effectively bisecting our tiny hushed laboratory cubicle. Once when we were alone, Larry confided that he believed that the $7000 American postdoctoral stipend we were each receiving was more than the pay of our famous British mentors.

Postdoctoral Work in Babraham

Merrill was very pregnant with our first child, and some weeks before the squid season was over we had to return to Cambridge to get settled before the birth. As our move approached, I asked Hodgkin if I might try my hand at the three-electrode muscle clamp in his laboratory. He said no, that nobody could touch the unused setup until the papers were published (3 years later). He announced that there was no laboratory space for me in Cambridge and I had better do a theoretical problem. Richard Keynes soon stepped in and proposed that I could continue to work with Larry at Babraham, which I did.

Back in Cambridge, it seemed prudent to get a telephone connection for Merrill to contact me from our row house in case labor was approaching. However, the authorities said that there were no extra phone lines in our area (a 20-minute walk from the Physiological Laboratory). If we were both medical doctors, we could take the phone from the flat down the street where there was only one doctor. Otherwise we would have to use the phone box in the next block like everyone else. Merrill tried that phone box and, finding the tones unfamiliar, she asked a passing 20-year-old to tell her if this was a ring tone or a busy signal. The passer-by apologized that she had never used a telephone and did not know. Although milk was delivered every day to our doorstep, we felt it would be useful to have a refrigerator in the kitchen.

The landlady didn't think there could be a reason to have one but finally agreed that if we bought a small under-the-counter fridge, she would buy it from us when we left. Erik Darwin Hille arrived on a snowy night in early January. Two weeks later, Merrill started her postdoctoral work in chick bone development with Dame Honor B. Fell, who directed the Strangeways Laboratory.

While waiting for Larry to return from Plymouth I decided to read more about optics to be sure we understood what we were doing. Optics books were shelved in the Physics Library. First I had to write a letter to Professor Sir Neville Mott, the respected Chair of Physics (1977 Nobel laureate), to request his kind permission to enter. After a few days, a formal letter of entry arrived. I enjoyed reading physics again and came away with clearer criteria for our experiments.

In the next 8 months, Larry and I completed several studies on optical changes in the electric eel electric organ and in crab nerves. Electric eels were large and very costly. They had to be obtained through shady characters in the Amazon basin, who wanted prepayment and did not always deliver. In Grundfest's lab I had seen *Electrophorus* used for many days by cutting a piece off the tail and cauterizing repeatedly, but in England the animal use laws required quickly dispatching the 150 cm fish before use. The tissue survived for only a few more hours. After we had used the available animals, Richard declared an end to the experiments, but one day A.V. Hill (of the Hill equation 1910, Nobel Prize1922) visited from London to see what was going on. He heard us lament that we had to stop our work since Richard did not want to buy more electric eels. Solemnly he pronounced that one should never change experimental animals in the middle of a successful series of experiments, and Richard was forced to buy us more in deference to this great hero of physiology—his uncle.

We characterized small changes in birefringence and light scattering during the action potential. Eventually I was discouraged to find that the optical signals did not seem a direct way to learn about the conductance mechanisms or ion channels of excitable membranes. Larry, on the other hand, devoted the rest of his career to light. Later at Yale, he exploited dyes to increase absorbance and fluorescence signals by up to six orders of magnitude, and by now it is common to use optical monitors of membrane potential and calcium in living animals and to use light to stimulate or silence neurons in the nervous system.

Richard Keynes extracted the $1000 that the Helen Hay Whitney Foundation had given Alan Hodgkin for expenses and kindly allowed me to take a trip. I made a pilgrimage to the node of Ranvier laboratories. At the University of Saarland (Homburg), Robert Stämpfli was a grandfather of the frog node of Ranvier. The Huxley-Stämpfli papers (1948–1951) and earlier ones from Ichiji Tasaki (1940–1944) had established saltatory conduction in brilliant experiments with single myelinated nerve. Stämpfli was an

extraordinary dissector. Wolfgang Nonner, still a medical student, was developing compact transistorized amplifiers and a new method for voltage clamping single nodes (Nonner, 1969; to this day the ultimate). He had realized that the impedance properties of the preparation required frequency-gain characteristics of the amplifier that *increased* at high frequency, quite the opposite from the simple roll-off of contemporary operational amplifiers. In Kiel, Hans Meves had just become the Professor, Werner Ulbricht was measuring the on- and off-kinetics of TTX and TEA and the actions of veratridine, and I got to know Werner Vogel, who had just finished his Ph.D. In Stockholm, Bernhard Frankenhaeuser was low key and more interested in how to design a stimulator than in thinking about ion channels. The labs in Homburg and Kiel were warm and friendly places that I visited many times afterward.

A Beginning Faculty Member

In September 1968, we left our postdoctoral work after a year in England and flew to Seattle. The agreement with Harry Patton at the University of Washington was that my salary would be $14,000, a lab room would be found, I would teach like everyone else after a short grace period, and, for startup equipment, I could rummage in a room full of unused old Tektronix pulse generators and oscilloscopes. No special start-up funds were provided in those days. When I arrived, the lab room was not yet available (that happens to everyone), but I found in my new mailbox an envelope saying that my NIH grant was awarded. The NIH has been a steadfast source of support. That same grant continues now in its 42nd year. We bought one house, two cars, and a piano and were broke. Three months later our second son, Jon Trygve Hille, was born. Merrill soon started her third postdoctoral fellowship, now in echinoderm developmental biology. We were fully engaged. I was still 27.

The Department of Physiology and Biophysics was chaired by Patton, who had taken over from Theodore C. Ruch, the founding (1946) Chair. Ruch had become the first Director of the Regional Primate Center in the next wing. The department had special strength in cardiovascular physiology, neurophysiology, and biophysical thinking. The orientation was classical. About six labs experimented on anesthetized cats (central nervous system), four on anesthetized dogs (cardiovascular and respiratory), and four on awake monkeys in the Primate Center (central nervous system). Most of the faculty were contributors to the widely used textbook, Ruch and Patton, *Physiology and Biophysics* (later called *Textbook of Physiology*). In addition to teaching medical, dental, nursing, and pharmacy students, the department offered an intensive 2-year across-the-board physiology series for our graduate students. Ten new graduate students arriving each year and all the more senior ones were supported by five generous departmental

training grants from the NIH. All students were to be able to teach every aspect of physiology "without embarrassing us." There was a foreign language requirement for the Ph.D., which for some years I administered. By today's standards, this was a large, demanding, old-school comprehensive disciplinary degree program that was extraordinarily successful.

The department had and continues to have an exceptionally friendly and supportive attitude. When I came, many of the faculty had been students or postdocs in the department. Other than my Chair, three faculty members were of greatest importance to me then. Chuck Stevens was a kindred spirit, turning then productively from neurophysiology to full-fledged biophysics of isolated neurons and frog neuromuscular junction under voltage clamp. He was pioneering fluctuation analysis with power spectra, making the first full Hodgkin-Huxley description of a spiking cell body, and determining the voltage-dependent open time of the nicotinic acetylcholine receptors of the neuromuscular junction. His tiny lab was full of excellent students and postdocs thinking well. Walter Woodbury was the senior membrane biophysicist steeped in the lore of impedance measurements (Cole), cables (Hodgkin and Ruston), and squid action potentials (Hodgkin and Huxley). Walt had learned Eyring rate theory from his mentor Henry Eyring and how to make intracellular recordings with glass electrodes from the inventor of the technique, Gilbert Ling. (Walt pulled pipettes over a Bunsen burner by hand.) He had worked on radar during the War. He did an extraordinary amount of teaching and ran the biophysics training grant. Walt had assembled his own LINC lab computer (at the MIT Lincoln Laboratory, together with Albert M. Gordon) and liked making equations, designing electronic devices, and above all helping others to move forward. He was a kind and generous mentor, still an inspiration to me. Finally, Theodore Kehl was director of an online computer facility, one of the few in the country. His group supplied me with laboratory computing power for 16 years until the personal computer revolution made it possible for everyone and every setup to have their own. They wrote enabling micro code and developed higher level languages anticipating spread sheets for data analysis; they designed interfaces and eventually minicomputers. I learned a lot about making digital circuits and programming through years of close interaction. The online computer across the hall from me was used for dog and cat experiments throughout the week, but it was made completely available to me for a long day of experiments every Saturday.

My doctoral studies and degree were called Life Sciences, and my classes had been dominated by a wide range of "pure" sciences. Science was abstract and undertaken for intellectual reasons, like playing chess. Through its adaptation to the environment, every organism offered unique puzzles worthy of investigation, and yet biology could recognize universal principles that held it all together. If research offered any benefits to society, they would emerge in due course but could not necessarily be predicted.

The University of Washington gave me my first experience in the culture of American medical schools. I encountered a more "applied" motivation. In this ideal, science was a quest to cure diseases of people. All other organisms were simply "models," practical "systems" that could model a human problem. When I wrote the next chapter on active transport for the *Textbook of Physiology*, I described the Cambridge school's classical experiments on squid giant axons. Professor Ruch asked me to use mammalian experiments instead. Science was divided. On our side of the street, the medical school side, funding came from the NIH, and across the street, the natural sciences side, from the National Science Foundation (NSF). Increasingly over the years we were asked to write in our proposals about the health and disease motivation of our work and to strive for "translational" impact from bench to bedside. For American society it is clear that Congress funds the NIH with enthusiasm because of the promise of longer and healthier lives. As biomedical scientists we are the beneficiaries of that vision, which has been much more generous for us than for life scientists funded by the NSF. Indeed, today I can think better about the potential health relevance of our research and can pass these ideas along to others. Still, rarely in my scientific life did I make major choices about my research direction because of their apparent health relevance.

In contrast to my experiences at The Rockefeller University, the department did not yet embrace the importance of molecular biology, biochemistry, and cell biology for functional understanding, a deficit in thinking and staffing first addressed by our third Chair, Wayne E. Crill, in the 1980s, and now fully changed after Stanley C. Froehner became the fourth Chair in 2001. Over the years, like many departments, ours became increasingly interdisciplinary, and our graduate program developed an emphasis on getting students quickly into the lab and not requiring so many disciplinary courses. Also, the number of training grant slots has dropped dramatically, so our departmental graduate program became smaller while interdisciplinary programs of Molecular and Cellular Biology and Neuroscience and Behavior grew.

Over the years our department has been fortunate to keep a strong ion channel and biophysical faculty. Fairly soon Chuck Stevens and Walt Woodbury left, but they were replaced by Wolfhard Almers and Peter B. Detwiler; Jonathan (Joe) Howard came; eventually Wolf and Joe left, but William N. Zagotta, Fred Rieke, Sharona E. Gordon, L. Fernando Santana, and Charles L. Asbury came, keeping us young and vigorous. Quite early, Edwin G. Krebs of Pharmacology brought in William A. Catterall, who replaced him eventually as Chair and brought in Bruce L. Tempel and Todd Scheuer. It has been wonderful to have these excellent colleagues and good friends. Between them and the strong intellectual descendents of Ed Krebs and Edmond H. Fischer (1992 Nobel laureates for the discovery of protein phosphorylation), there have always been people to provide intellectual and

technical guidance as we ventured into new areas of signaling (Bill Catterall, Neil N. Nathanson, H. Stanley McKnight, Joseph A. Beavo, Daniel R. Storm). I was never tempted to go to another institution and never allowed any offers to be developed for more than a polite 10 minutes.

Ion Channel Biophysics: Starting with Permeation

In my empty laboratory room, everything was missing! Could I make a voltage clamp from operational amplifiers? Fortunately, operational amplifiers had not yet become integrated circuits so I could expose the inner printed-circuit board to remove the capacitative feedback elements that rolled off the frequency response and thus restore the bandwidth needed for a node clamp. This required no understanding of de novo transistor circuit design. Ted Kehl explained to me about the "new" digital large scale integrated (LSI) circuit chips with NOR gates, NAND gates, flip flops, and even decade counters. With these I designed my first digital circuit, a digital timer for the stimulator. Also light-emitting diodes (LEDs) were new in the toolbox, so I added a few to show when each pulse was active. Frankenhaeuser had shown me a stimulator he designed, and I could copy his analog output. A plate glass company had half-silvered glass I could use to make a mirror for photographing the oscilloscope with a Grass camera. And so it went in small steps, much aided by the skills and experience of our departmental electronics shop and machine shop.

I was lucky that Denis Noble as editor of the series *Progress in Biophysics and Molecular Biology* had asked me to write a review, undoubtedly at Hodgkin's suggestion. This gave me a fun task to interleave with the slow assembly of equipment. My essay "Ionic Channels in Nerve Membranes" (1970) addressed two questions: "(1) Do several permeant ions share a common pathway through the membrane or are the pathways for different ions different? (2) Is the ionic permeability a diffuse property of broad areas of membrane or is it located in discrete specializations?" I argued for discrete Na^+, K^+, and leak channels and attempted to calculate plausible limits on single-channel conductance. Not everyone agreed fully with the thesis: to his helpful stylistic comments on a draft of the chapter, Kenneth S. Cole added a note, "I am worried that you may be pushing some of your channel arguments pretty far." This was my first review and my first book chapter. I learned that I like to write reviews and that multiauthored book chapters appear in print only a distressingly long time after the original deadline.

For the first 15 years in Seattle, my work was unabashedly membrane biophysics. Every new lab seems to get started too slowly. My first research project was to determine the conductance of a single Na^+ channel by looking for a reported quantization of the subthreshold voltage responses of the node of Ranvier. It was a total disaster and made me despair of ever succeeding in research. For a year I could see apparent quantization in histograms

that in the end I found was what you could get with a random number table!

Then I moved to the mechanisms of ionic selectivity. Could one learn about the pore by probing it with different ions? Several papers had described the permeability to NH_4^+ both in Na^+ and K^+ channels, and Chandler and Meves (1965) had measured the alkali ion selectivity of the Na^+ channel in squid axons. Papers by Tasaki and others had shown that nerve fibers could conduct action potentials in Na^+-free solutions containing organic cations such as guanidinium. (He presented such work as refuting Hodgkin's "Na^+ theory" of action potentials.) I felt that before one could speculate about permeation mechanisms, one should identify every ion that went through and several similar ions that did not. Maybe then, as in studies of the active site of enzymes and drugs, one could postulate some steric and chemical properties of the pore. Within a few years, I found 11 cations were clearly permeant in Na^+ channels, and 4 in K^+ channels of the node of Ranvier. Strikingly, every cation with a methyl group was impermeant. Thus, in Na^+ channels "big" hydroxyguanidinium, $(NH_2)_2C^+NHOH$, was permeant, whereas "little" methylammonium ($CH_2NH_3^+$) was not! Using molecular models and reading about alkali metal crystal radii and the length of hydrogen bonds, I proposed in a series of papers that the Na^+ channel offers an oblong 3.1 x 5.1 Å slit to permeating cations lined by hydrogen-bond-accepting oxygen atoms acting as surrogate water molecules (e.g., Hille, 1971). I called this the "selectivity filter." Graduate student Donald T. Campbell and I developed a new voltage clamp method for frog skeletal muscle that used the same vaseline gap setup as the nodal clamp (Hille and Campbell, 1976). For dissection, it required only snatching a short segment of one muscle fiber from the muscle with watchmakers forceps. The ionic selectivity of the muscle Na^+ channel was identical to that of the node (Campbell, 1976), although we now know that the Na^+ channel is coded by a different gene. Similarly, Bezanilla and Armstrong (1972) and I (1973) proposed that the K^+ channel is a round 3.0–3.3 Å hole lined with oxygens. The K^+ channel prediction was elegantly confirmed by the first K^+ channel crystal structure from Roderick MacKinnon (Doyle et al., 1998), but for the Na^+ channel there are still no structures after almost 40 years. Now we know of many Na^+ and K^+ channel genes. All these channels show remarkable near identity in selectivity sequence, reflecting a strong conservation of the selectivity filter.

In selectivity studies, one can define the relative permeability of two cations, either from the reversal potential in a biionic experiment or from comparison of the magnitude of the currents the ions can carry. Hodgkin and Huxley's (1952) independence principle states that these two measures would be equivalent, but in my measurements they were not. Apparently ions did not move independently. I wrote, "[Some] organic cations may leave the pore more slowly than sodium ions do. Slow leaving could explain the reduction of currents below the size expected from the independence

principle . . . for the cation might 'tie up' the channel so long that other ions are prevented from entering" (Hille, 1971). We began to recognize a subtle gradation from permeant ion to blocking ion. Extending this idea, I found that even Na^+ ions could saturate the pore at high enough (nonphysiological) concentrations (Hille, 1975).

Ann M. Woodhull, my first graduate student, revisited my earlier observation that extracellular protons could block the Na^+ channel pore and discovered that the block was relieved by depolarizing the membrane potential further. This was well fitted by an Eyring rate theory model in which extracellular protons were hopping into a binding site, the acid group, within the pore with rate constants that depended on the membrane potential (Woodhull, 1973). While protonated, the pore was not Na^+ permeable. The model became very widely used, cited 1330 times. The idea was that, since the acid group was part way through the pore, the protons would experience a small fraction of the membrane potential drop in getting to it. Ann's results put the acid group 25% across the membrane field from the outside. It was natural therefore to go on to describe saturation, block, and the deviations from independence for permeant ions by similar hopping models. An Eyring rate theory model with three Na^+ "sites" and four barriers in a row in the permeation pathway, gave a reasonable fit to the deviations from independence (saturation) for Na^+ channels when only one ion was allowed in the pore at a time (Hille, 1975). Although a general rate-theory description of diffusion had been presented years before by Eyring and his colleagues (including Woodbury), the new features of our models were judiciously placed barriers and wells, combined with saturability of permeation sites. Other early biophysical work in my lab was done by graduate students Rosalia Ridaura, Shing-Yan (Bill) Chiu, Michael D. Cahalan, Kenneth R. Courtney, and Bruce C. Spalding and by postdoc Ted B. Begenisich.

At this time our family took a sabbatical year in Germany. I joined Robert Stämpfli's lab at the University of Saarland. Our two boys went to a German elementary school and Merrill used the library to prepare grant proposals for her new faculty position in Zoology at the University of Washington. In a fun collaboration with Robert Stämpfli, Wolfgang Nonner, Berthold Neumcke, and Franco Conti from Italy, we measured the single-channel conductance of the Na^+ channels of the node of Ranvier by stationary fluctuation analysis (Conti et al., 1996). We got 7–9 pS. The method required measuring the noisy small stationary currents remaining after a few hundred milliseconds of depolarization, calculating their power spectral density, subtracting a background noise spectrum, and fitting the remainder to the sum of Lorentzian power spectra predicted from a Hodgkin-Huxley-like description of the macroscopic currents. It was an interesting exercise. Fortunately the others were in full mastery of the analytical and theoretical sides of the study, and Robert Stämpfli dissected all the nodes. Five years later Franco and Wolfgang and I could get together in Seattle

where we measured nonstationary fluctuations from K^+ channels and, by extensive autocorrelation analysis, Wolfgang was able to extract kinetic details of several K channels in the node (Conti et al., 1984).

Hodgkin and Keynes (1955) had done isotopic K^+ flux experiments in *Sepia* axons, which they interpreted in terms of a K^+ channel pore that contained several K^+ ions in a row, moving in single file. Wolfgang Schwarz and I modeled this more complicated "long-pore" effect with a multi-ion hopping model, again using a saturable rate theory (Hille and Schwarz, 1978). It reproduced the results of Hodgkin and Keynes (no surprise) and went on to show that such a multi-ion pore could be blocked with exceptionally steep voltage dependence by an internal ion that cannot cross the outermost barrier. This gave further credence to the earlier brilliant hypothesis of Clay Armstrong (1969) that inward rectifier K^+ channels are pores that are blocked by an impermeant TEA-like particle coming from the cytoplasm when trying to pass outward K^+ current. Only in the late 1980s were cytoplasmic Mg^{2+} and organic polyamine compounds shown to be the endogenous voltage-dependent blockers underlying inward rectification. As a sign that the channel hypothesis was still not universally accepted in 1978, our theoretical paper had difficulty in the review process. Before it was finished, there were five reviews, three of which were negative for numerous reasons: Theoretical work "did not belong in the *Journal of General Physiology*"; it "does not unify a number of disparate findings into a single theoretical framework . . . rather it attempts to support a molecular notion of a potassium channel"; the arguments were "salesmanship" and "uncritical"; evidence that the delayed rectifier is a multi-ion pore "is thin, almost to the vanishing point"; the inward rectifier is probably a carrier and "the argument that inward rectifier channels are pores because delayed rectifier channels are likely to be pores has no force and should be deleted." Fortunately, the editor, Paul F. Cranefield, accepted the paper with relatively small modifications. This paper has been cited 660 times. A few years later, graduate students Andrew (Andy) L. Blatz studied proton block of inward rectifiers and Paul J. Pfaffinger studied regulation.

We moved to permeation in ligand-gated ion channels. Postdocs David J. Adams and Terry M. Dwyer heroically measured reversal potentials for 75 (!) cations at the nicotinic acetylcholine receptor channel at the frog neuromuscular junction. They found that 40 cations have a permeability relative to Na^+ that is larger than 0.1 (Dwyer et al., 1980; Adams et al., 1980). The pore was large, 6.5 x 6.5 Å. With such a large pore, the small alkali metal cations would rarely touch the walls in passage and the channel accepts them all with little discrimination. Postdocs Jorge Sanchez, John A. Dani, and Detlef Siemen continued with competition among slow permeant ions in the nicotinic acetylcholine receptor. With a smaller subset of ions, graduate student Jian Yang found that the selectivity of the 5-HT_3 (serotonin-gated) receptor channel closely matches that for nicotinic receptors

(Yang, 1990). Today we know that nAChRs and 5-HT_3 receptors have very close sequence similarity, and that more generally, the postsynaptic excitatory and inhibitory neurotransmitter-gated channels all seem to have relatively wide, poorly selective pores. We still lack high-resolution crystal structures of open postsynaptic channels, so the pore sizes estimated from permeation have not been tested directly. In our last channel-characterizing studies, Paul S. Taylor determined the ionic selectivity of snail A-current channels, Karl Woll studied a high-conductance Cl^- channel, Steven Barnes described Ca^{2+}-activated Cl^- channels and hyperpolarization-activated I_h channels in salamander rod photoreceptors (Barnes and Hille, 1989), and Lonnie P. Wollmuth characterized the ionic selectivity and block of those I_h channels (Wollmuth and Hille, 1992; Wollmuth, 1994)—now called HCN channels.

Ion Channel Biophysics: Pharmacology

In my Ph.D. thesis, I considered drugs worth studying because they would inform about the channels I wanted to understand. There were already spectacular results from Clay Armstrong. We continued this approach in Seattle. Some examples are given here.

Early on, when it was discovered that myelinated axons could be loaded with TEA in the cytoplasm by cutting the ends of the fibers in the desired internal solution (Koppenhöfer and Vogel, 1969), Clay Armstrong called to suggest that we should use his TEA analogs to show that the TEA receptor I had studied (Hille, 1967) was inside the axon rather than outside as I had thought. We had a couple of very enjoyable weeks together in Seattle, and I continued a few more experiments afterward. The upshot was that at the inner mouth of the nodal K^+ channel there was indeed a TEA receptor guarded by the activation gate that duplicated all the properties seen in the squid (Armstrong and Hille, 1972). Thus, for this channel too the activation gate is on the cytoplasmic end. However, on the outside there was another TEA receptor not seen in squid that did not depend on gating. This is the one I had studied before. We now know that K^+ channels differ widely in their TEA affinity at the outer TEA site, depending on whether there is a ring of four tyrosines (highest affinity) or other residues in the mouth.

Many people in the lab studied local anesthetic action on Na^+ channels (Gary R. Strichartz, Ken Courtney, Wolfgang Schwarz, Phillip T. Palade, and me). Again we were greatly helped by Bertil Takman who suggested key lidocaine analogues. Gary, my first postdoc, discovered that membrane-impermeant quaternary derivatives such as QX-314 applied inside the axon via the cut end blocked Na^+ channels best with large depolarizations (Strichartz, 1973). The phenomena suggested that Na^+ channels have an activation gate at the cytoplasmic mouth that reveals a large inner vestibule with a local anesthetic binding site, just as Clay had deduced before for TEA

in K^+ channels. Evidently, Na^+ and K^+ channels shared significant structural similarities. With ionizable amine local anesthetics, we found the phenomenon of use-dependent block, a growing accumulation of blocked channels during repetitive depolarizations. It arises when the local anesthetic is driven into its receptor within the pore by depolarization and is slow to escape afterward. Using pH changes and analogs of different hydrophobicity, I concluded that local anesthetics approach their binding site in the channel vestibule along two paths, the hydrophilic path found by Gary for charged species when the gates are open and a hydrophobic path sneaking perhaps from the lipid right through the substance of the channel into the vestibule even when the gates are closed (Hille, 1977a). By exploring various voltage protocols, I also found another dependence on gating. The local anesthetics had higher affinity for inactivated channels than for noninactivated ones and thus made inactivation more likely (Hille, 1977b). These ideas were dubbed the modulated receptor hypothesis and have been cited ~1400 times. Interesting medical implications became apparent to me only after the experiments were over: the use-dependent block meant that local anesthetics would block high-frequency firing (as during a noxious stimulus) much better than low-frequency firing; and the modulated receptor meant that related molecules used as cardiac antiarrhythmics would beneficially prolong the refractory period of cardiac muscle, eliminating premature firing of action potentials. The latter idea was made clear in a closely similar model of antiarrhythmics given in a review by Hondeghem and Katzung (1977). These medical implications were said to be instrumental in my election 25 years later to the Institute of Medicine.

Michael Cahalan (1975) studied action of a *Centruroides* scorpion venom on Na^+ channels. Depolarization promoted a reversible modification of activation gating that shifted the voltage dependence of activation in the hyperpolarizing direction by an enormous 40–50 mV without changing inactivation gating. Thus, activation and inactivation gating can be severely decoupled. Again the drug–receptor interaction depended on the gating state of the channel—foot in the door. In the end, as with many other scorpion venoms, the axon fires excessively, which can produce pain, exhaustion, and arrhythmias. Now we understand that scorpion toxins bind directly to the extracellular side of one of the Na^+ channel voltage sensors.

Ann Woodhull, Bert I. Shapiro, and I (Hille et al., 1975) revisited shifts of the voltage dependence of activation in Na^+ channels due to divalent ions (Hille, 1968a). We measured shifts under a wide range of extracellular divalent ions, pH, and ionic strength and argued that all changes could be understood as the electrostatic effects of solution cations interacting with the local negative surface potential of the membrane (an idea that traces back to A. F. Huxley) and binding to surface acidic groups. We successfully modeled all the shifts with a full Gouy-Chapman-Stern surface-potential theory from

colloid chemistry, including surface charges, ion atmospheres, and binding. Our work was inspired by a clever compact paper with the same variables studying activation of K^+ channels in the node from our Leningrad colleagues Galina Mozhayeva and Alexander Naumov (Mozhayeva and Naumov, 1970). Clay Armstrong never accepted biasing voltage sensors by surface potentials. Instead he favored models with discrete binding complexes on the ion channel protein and no electrostatic component. I imagine that both mechanisms apply. They have conceptual overlap. Today one would model these effects as occurring on individual voltage sensors.

As a last exercise in gating pharmacology, we returned to the Na^+ channel openers, veratridine, pyrethroids, DDT, and batrachotoxin. Graduate student Jeffry B. Sutro, postdocs Steve Barnes and Mark D. Leibowitz, and visiting faculty Jürgen R. Schwarz contributed to a story that these "toxins" all modify Na^+ channels in a reversible use-dependent manner. A foot-in-the-open-door step severely shifts the voltage dependence of activation gating and shuts down inactivation such that the channels remain open (but with lowered conductance) vastly longer than normal after a brief opening depolarization (summarized in Hille et al., 1987). Thus, insects die of DDT and pyrethroid poisoning because of a continuous massive discharge of their nervous system. They may lie on their backs thrashing their limbs to exhaustion.

On Writing

My channel biophysics period culminated in writing a book on ion channels. Writing numerous review articles and several book chapters had confirmed the painful delays experienced when waiting for other authors. I had taught medical students the basics of nerve and synapse. I had taught graduate students, both in basic biophysics and in an advanced course that changed topics every year and had included whole quarters on, for example, gating currents, lipid bilayers, pore theory, or calcium signaling. I had a grant, was a professor, and was serving 4 years on an NIH Study Section. Inspired also by my father, who had written 13 mathematics texts in his lifetime, I decided I was ready to write. My training in biology had made me uneasy with the direction that channel biophysics was taking. Did it matter for biology whether a Na^+ channel has 8 gating states or 31? Why was channel biophysics out of the mainstream and usually written in an electrical and mathematical language inaccessible to biologists? When biochemically minded people had tried to approach ion channels, they often were not aware of the wonderful things that were already known and the precise vocabulary that we had developed. Couldn't one make this field I loved more understandable and summarize the extensive body of knowledge in simpler language, so more people would be excited and interested to bring the techniques and

concepts of their discipline to bear on the ion channels? Would this allow a new era of advances in ion channel genetics, evolution, development, cell biology, protein chemistry, structure, neurobiology, and disease?

Writing the original *Ionic Channels of Excitable Membranes* book (1984) took 3 hard years. Each chapter would introduce only a few fundamental concepts, getting progressively deeper. I wanted to base the story on the original observations and the original data records. The information would not be comprehensive but rather clear and correct, yet it would form a baseline from which professional training could continue. Electrical words, including conductance, and equations would be minimized. There would be no integrals or Laplace and Fourier transforms, and even algebra would be minimal. There would be no technical information on how to do electrical experiments, series resistance, or electrodes. The examples would be those I thought could survive indefinitely. I had realized that some fields were looked down upon because the investigators could not agree, so I eschewed controversy. I wanted readers from all countries, so I stuck to a basic Anglo-Saxon English. My goal was to write a paragraph a day. A chapter would have to be divided if it took more than an hour to read out loud. I wanted readers to feel the satisfaction of reading a whole chapter in a day without giving up—and to find it interesting. Many colleagues made good suggestions on drafts, and even my mother ruthlessly removed superfluous words.

The next two editions of my book (1992 and 2001) again took 3 full years to write. Each time the literature had grown more than three-fold. There were 7000 papers on ion channels by 1984, 30,000 by 1992, and 100,000 by 2001. The first time, personal computers were in their infancy and the first word processors were appearing only at the end, so my dedicated, precise, enthusiastic, and invaluable secretary, Lea M. Miller, typed the whole thing by hand numerous times. The next two times, Lea could use a word processor. Only for the last edition did we have the great advantage of easy e-mail and Pubmed and could the publisher Andy Sinauer set type automatically from the digital files. I am very grateful to Andy for full trust on the direction and content of what I wrote, although in retrospect I wish someone had noticed that the last edition became inordinately long. It no longer allowed a quick, cover-to-cover read. The last edition had a revised title, *Ion Channels of Excitable Membranes*, at Christopher Miller's urging. He even organized a vote at a Gordon Research Conference about "ion" versus "ionic." The vote was overwhelmingly in favor of ion! Still needed today would be a short book for introductory teaching that is much more basic than my first edition.

Writing the book led to a new status. I became recognized by many as the author of their text rather than as a practicing scientist. It also led to more invitations and changes in my alliances. I was elected to the National Academy of Sciences. After I was asked to give a Presidential Lecture (1984) and a Grass lecture (1989) to the Society for Neurosciences, I finally moved from thinking of myself solely as a membrane biophysicist to adding the

label neuroscientist. Only then did I become a member of the SfN and begin to go to their meetings as well as those of the Biophysical Society.

As an aside on writing, I mention authorship. In an older tradition that was common in physiology and in British laboratories, Ph.D. students worked by themselves and published by themselves. It was true of my own thesis, and I have tried to make this possible for most of my own students as well. They acknowledged my help and my grants, and that was enough. However, as research has become increasingly interdisciplinary, we have often had to add names from other laboratories for tools and data provided. In those cases, I have added my own name as well. For 15 years, my own papers were also largely sole-authored as they represented work done by me on Saturdays in an otherwise empty laboratory. However, eventually I stopped direct research, and very fine postdocs became an extension of my personal research. Then my name would be at the end. I have continued programming, data analysis, and model building but not actual work with the cells. When someone in my lab has contributed to a paper from a lab of an untenured faculty member, I try to keep my name off the paper, as it confuses the subsequent review for tenure to see the name of a more senior principal investigator and to not to be sure whether the junior or the established author should take credit for the writing, insights, and discoveries.

People often lump scientists and engineers into a language-deprived category. High verbal scores qualify people for the humanities and the arts. Wrong. Writing grants, articles, e-mails, reports, and evaluations of grants, manuscripts, and people as well as reading or editing what other people write account for the majority of my day. I would say that writing is power. It is a skill that can be sharpened by study and practice and becomes a lot of fun. Read books of style. Read good writing and think about what makes it good rather than just the content. One needs to parse sentences, paragraphs, and essay structures in the same way that we critique science. If you don't want to do it alone, there are courses and editors for people at any level of accomplishment. The sooner you start, the better you get and the more you enjoy it. Some people have writer's block and would rather do an experiment than write it up. They should remember that all of our support comes from taxpayers, foundations, and companies who don't care about doing experiments for fun; they want only the published, accessible, peer-reviewed results, often for the benefit of society. The contract is not met without the final vetted writing. In our lab, writing the paper becomes a significant learning experience in research.

G Protein–Coupled Regulation of Ion Channels

While the book was being written, the biophysics scene changed dramatically. Three major breakthroughs had occurred in 1981 and 1982: the patch clamp invented by the Neher-Sakmann lab (Hamill et al., 1981), the IBM

personal computer, and the first ion channel cloning (Noda et al., 1982). The patch clamp meant that reliable voltage-clamp records could be obtained in any cell type. No longer did one need special giant cells. Even the central nervous system and somatic tissues became accessible. I built four patch clamps early on, but soon they could be bought off the shelf from several manufacturers. Within 15 years, over 10,000 patch clamps had been sold, all capable of recording the conformational changes of single ion channel proteins in the submillisecond time frame. All users could forget about how electronic circuits are designed. The IBM PC meant that every investigator and setup could have a capable computer—every day of the week. Our lab, particularly postdocs Mark D. Leibowitz and Donald W. McBride, wrote good online data acquisition systems for the PC combining C and BASIC languages, which we used for 15 years at each clamp setup. However, again before the decade was over, everyone was buying excellent commercial acquisition and analysis software bundled with their patch clamp. Quantitative electrophysiology became accessible to the masses both in academia and industry. With the later advent of the Microsoft Windows operating system, the style of real-time programming we had been using in Microsoft DOS became obsolete. Some channel biophysicists stopped programming for themselves. Cloning of channel subunits meant that the chemical information we had inferred so indirectly began coming out in quantity, and, later, that many people turned from adult differentiated nerve and muscle to expression systems using transfection in cell lines to study their favorite ion channel in isolation. Eventually patch clamp could be done in high-throughput, 96-well plates.

I was eager to start in new directions of research that might be more biological. We stumbled into a new area quite by accident. In the older vein, I had suggested to graduate student Paul Pfaffinger that he consider a thesis on the properties and pharmacology of inward rectifier K^+ channels. It was characteristic that I selected problems in part because nobody else was doing them at the time. I haven't liked to work in competitive areas. Since this was 1984, still before the cloning of K^+ channels, it was necessary to find a suitable excitable cell, so I sent Paul to visit Neil N. Nathanson in Pharmacology, who was doing pharmacological work on chick atria. We learned that Neil's lab was using isotopes of K^+ to test whether muscarinic agonists increased K^+ permeability via a receptor coupled to a G protein. I knew little about G protein–coupled receptors (GPCRs), which at the time were just emerging as a concept, but we were sure that patch clamp would be a clearer way to study the K^+ permeability change. At that time, the physiologists who had studied the acetylcholine-activated inward rectifier of heart were suggesting that the muscarinic receptor was mechanistically like the nicotinic receptor, a channel, but taking 100–200 ms instead of <1 ms to open in response to acetylcholine. Indeed, according to nice experiments by Soejima and Noma (1984), no intracellular diffusible second messenger was involved.

Neil tutored us in the basics, and soon in a wonderful collaboration, Paul showed that GTP was needed and a pertussis-toxin-sensitive G protein (G_i) coupled the muscarinic receptor to the channel (Pfaffinger et al., 1985; also Breitweiser and Szabo, 1985), challenging the prevailing view of cardiac electrophysiologists. A couple of years later Diomedes Logothetis, in David Clapham's lab, showed that the G protein βγ subunits were the membrane-delimited direct signal between receptor and channel in this signaling without a cytoplasmic second messenger (Logothetis et al., 1987). This was one of the first clear examples of signaling by Gβγ subunits, so novel that it met with resistance. The ion channel was soon dubbed GIRK (G protein–coupled inward rectifier K^+ channel, now the Kir3 channel family).

Paul Pfaffinger's discoveries were made so fast that he still needed a thesis project. Perhaps he could generalize our conclusion to another K^+ channel controlled by a muscarinic receptor. He turned to the suppression of the noninactivating M-current in sympathetic neurons by muscarinic and other agonists (Brown and Adams, 1980). As he found, however, this was hardly parallel to the cardiac example. The channel was turned off rather than on by muscarinic agonists. The inhibition and recovery were much slower. He showed that a G protein and GTP were clearly involved, but it was not pertussis toxin sensitive. Around that time, muscarinic receptors were cloned and it became evident that they are all GPCRs. The M_1, M_3, and M_5 receptors couple through the G protein later called G_q, whereas the M_2 and M_4 receptors couple through pertussis toxin-sensitive G_i/G_o. The GIRK channel was activated by cardiac M_2 receptors, and the M-current on the other hand was suppressed by neuronal M_1 receptors. It took us another 15 years to elucidate the mechanism of M-current suppression.

We had become hooked on a new direction of research involving GPCRs and ion channel modulation. We left the frog node of Ranvier for good and soon switched to rodent neurons with an eye to the greater medical "relevance" of mammals. Whole-cell patch clamp became our technique of preference. Out of a lingering affection for frogs, I still give each departing lab member a frog statuette from places like Mexico, China, or Africa, where they are properly revered.

It was a little unnerving to go from a field that is as precise and well studied as channel biophysics to a new much more biochemical field that was just emerging and had no precise answers, but it has been very satisfying and productive. By good fortune, a generous mid-career grant from the McKnight Foundation allowed us to retool our lab toward the new patch clamp methods and to get plausible experience for federal support. We could write that disorders of monoamine GPCR agonists are considered major contributors to Parkinson disease, schizophrenia, and depression, that many drugs of psychiatry and drugs of abuse act on GPCR signaling, and that GPCRs control our mental state—although we still worked with only a single cell at a time. Our group became larger. Because of the interdisciplinary

nature of the new work, our papers tended to have more authors. People who worked in the lab on these problems included Paul Pfaffinger, Mark D. Leibowitz, Martha M. Bosma, Ken Mackie, Laurent Bernheim, Alistair Mathie, David J. Beech, Mark S. Shapiro, Felix Viana, Jiuying Zhou, Lonnie P. Wollmuth, Duk-Suh Koh, David E. Garcia, Jeffry B. Isaacson, John P. Roche, Humberto Cruzblanca, Byung-Chang Suh, Lisa F. Horowitz, Wiebke Hirdes, Keith W. Dilly, Jane E. Lauckner, Jill B. Jensen, Björn H. Falkenburger, Eamonn J. Dickson, and Martin Kruse.

While we were leaving channel biophysics for receptor signaling, Merrill was also moving from the classical sea urchin to the newer zebrafish. We admire scientists who can pursue a single problem to exquisite depth in a long career, yet sometimes it is good to change. The old problem really may be finished, or it may seem no longer as scientifically interesting as some new problems—or perhaps the problem would advance better with fresh perspectives of other minds. It is also personally refreshing and exciting to think about new challenges.

Cell Biology of G Protein Signaling

When we switched to mammalian neurons, we began to follow up on published observations that voltage-gated Ca^{2+} channel currents could be reduced by a variety of GPCR agonists (Dunlap and Fischbach, 1978; Marchetti et al., 1986). We and others found a dozen receptors that could decrease the N-type Ca^{2+} channel currents of all large superior cervical ganglion cells (Hille, 1994). Their action took <1 s to develop and was almost as fast to reverse. Many were pertussis-toxin sensitive and insensitive to intracellular Ca^{2+} chelators. One lesson from this was that each individual neuron is likely to have a large repertoire of different GPCRs coupled to some common effectors, as Roger Nicoll had argued (Nicoll et al., 1990). In the end, the rapid inhibition was another direct $G\beta\gamma$ action, this time on Ca^{2+} channels, with no cytoplasmic intermediates (Herlitze et al., 1996; also Ikeda, 1996).

In a parallel line of study, Ken Mackie came to our lab during his fellowship training for Anesthesiology. We soon heard about the identification of a new GPCR common in the brain for cannabinoids, later called the CB_1 receptor. It inhibited adenylyl cyclase, so we guessed it should be able to inhibit Ca^{2+} channels and activate GIRK channels like other G_i/G_o-coupled receptors. Furthermore, like the stunning success story of one decade before with opiate receptors, maybe endogenous agonists would be discovered and a new field would blossom. As anadamide was announced, Ken confirmed the predictions for Ca^{2+} and GIRK channels, giving the first electrophysiological mechanism for endogenous cannabinoid actions (Mackie and Hille, 1992; Mackie et al., 1993; together cited >800 times). The field took off, and Ken with it. Ken remained as faculty in Anesthesiology for many years, so

we could share and publish many fun studies together. His lab adopted molecular biological approaches quickly and gave us a lot of help in working with the constructs we wanted to express.

Continuing with the sympathetic ganglion neurons, we and others also recognized four receptors that decreased currents in three ion channels: N- and L-type Ca^{2+} channels and M-type K^+ channels (Hille, 1994). These actions took 10 s and were slow to recover. They were blocked by Ca^{2+} chelators inside the cell but were not pertussis toxin sensitive. This slow pathway, as we called it, had to wait another decade to be understood.

A key technical breakthrough came when the genes underlying M-current were finally identified as KCNQ2 and KCNQ3. Mark Shapiro in our lab quickly set them up in a mammalian cell expression system with coexpressed M_1 muscarinic receptors (Shapiro et al., 2000). At last we could study M-current modulation in cells that were readily transfected, and we could dispense with using animals. We returned to elucidating the signaling of the slow pathway from M_1 receptors to KCNQ K^+ channels. For a couple of decades, several labs had asked in vain, what is the inhibitor of the KCNQ channels in this pathway. Fortunately, postdoc Byung-Chang Suh realized that this might be the wrong question. He proposed instead that the channels might turn off because of the destruction of a channel activator. Stimulation of M_1 receptors is communicated to G_q, which in turn activates the enzyme phospholipase C (PLC). PLC then cleaves the rare plasma membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2). Suh's hypothesis was that the channels need PIP_2 as a cofactor to function and the depletion of this lipid during receptor activation turns them off temporarily until PIP_2 is resynthesized. This notion seems to be correct: channels turn off when PIP_2 is depleted, and they require PIP_2 resynthesis to recover (Suh and Hille, 2002; Suh et al., 2006). These discoveries were made possible because we could use an expression system, but the reviewers asked that they be verified in "real" neurons.

In my early career it was hard work to keep an audience focused on ion channels as proteins and to explain that the lipid bilayer is just a passive supporting dielectric insulator that allows channel proteins to do their jobs. Now I began to read and lecture about the importance of phosphoinositide phospholipids! In reviewing the literature, we realized that many plasma membrane proteins are sensitive to the level of PIP_2 (Suh and Hille, 2005). Donald W. Hilgemann had pioneered with a report that one cardiac ion channel and one ion transporter required PIP_2 to function (Hilgemann and Ball, 1996). We showed that regulation by receptors in neurons could be explained this way. As biophysicists, we developed quantitative kinetic descriptions with informative models that included the known biochemical steps of G protein signaling and phosphoinositide metabolism (Suh et al., 2004; Jensen et al., 2009; Falkenburger et al., 2010a, b). We became lipocentric. We added confocal microscopy and a wide range of fluorescent probes,

including pairs of probes for measuring molecular proximity by fluorescence resonance energy transfer (FRET). This has been an unexpected and satisfying shift of direction. The cell biology of phosphoinositide signals is a fascinating subject in its own right.

Biophysical Excursions into Other Fields

Biophysical methods, especially those resulting from the patch-clamp revolution, can contribute to many outstanding problems in cell biology. Our lab has had a significant number of publications in several overlapping nonneuronal fields: *(1)* anterior pituitary endocrinology, *(2)* sperm activation, *(3)* calcium signaling, and *(4)* regulation of exocytosis. Some would say this shows lack of focus, but the problems are all approachable by similar methods and the questions are ones that in my dilettante undergraduate and graduate biology training were of great interest to me.

Our endocrine signaling studies included postdocs, Martha M. Bosma, Amy M. Tse, Frederick W. Tse, Akiko Iwata, James B. Herrington, Alexander P. Naumov, Solveig Hehl, and Andre Golard, and graduate student Julia Billiard. Most of this work was related to reproductive biology and done during a long and very satisfying association with other investigators in an NICHD-funded population center concerned with male contraception. This goal was one that I could readily identify with, given the enormous overpopulation of our planet. In good collaborations with Wolf Almers downstairs, we learned ratiometric measurements of calcium dyes and measurements of exocytosis by membrane capacitance increases, bringing me back to my postdoctoral days of photometry with photomultipliers. One experimental approach to contraception is to stop release of the gonadotropins LH and FSH from the anterior pituitary. We decided to analyze the GPCR signaling underlying gonadotropin release. Amy Tse set up identification of pituitary gonadotropes from dissociated rodent pituitaries. We traced the intracellular events during stimulation by gonadotropin-releasing hormone (GnRH). In brief, the GnRH receptor acts through G_q to stimulate phospholipase C (PLC), generating inositol trisphosphate (IP_3) and diacylglycerol. The IP_3 releases Ca^{2+} from intracellular stores in an oscillatory manner, with plasma membrane Ca^{2+} channels making almost no contribution to the Ca^{2+} signal (Tse and Hille, 1992). The Ca^{2+} stores are only partly emptied and then refill in each cycle (Tse et al., 1994). At the high point of each cycle of a Ca^{2+} oscillation, a hundred secretory granules containing the hormones LH and FSH are secreted by exocytosis (Tse et al., 1993). Thus, the secretion from an individual cell occurs in repeated little bursts, 15–20 s apart. The secretion is further potentiated by protein kinase C activated by the diacylglycerol (Zhu et al., 2002). During each Ca^{2+} elevation, the cell hyperpolarizes (via K_{Ca} channels) and it secretes. With the exception that the exocytosis is Ca^{2+} regulated, this pathway is in almost all respects quite

unlike that which regulates secretion in neurons. This made me more interested in the cell biological differences between neurons and other secretory cells. To my surprise when we tested how growth hormone releasing hormone (GHRH) releases growth hormone from somatotropes, another class of anterior pituitary endocrine cells, there was little in common with the gonadotrope story. It was a little more like that in a neuron. The mechanism involved synthesis of cAMP, phosphorylation of some target channel, depolarization of the cell, Ca^{2+} entry via voltage-gated Ca^{2+} channels on the plasma membrane, and Ca^{2+} regulated exocytosis (Naumov et al., 1994). In the same cells, the famous pertussis-toxin-sensitive inhibitory actions of somatostatin could be explained by hyperpolarization due to opening of GIRK channels and simultaneous inhibition of adenylyl cyclase due to G_i.GTP.

Gradually we realized that regulation at the pituitary would at best take months to stop and to restart male fertility, so we then turned more directly to the physiology of spermatozoa. The team spearheaded by Donner F. Babcock included Gunther Wennemuth, Andrew J. Harper, Anne E. Carlson, Sonya M. Schuh, and Lindsey A. Burnett with significant collaborations with other labs providing knockout mice. Many experiments were based on a new technique that Donner established to get time-lapse movies of the flagellar beat and to analyze the flagellar waveform quantitatively. They studied factors in the female tract that can activate the flagellar beating of sperm, which include bicarbonate, adenosine, and catecholamines. They found that a soluble (not membrane) sperm adenylyl cyclase (SACY) and protein kinase A were essential both in this activation of beating and in enhancing Ca^{2+} entry in response to alkaline depolarizing solutions. Although sperm seemed to have immunoreactivity for numerous kinds of standard voltage-gated Ca^{2+} channels, the Ca^{2+} entry evoked by depolarization required instead a unique sperm Ca^{2+} channel, CatSper, discovered in David E. Clapham's lab. The slow transition of sperm to hyperactivated swimming some time after ejaculation also required the CatSper channel. Perhaps such findings can contribute to future approaches to family planning and fertility.

Finally, our calcium signaling and exocytosis work has been done by Dok-Suh Koh (and his several Korean students), Donner Babcock, Jim Herrington, YoungBae Park, Edward J. Kaftan, Ronald F. Abercrombie, Gunther Wennemuth, Tao Xu, Liangyi Chen, and Joseph G. Duman, with good collaboration from Toan T. Nguyen in pancreatic duct epithelium. One line of work concerned Ca^{2+} clearance from the cytoplasm after a modest load. Using chromaffin cells, we found to our surprise that 80%–85% of the Ca^{2+} load disappeared in seconds into the mitochondria, from which over a minute or two it was transported back into the cytoplasm while the plasma membrane transporters gradually exported it from the cell (Babcock et al., 1997). We wondered whether mitochondria are always the most powerful Ca^{2+} clearance mechanism. In comparing a number of different cell types,

we found that the fastest clearance could be via mitochondria (chromaffin cell), via the intracellular SERCA pump (pancreatic β-cell), both (gonadotrope), or via the plasma membrane transporters (sperm). Apparently, each cell is specialized for a different agenda. Another line we pursued was stimulation of exocytosis in the absence of Ca^{2+} signals (Hille et al., 1999). In some secretory epithelia, the physiological regulation of exocytosis is primarily through receptors that stimulate protein kinase A and protein kinase C without the Ca^{2+} rise that is so prominent in neuronal synapse. The resulting phosphorylation also potentiates Ca^{2+}-induced secretion (Zhu et al., 2002; Jung et al., 2010).

A Life of Academic Science

We are lucky to be academic scientists since we get to think all day about questions that fascinate us, we direct ourselves, we interact continuously with smart and like-minded people, and we are invited to travel to many fascinating parts of the globe. This addictive job comes with responsibilities. For research, we need to run a responsible and reliable research group that publishes its discoveries after suitable critical review and is accountable for where the money went. For teaching, we need to offer classes that inspire students and communicate the concepts, process, and content of our science. For administration, we need to help academia and the institutions of science and government to provide a system, finances, and facilities for research and education, for the improvement, prosecution, dissemination, and use of science in our society.

The high standards of today's science are maintained by peer review. Peer review is key to the success we have and to our feeling of relative autonomy—academic freedom. It should be vigorously defended and responsibly executed. After we have benefited from funding of our research proposals and publication of our papers for a while, we owe our peers our best judgment by serving on review panels and editorial boards. Sometimes the requests come in too early. I suggest junior scientists accept only a single session of study section to learn more about what makes proposals successful and to gain some confidence in the peer review system. Then, I would stay away until the lab is well established and you have tenure before committing to a several-year term. It is hard work. I first served a full term on an NIH study section in 1980-1984. The success rate was high then, but I favored a more elitist approach with more money going to the most productive and trendy scientists and less to those whose science was being done well but just seemed less exciting. However, subsequently I realized that the scientists I would have cut off were specialists, even sleepers, in some field that suddenly became necessary and important for a new development. For example, when molecular biology discovered new genes to test, certain seemingly esoteric biophysical assays became the best way to study their roles,

and valuable science was rapidly advanced. Natural populations are strongest if they offer a wide range of genetic variation to adapt to changing conditions. I conclude similarly that the scientific enterprise is strongest if a broad range of specialties is maintained that can be called on as science advances. Equally, a review committee is strongest when it has a variety of viewpoints. Later I served for many years on the Scientific Advisory Boards of the Biomedical Scholars program of the PEW Charitable Trusts and of the Investigators Program of the Howard Hughes Medical Institute. These programs were oriented more toward supporting the best investigators rather than some specific project. These boards were very satisfying to serve on because of the high quality of the science, the investigators, the members of the Board, and often the venue.

Another debt that we owe is in mentoring. By the time we reach seniority, many people have helped us greatly along the way. Early on, we are supervisors of those who work in our laboratories, and our orientation is typically, how can I get the most progress out of them for my advancement? Later we need to pay back more fully, with a new attitude: My job definitely is to advance the career of each person in the lab but also, where possible, to assist faculty colleagues. The university often does not provide much of a contract, if any, to its employees, but I would say that it acquires an unwritten obligation to each new junior faculty member. The faculty member should provide research, teaching, and other service, and the university should make every effort to facilitate growth and nurture careers and satisfaction of its junior faculty. Junior faculty are expected to do many things they were not trained to do, things that we senior faculty have seen endless times. It is easy for us to provide guidance and suggestions on these familiar topics to relieve the stress. Soon the junior faculty grow into stronger senior faculty.

The public thinks of universities, especially state universities like mine, as institutions that exist to teach students. We may be promoted on the basis of published research, but we are hired and paid to teach. This is a major role of professors. Fortunately, the subjects we teach are usually directly interesting to us and the rewards of imparting understanding, sophistication, and success to junior minds are great. Teaching is taking students from wherever they are to a higher level. I am best at lectures. The effective lecture has to be a form of entertainment: interesting, relevant, understandable, and snappy. It must be progressive, developing concepts in a logical order. I count on needing 12 hours to make a completely new lecture, so it can't be done on the fly while already giving the course. Fortunately today, PowerPoint keeps a good record of what you did that is easily edited so next year is much easier. For research seminars, I see the same criteria of theatre, progressivity, and so forth, but the subject can go further because of the sophistication of the audience. Nevertheless, I like to assume as little specialized knowledge as possible. Define terms, explain abbreviations,

use them minimally, and bring people up to speed with some didactic development. Stick with points that are needed to get to the punch line. Too many opaque professional talks are given that could have been understood and would have been interesting to everyone had the speaker only taken care to bring them on board. It seems that many people develop their style in lab meetings where maybe everyone already knows what you are talking about. I treat research lectures very much like teaching lectures. Finally, both in lectures and in publications, the slides and figures are important. They need clarity, visibility, simplicity, and grace—even beauty if possible. In our lab we rehearse lectures and fine-tune graphics.

Science is a world activity. In every country people are thinking deeply and refining our body of knowledge. We are invited to many places where we are warmly accepted by colleagues we have not met before. For example, for me significant early visits were associated with the 1972 IUPAB International Biophysics Congress in Moscow. I went to the three great Soviet laboratories of ion channel biophysics: Boris I. Khodorov in Moscow, Galina N. Mozhayeva with Alexander P. Naumov in Leningrand (now St. Petersburg), and Platon G. Kostyuk with Oleg A. Krishtal in Kiev, Ukraine. Through the later dissolution of the Soviet Union (1991), the mass emigration of many Soviet scientists, and the disastrous drop of their economy and support for science, these three labs continued to train extraordinary scientists. Their heads have remained apparently as active as ever, still leading their groups today with the highest standards and vigor well after they passed their 80th year. Their perseverance in adversity and continued accomplishment as octogenarians are lessons in the strength of the human mind and of a life in science. I have gotten to know and respect these scientists and many others. They exemplify the spirit of scientists around the world.

Like the arts, science needs patrons to support it, and fortunately our society has supported science generously for 60 years. Where would the arts and humanities be now had they been as highly regarded by governments! Unlike the arts, science is a collective and incremental activity. We can easily name dozens of individual artists, authors, and philosophers of each of the last five centuries and say that we have seen, heard, or read their original works. However, nobody but a historian reads science done 100 years ago, and as ideas are refined each decade, we do not remember the past contributors. Peer review that I praised before is a critical process that constantly looks for deficits in every new work. If we do not remember the contributors and we are used to finding faults, where is the recognition for the scientists? We must all remember to praise now. Especially as we become more senior, it is our turn to remember to give encouragement, to highlight good research, to credit our sources, and to nominate people for awards and honors. Every paper will be improved upon by someone else tomorrow, but that is not a reason to criticize the previous authors. It just means that the scientific method is healthy and science continues to progress as it must.

A significant part of academic life is family and what happens outside the workplace—the Greek idea of a balanced mind and body. As cell scientists, Merrill and I do enjoy discussing lectures and ideas we have heard, and we do a lot of reading and writing at home. But very important are hiking, backpacking, snowshoeing, skiing, going to concerts and art exhibits, bird watching, gardening, and other activities that we often did with our sons as they were growing up, but now also with our grandsons. Erik became an actuary, and Trygve, a chemical engineer. They each have a wonderful spouse and each a son. The generations continue.

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