I was born in Auckland, New Zealand, in 1933. Hitler was on the rise in Germany, but this was of little concern to most of the inhabitants of this beautiful country so far from the political, economic, and cultural centers of the world. During my early years, my family lived by the seaside, and my sister and I led an idyllic existence playing on the beach, exploring rock pools teeming with life, and climbing the cliffs to see the nests of ocean birds. My exposure to the unique flora and fauna of New Zealand engendered an early interest in biology. I left all this behind when my family moved to the city, where my education began in earnest. This was when atomic bombs were dropped on Japan to end World War II. I did not recognize the moral issues involved in this act, but it stimulated my intense interest in nuclear physics. I was probably one of few elementary school pupils in New Zealand who read about Madame Curie, Niels Bohr, Max Planck, Ernest Lawrence, Enrico Fermi, Walter Heisenberg, Erwin Schrödinger, and, of course, New Zealand’s own Ernest Rutherford.

For my secondary education, I was sent to a Church of England preparatory school that was patterned after an English public school. Discipline was severe, with the cane being liberally applied and bullying rampant. However, my parents did not pay high fees just for the infliction of pain on their son, but for an excellent academic program in which Latin was mandatory and Greek recommended. This classical education was broadened by exposure to history and literature, almost exclusively British, and to mathematics and the sciences. For sport, we were required to play cricket and rugby football whatever our competence, which in my case was low. The chemistry teacher frequently put on dramatic explosive displays of chemical reactions, causing us to crouch behind our desks while he dropped solid sodium or potassium into concentrated nitric, sulfuric, and hydrochloric acids!

In New Zealand, there is no college system, and at that time, relatively few high school graduates proceeded to university. I was fascinated by both chemistry and biology and had to choose between a degree in medicine or the sciences, with my teachers strongly recommending medicine. So I took the two-day journey from Auckland in the North Island to Dunedin in the South Island, where the only medical school at that time was located at the University of Otago. I had led a rather sheltered life and was initially shocked by the riotous and decidedly unintellectual behavior of my fellow students. Dunedin was founded by dour Scottish settlers, many of them Calvinists, but somehow they tolerated the frequent and outrageous student pranks. One of Dunedin’s greatest assets is its proximity to some of New Zealand’s most beautiful lakes and mountains, and groups of us would spend weekends and other breaks hiking in the spectacular scenery.

Medical training in New Zealand takes six years, with almost two years devoted to anatomy, physiology, and biochemistry. Unlike the vast majority of students, I was fascinated by biochemistry because it fused my interests in chemistry and biology. Another factor was that it was taught by Norman Edson, one of the unsung heroes of New Zealand Science. He had worked with Hans Krebs in Gowland Hopkins’ laboratory in Cambridge, England, where he had studied the regulation of ketone body production in the liver. He had an encyclopedic memory and kept up with the latest advances in the U. S. and U. K. I decided to take a year out of my medical training to do biochemical research and studied the breakdown of pyrimidines. At that time, there was contro-
versy about the degradation pathway, but I showed that the scheme proposed by Robert M. Fink and co-workers was correct. This involved the intermediate formation of β-ureidopropionic acid from uracil and β-ureidoisobutyric acid from thymine (1). There was an initial reduction of the pyrimidines to dihydropyrimidines, and I set about to find the dehydrogenase responsible. This was unsuccessful because the only reduced nicotinamide nucleotide commercially available at that time was NADH, and the enzyme was later shown to use NADPH. I continued with my medical studies, and my social life greatly expanded when I met my future wife, who was a great companion on our rugged hikes and sailing adventures on the icy waters of Otago Harbor. She was also a student at the University of Otago and came from a sheep farming community in the North Island with the exotic name of Ongaonga. Her father often employed me on the farm during vacations. Being a city slicker, I was of doubtful use to him except during lambing, when my obstetrical skills learned on the sturdy Scottish women of Dunedin were useful when the ewes were in difficulties. I suspect he tested me as a suitable son-in-law by sending me out to the endless task of draining a large swamp by hand shovel. I must have passed muster because he later gave his daughter in marriage when I was a final year student.

During my student years at Otago, I supplemented my scholarship funds by working in a variety of occupations during the summer breaks. These included working in an ice cream factory, as a surveyor’s assistant, in a packing house, in a wool store, and as a longshoreman. These gave me the opportunity to interact with a wider socioeconomic group than my middle class family and friends and encouraged my thinking away from the relentless conservatism of my parents!

At several times during my training, I contemplated switching to a Ph.D. in Biochemistry, but Norman Edson advised me strongly to continue in medicine, a course I have never regretted, particularly because there were aspects that I really enjoyed, and it gave me fortitude when I later had to lecture to medical students. After completing medical studies and an internship in Auckland, I returned to Dunedin to do a Ph.D. in Biochemistry under Norman Edson. The thesis topic was the metabolism of isolated rat liver cells. Michael Berry, an energetic student in the laboratory, had discovered how to prepare these cells, and my project was to study their carbohydrate and lipid metabolism and to understand why they produced large amounts of ketone bodies (2, 3). This was traced to a limitation in the citric acid cycle such that the fatty acids were preferentially oxidized to ketone bodies. At that time, the medical school had only one electron microscope, and when the cells were examined later, it was found that their plasma membranes were disrupted and their mitochondria distorted. Nevertheless, the findings were reported in the Biochemical Journal because studies of isolated mammalian cells were unusual at that time. A visitor from the U.S. at that time was Harland Wood from Western Reserve, who came to study pyruvate metabolism. I am sure an additional attraction for him was the proximity of great deer hunting to Dunedin. Because of the great fear of radioactivity in the 1960s, Norman Edson built a small laboratory for him on the roof of the medical school. Imagine his chagrin when Harland loaded a syringe with [14C]pyruvate and squirted part of it into the air to expel an air bubble!

When my Ph.D. was nearing completion, I looked around for possible postdoctoral positions. The population of New Zealand was then not much more than three million, and Ph.D. and medical graduates desiring further training had to look overseas. Britain was the traditional place, and relatively few went to America. I sought a fellowship to Trinity College Oxford to work on a D.Phil. under Hans Krebs, although why I needed an additional degree escapes me now. In case I was not successful in the fellowship, I looked into several possibilities in the U.S. The most attractive of these was the Department of Physiology at Vanderbilt University under Charles R. (Rollo) Park, whose group had just published an impressive series of papers in the Journal of Biological Chemistry dealing with the effects of insulin, diabetes, and other hormones on glucose transport and phosphorylation in heart muscle. The rigor of their data and their in-depth analysis impressed me greatly.

I did win a fellowship to Oxford, but when the details arrived from the British Council, I was frankly outraged. At that time (1963), my family had grown to two children, and the Council secretary advised me to leave my family in New Zealand for two years because the stipend could support only a single person. Furthermore, I could travel only at the lowest level on the ship to Britain. On the other hand, a letter came from Rollo Park offering to pay the travel expenses of the whole family, so I outraged the British by refusing their fellowship to Oxford, which may never have been done before by a mere colonial and certainly did not please Krebs!

We went by ship to the U.S., stopping at such exotic and erotic places as Tahiti, Panama City, and Jamaica and disembarking at Port Everglades in Florida. We hired two Cadillac limousines to take our large pile of baggage to the train station and then took one of them on a tour of Miami.
It seemed that we were living the American dream a few hours after arrival. We took a train to Nashville, but it had been rerouted, and no one on the train knew which station was Nashville! We discovered for sure only upon alighting on the platform! Rollo turned out to be as generous as his correspondence indicated and told me that the scientific environment of the department had recently been enhanced by the arrival of Earl Sutherland’s group from Western Reserve. Earl had achieved great recognition as the discoverer of cyclic AMP, the second messenger of many hormones.

My project was to look at direct effects of insulin and other hormones on the liver using the isolated perfused rat liver preparation. However, the existing perfusion system left a lot to be desired in that the oxygenation system was immersed in a water bath for temperature control and frequently leaked. The temperatures and flow rates of control and experimental livers were often not the same, and there were other problems. When I arrived, a postdoc was running the experiments, and he overlooked any perfusion disasters by ignoring the apparatus while reading Life magazine. When it was discovered that he did not actually have a Ph.D., he was dispatched back to England posthaste, where he opened a candy store! The first order of business was to develop a better perfusion apparatus, and this was done with the assistance of Howard Morgan and Bailey Moore, the skilled head of the apparatus shop. Howard had a very pragmatic attitude and later became Chair of Physiology at Penn State, President of the American Physiological Society, and President of the American Heart Association. With the problems of the perfusion system solved, I began a lengthy series of studies of the hormonal control of gluconeogenesis and glycogen metabolism in the isolated liver. In an interesting turn of events, this put us in competition with the laboratory of Hans Krebs.

Gluconeogenesis occurs principally in the liver and involves the formation of glucose from non-sugar sources such as lactate, pyruvate, glycerol, and certain amino acids. It is essential for life during starvation and recycles lactate formed during exercise back to glucose (the Cori cycle). My work with Rollo Park showed that physiological increases in amino acids and other substrates could alone increase gluconeogenesis, and measurements of metabolic intermediates in the pathway revealed that the major rate-limiting step was the substrate cycle between pyruvate and phosphoenolpyruvate (4, 5). Later, we showed that hormones such as glucagon and epinephrine could directly stimulate gluconeogenesis in the liver by acting on this cycle and that their effects were antagonized by insulin. Work in several other laboratories subsequently showed that the enzyme affected was pyruvate kinase. Diabetes also stimulated the process, as did glucocorticoids, which also exerted a permissive effect on the actions of glucagon and epinephrine (5, 6). In collaboration with the Sutherland group, we determined that cyclic AMP played a major role in the regulation of gluconeogenesis at the level of the pyruvate-phosphoenolpyruvate cycle (7, 8). This work was conducted with the help of two excellent associates, Leonard (Jim) Jefferson, a graduate student from Kentucky who drove a Cadillac and ultimately became Chair of Physiology at Penn State Medical School and President of the American Physiological Society, and Tom Miller, a perpetually good-natured technician who came from a small town in West Tennessee and became Professor and Dean of Graduate Studies at the University of Massachusetts Medical School. These were superb experimentalists, although I had to avoid scheduling complex experiments on mornings after they had enjoyed the bars of Nashville.

Another important contributor was Sandy Harper, who truly qualified for the title of super-tech. Supervising all this work was the tall aristocratic Rollo Park, with his impeccable heritage of clerics, generals, and academics. He provided invaluable insights and conveyed an urbane relaxed attitude in which issues such as research funding and priority in publication were not of much concern. When I complained that one of my papers was taking over 17 months to be submitted because of constant revising, his response was, “In the end, the best paper wins.” Rollo sent me out to look at many departmental chairmanships despite my relatively young age because he believed that any candidate older than 40 was over the hill!

A year after my arrival in Nashville, we were joined by J. G. T. Sneyd, who was also from the Biochemistry Department at Otago. He was nicknamed Sam after the golfer Sam Snead, although he never played golf. He was a brilliant guy but an inveterate prankster. A list of his shenanigans would occupy this entire essay, but I will report only the episodes when he served rat liver pâté to his mother-in-law and staged a cricket match inside his house. The ultimate Sneyd story was when his wife put an advertisement in the local newspaper in which she tried to sell this work was the tall aristocratic Rollo Park, with his impeccable heritage of clerics, generals, and academics. He provided invaluable insights and conveyed an urbane relaxed attitude in which issues such as research funding and priority in publication were not of much concern. When I complained that one of my papers was taking over 17 months to be submitted because of constant revising, his response was, “In the end, the best paper wins.” Rollo sent me out to look at many departmental chairmanships despite my relatively young age because he believed that any candidate older than 40 was over the hill!

A year after my arrival in Nashville, we were joined by J. G. T. Sneyd, who was also from the Biochemistry Department at Otago. He was nicknamed Sam after the golfer Sam Snead, although he never played golf. He was a brilliant guy but an inveterate prankster. A list of his shenanigans would occupy this entire essay, but I will report only the episodes when he served rat liver pâté to his mother-in-law and staged a cricket match inside his house. The ultimate Sneyd story was when his wife put an advertisement in the local newspaper in which she tried to sell him plus his TV (both in working order) because of his addiction to football during the holiday season. This was taken up by countless news sources in the U.S. and throughout the world and occasioned many TV and radio interviews in which his wife played it straight!

The presence of Earl Sutherland’s group gave a great boost to my research, but discussions with him frequently involved mental gymnastics because he would often change topics mid-sentence. I remember talking about
glycogen metabolism when he suddenly mentioned TG. For the life of me I could not see the relevance of triglyceride, but he was referring to transglucosylase, an uncommon name for glycogen synthase! He was quite convinced that cyclic GMP was the second messenger for insulin, and this seemed quite logical. However, when Joel Hardman, subsequently Chair of Pharmacology at Vanderbilt, and I put this to the test in the perfused liver, the results were negative (9). Everyone in the department recognized the fundamental importance of Earl’s discovery of cyclic AMP and waited each year for the call from Stockholm. However, when a crew from Swedish TV arrived in late October 1971 to film him and his laboratory, we knew his time had finally arrived.

In 1968, I was promoted to Associate Professor and also appointed (anointed) as an Investigator of the Howard Hughes Medical Institute. At that time, Investigators received only a stipend and a travel allowance, which would get me to California and back. Nowadays, it would get me only a one-way ticket to St. Louis! In 1976, under pressure from the Internal Revenue Service, which was perpetually suspicious of the Institute, it was compelled to disburse more funds, and a cornucopia of equipment, supplies, and support for postdocs and technicians descended upon my grateful head! The Institute even renovated the lab using only first-rate materials. At that time, Hughes Investigators lived in a form of research paradise that was even better than it is now. Oversight was not very rigorous and the annual reviews perfunctory. The Medical Advisory Board believed that once Investigators were approved, they should be left to their own devices. All this changed in 1978, when the reclusive Howard Hughes died, and the Institute was restructured. Now, the review process became much more rigorous (terrifying), and they actually terminated some Investigators. I survived the review process and retired in 2004 as the longest tenured Investigator. I felt like a living fossil among all the young Investigators!

In 1970, I spent a sabbatical in Geneva at the Institut de Biochimie Clinique, headed by the renowned diabetes researcher Albert Renold. I worked primarily with his gifted associate Bernard Jeannaud, and I was greatly impressed by the charming and multilingual Albert and the handsome and debonair Bernard. I studied the hormonal regulation of glycogenolysis and gluconeogenesis in mouse liver, working with a superb graduate student, Françoise Assimacopoulos-Jeannet. I depended on her skill in adapting our procedures to this small animal. We rented a nice villa in Geneva and took frequent trips to France, Italy, and the rest of Switzerland to explore the countryside and culture. One of my first conferences was at a Swiss ski resort, where the Nobel Laureate Feodor Lynen sat at the rear of the room on a large throne-like chair and made audible derogatory comments about most of the talks. I approached him with some trepidation after my talk, but, thankfully, he was complimentary.

While I was in Geneva and after I returned, studies on the regulation of gluconeogenesis by glucagon and insulin continued using the perfused liver system. Some of this work focused on amino acids as gluconeogenic substrates and was carried out by Larry Mallette, a brilliant M.D./Ph.D. student, and Michio Ui from Japan, who would later become famous through his work on a Bacillus pertussis toxin that inactivates G_{i} through ADP-ribosylation. Other work studied the antagonistic action of insulin on the effects of glucagon in the liver and revealed the primacy of cyclic AMP changes in this interaction. A key player in this work was Steve Lewis, an M.D. with enormous energy who would try any experimental approach including perfusing the livers backwards or using distilled water as the perfusion medium! It was difficult to restrain him from Geneva.

Soon after my return to Nashville, I switched our experimental system from the liver perfusion procedure to the use of isolated rat liver parenchymal cells. After years of persistence by my former New Zealand colleague Michael Berry, structurally and metabolically intact liver cells could now be prepared using collagenase. Now the many perfusion apparatuses were used only to prepare these hepatocytes. Although the use of hepatocytes greatly increased the throughput of experiments and permitted better controls, I was faced with a dilemma as follows. Whereas all our data obtained using the hepatocytes supported a role for cyclic AMP in the action of glucagon on gluconeogenesis and glycogen breakdown, this was not the case for epinephrine. Try as we could, we were unable to correlate the changes in these processes induced by this catecholamine with increases in cyclic AMP (10–12). Our collaborator was Al Robison, a member of the Sutherland group who was a nocturnal worker and subsequently Chair of Pharmacology at the University of Texas Medical School at Houston. We would leave our samples on the desk, and in the morning, the cyclic AMP measurements would appear like magic! Our findings caused some consternation in the Sutherland group because the original work leading up to the discovery of cyclic AMP involved the effects of epinephrine on glycogen breakdown in dog liver. This challenge to the dogma was resolved when it was realized that there was a species difference. In dog liver, the effects of epinephrine are mediated by β-adrenergic receptors acting via cyclic AMP, whereas in rat
liver, they are mediated by $\alpha_1$-adrenergic receptors acting via Ca$^{2+}$. The work with epinephrine had a very long heritage starting with Claude Bernard, who found in the 19th century that stimulation of the brain increased the blood glucose level (piqûre hypoglycemia), and Walter B. Cannon, whose equally classic work ("fight or flight response") showed that increases in blood glucose and other changes occurred in response to activation of the sympathetic nervous system. When Mahmoud El Refai, an Egyptian postdoc, found that the rat liver receptors involved in glycogenolysis were of the $\alpha_1$-type, a competing group tried to prevent us from publishing because it was contrary to their incorrect findings! A collaborator in some of this work was Craig Venter, who had not yet developed his reputation as the enfant terrible of the sequencing world.

Work by Jim Putney, then at the Medical College of Virginia, and other investigators utilizing salivary gland, smooth muscle, and other tissues indicated that the $\alpha_1$-adrenergic mechanism involved a rise in intracellular Ca$^{2+}$, and we soon confirmed this for rat liver. However, a problem arose because it was then believed that the increase in Ca$^{2+}$ came from an influx through channels in the plasma membrane, but our data indicated that it came from an internal pool (12). A key collaborator in this project was Peter Blackmore from Australia, who was nicknamed "Quokka" after the small Australian marsupial. He was a remarkably adept experimentalist and is now a Professor of Physiological Sciences at the East Virginia Medical School. Also involved were Nancy Hutson, a smart and very personable graduate student who is now an executive at Pfizer, and Francoise Assimacopoulos-Jeannet from Geneva, who is now a Professor Titulaire at the Centre Médical Universitaire there. The role of Ca$^{2+}$ in the actions of $\alpha_1$-adrenergic and related agonists was demonstrated unequivocally by the use of Quin-2, a reagent developed by Roger Tsien that measured free cytosolic Ca$^{2+}$ (13). The postdoc involved in this work was Bob Charest. He was a chain smoker, and I worried about the effects of cigarette ash on the Ca$^{2+}$ measurements!

During this period, we also engaged in some experiments dealing with the effects of epinephrine and insulin on glycogen metabolism and glucose uptake in skeletal muscle using the isolated perfused rat hind limb preparation, and also the modulation of these metabolic processes by adrenalectomy and thyroid status (14, 15). These studies were of value in the interpretation of related in vivo experiments in man and experimental animals because they were not confounded by secondary effects. The importance of studying direct effects of hormones uncomplicated by in vivo changes also applied to our rat liver perfusion studies. Interestingly, later experiments with in vivo models largely confirmed our findings in the perfused liver, thus establishing the validity of our system. Jean-Louis Chiasson, an energetic researcher from Montreal, Melissa Dietz (Lojek), a graduate student and ballet dancer, and Mike Caldwell, a surgeon, were key to these experiments.

With the ready availability of hepatocytes, we also conducted many studies examining factors that modulated hormone effects on the liver. In an extension of our earlier work indicating a species difference in the adrenergic receptors involved in epinephrine action in the liver, we found that age, adrenal cortical status, and gender also influenced the extent to which epinephrine acted through $\alpha_-$ versus $\beta$-adrenergic receptors (16). Another finding was that agonists acting through cyclic AMP enhanced $\alpha_1$-adrenergic responses, whereas insulin and phorbol esters inhibited them (17, 18). These observations not only cleared up some confusion in the literature, but also involved some rather colorful postdocs. For example, there was the energetic, likeable, but budget-busting Chris Lynch, now a Professor of Physiology at the Hershey Medical School. His philosophy was to order what he might possibly need rather than what he actually needed. Others were Bernie Hughes, an Australian with an inexhaustible store of risqué stories, the charming Bernard Bouscarel from Toulouse, who was notable because his girlfriends sent him flowers, and Jean-Paul Dehaye from Brussels, who was nicknamed "The Pope" after the pope at that time, Jean Paul II, although he was not as infallible as the pontiff! These characters were balanced by Tim Chan, now a Dean at the University of Southern California, and Noel Morgan, a steadfast Englishman who is now Head of Biochemistry at a medical school in Cornwall. At this time, we were visited by Fatima Bosch from Barcelona, whose energetic pursuit of research and stylish clothing impressed us greatly.

Studies of the mechanisms involved in the actions of Ca$^{2+}$-mobilizing agonists reached an exciting phase in which the hunt was on for the signal that came from the receptor in the plasma membrane to the internal calcium pool. Here we became misguided by our findings and thought that the pool was in the mitochondria, a well known source of Ca$^{2+}$. Other workers deduced correctly that the pool was in the endoplasmic reticulum, and I had to endure some abuse for our sins at several meetings. We were also skeptical of the emerging phosphoinositide hypothesis as the basis of signaling to the internal Ca$^{2+}$ store. This posited that the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP$_2$) induced by certain ago-
nists generated a second messenger that released the Ca$^{2+}$. Because we found that an increase in Ca$^{2+}$ stimulated the breakdown of this phospholipid in liver cells and plasma membranes, we felt that the hypothesis was wrong. In point of fact, we did not realize that there was a much earlier phase in the action of the agonists when PIP$_2$ breakdown preceded a rise in Ca$^{2+}$. Our questioning of the phosphoinositide hypothesis raised the ire of the English scientists, who were the main proponents. With the recognition of inositol 1,4,5-trisphosphate (IP$_3$) as the intracellular messenger by Robin Irvine, Michael Berridge, and associates, the controversy went away, except for some diehards with lingering resentment!

Much evidence was accumulating that a G protein was involved in the actions of many agonists to stimulate the breakdown of PIP$_2$ with the resultant mobilization of Ca$^{2+}$ from the endoplasmic reticulum (19, 20). This early work was done by three American postdocs and one from Croatia with very different personalities and communication skills: Thom Fitzgerald, who barely said a word, Janet Atkinson (Colbran), who exuded Southern charm and volubility, Ron Uhing, who was not much more talkative than Thom, and Vera Prpic, whose accent did not prevent a marriage with Ron. Because none of the known G proteins was found to be involved in the breakdown of PIP$_2$, the hunt was on for a novel G protein. We were successful in isolating and purifying a G protein that activated the phospholipase C that hydrolyzed PIP$_2$ (PI-PLC), but could not identify it (21). At the same time, the group of Paul Sternweis at the University of Texas Southwestern Medical School at Dallas had raised antibodies to two novel G proteins, but could not demonstrate that they activated PI-PLC. So we joined forces to demonstrate that the novel G proteins were in fact activators of the phospholipase and hence the mediators of hormone action on intracellular Ca$^{2+}$ mobilization (22). The two proteins were named G$_q$ and G$_{11}$, and I am often asked why the G$_q$. It turns out that a postdoc involved in the research in Paul’s laboratory was an avid reader of the Gentleman’s Quarterly! A key player in this research was Steve Taylor, a taciturn English postdoc who preferred to fly on British Airways because of the quality of their gin and tonics. Karen Shaw, a spunky English postdoc, was also involved in this work, as was Iro Georgoussi from Athens, whose vibrant personality and social skills livened up the social life of the laboratory. In a later extension of this work, Gita Venkatakrishnan localized the residues in the G$_q$ α-subunit that were specifically involved in the activation of PI-PLC.

Two interesting side projects arose from this work. The first was the confirmation by Jonathan Blank, another English postdoc, of the finding of Peter Gierschik at the University of Heidelberg that the βγ-components of G proteins could activate some forms of PI-PLC (23), a finding also reached by Ken Harden’s group at the University of North Carolina. At that time, the idea that βγ-subunits of G proteins could be involved in cell signaling was an issue of great controversy, sometimes vicious, and Gierschik had had great difficulty in publishing his findings. Another side project involved the discovery by Jonathan Blank and Gabriel Berstein in Elliott Ross’ laboratory at UT Southwestern that PI-PLC could stimulate the GTP hydrolase (GTPase) activity of G$_q$ (24). In other words, the phospholipase could inactivate its G protein activator, indicating a novel feedback mechanism. All this and other work extending to 1999 were supported by the efforts of Annette Ross, a truly superb technician. Three very competent secretaries, Penny Stelling, Carolyn Sielbeck, and Judy Childs (Nixon), aided immensely in the preparation of our publications during my tenure as a Hughes Investigator. After two terms on the Editorial Board of the Journal of Biological Chemistry, I was appointed an Associate Editor in 1988. I was again lucky to have the superlative services of two editorial assistants: Carolyn Sielbeck, doing double duty as my secretary, and Carolyn McDonald, whose gentle Southern voice calmed many an agitated author.

The breakdown of PIP$_2$ generates 1,2-diacylglycerol (DAG) as well as IP$_3$, and if the theory is correct, these should be produced in equimolar amounts in cells in which PI-PLC is activated by agonists. Imagine our chagrín when Steve Bocckino, another great postdoc, measured these compounds chemically and found that, in vasoressin-stimulated liver cells, the production of DAG greatly exceeded that of IP$_3$ and was much more prolonged (25). Now we were at odds with the experts in the protein kinase C (PKC) area because it was believed that the DAG that activated this kinase came from the breakdown of PIP$_2$, making a nice story in which second messengers (IP$_3$ and DAG) were simultaneously released in a bifunctional signaling system. Further work confirmed that PIP$_2$ was breaking down but that another phospholipid was being hydrolyzed to a greater extent. Measurements of choline release by Helen Irving, an Australian postdoc, and analyses of the fatty acid composition of the DAG by Steve Bocckino and Guy Augert, a dashing postdoc and fearless skier from the Haute-Savoie region of France, indicated that the phospholipid was phosphatidylcholine (PC) (26, 27). This was a surprise because PC is the major phospholipid of cell membranes, and its breakdown would be expected to have deleterious effects on the cell.
However, chemical measurements of PC later showed that the decrease induced by agonist stimulation was barely detectable, although the resulting increase in DAG was sufficient for signaling.

The next surprise came when we set out to determine the phospholipase responsible for the breakdown of PC. By analogy with the phosphoinositide signaling system, we expected the phospholipase to be of the C type. However, chemical measurements showed that the initial product of PC breakdown was phosphatidic acid, which was then converted to DAG. In other words, the phospholipase was of the D type (PLD) (28). This was somewhat disconcerting because it was generally believed that this enzyme was present in plants, but not animals! Julian Kanfer’s group in Winnipeg had earlier obtained evidence of PLD in mammalian tissues, but their work had been largely ignored. Once again, these findings were not welcomed by the aficionados of the phosphoinositide hypothesis of cell signaling. However, further work by others, including Claire Allan, a Scottish postdoc with a delightful accent, showed that both systems operated, i.e., agonists caused an initial hydrolysis of PIP₂, followed by a larger and more prolonged breakdown of PC.

Reverting back to our studies of PLD, it became clear that a G protein played a major role in its regulation, but none of the known heterotrimeric G proteins could be shown to affect its activity. Then a report from the group of David Lambeth at Emory appeared showing that RhoGDI, an inhibitor of the activation of the small G protein Rho, inhibited the activation of PLD by guanosine 5′-O-(3-thiotriphosphate) (GTPγS) in membranes, implying a role for members of the Rho family. Ken Malcom, an environmentally conscious postdoc, used recombinant forms of these proteins to show that they could activate PLD directly (29). An important collaborator in these studies was Marc Symons, then at Onyx Pharmaceuticals. There were also reports from the Sternweis and Cockcroft laboratories that another small G protein, ADP-ribosylation factor (ARF), could also activate partially purified PLD or when tested in permeabilized neutrophils. We confirmed this and focused on factors involved in the action of ARF. This led to the discovery of the novel proteins arfaptins 1 and 2 (30) and arfophilin (31). Interestingly, both arfaptins and arfophilin were found to bind other families of small G proteins, implying the existence of novel signaling networks. Two gifted postdocs, Hiroyuki Kanoh from Japan and Ok-Ho Shin from Korea, were responsible for this work.

Because Rho was a major regulator of PLD1 in vitro, we were interested in examining its role in vivo. Steve Plonk, an M.D./Ph.D. student, showed that introduction of the C3 exoenzyme of Clostridium botulinum, which inhibits Rho, inhibited the activation of PLD by lysophosphatidic acid (LPA) and other G protein-linked agonists, but not platelet-derived growth factor (PDGF) (32). On the other hand, Jean Hess, a postdoc now living in rural Vermont, found that Rac mediated the effect of growth factors. It was also found that LPA induced the membrane translocation of Rho, but this did not occur with PDGF (33). The G protein involved in the activation of Rho was shown by ourselves and others to be G₁₃ and not G₂₅ illustrating a new signaling paradigm (34). Because heteromeric G proteins do not link directly to monomeric G proteins, we next searched for the guanine nucleotide exchange factor (GEF) involved in agonist activation of Rho family proteins. In collaboration with John Collard’s group in Amsterdam, we showed that Tiam1, a GEF for Rac, was translocated to membranes and phosphorylated on threonine in cells treated with LPA (35, 36). Both PKC and Ca²⁺/calmodulin-dependent kinase II were shown to phosphorylate Tiam1, but only the calmodulin-dependent enzyme induced activation (36). These findings did not explain how G₁₃ activated Rho (another group showed the involvement of other GEFs), but pointed to a mechanism for Rac activation. The postdocs principally involved in this work were Ian Fleming, not related to the author of the James Bond series, and Greg Buchanan, the ultimate University of Tennessee fan who wore orange almost continuously!

Many groups had obtained evidence that PKC could activate PLD in vivo. This included evidence by Eui-Ju Yeo, a Korean postdoc in my lab, that PKC mediated the effect of epidermal growth factor on the enzyme. However, a big surprise came in in vitro experiments showing that PKC could directly activate PLD but that this did not involve phosphorylation, but merely protein-protein interaction (37). This nontraditional view of how PKC acted was supported by the findings of several other groups, but was not accepted by others. The lead postdoc in this work was Kevin Conricode, who was notable in having seven brothers but no sisters. Our studies also showed that the activation of PLD1 by PKC involved the conventional α- and β-isozymes, but not the other isozymes. Later work by two Chinese postdocs, Tianhui Hu and Jun-Song Chen, showed that PKC could phosphorylate PLD but that this caused inactivation. This work on PKC led us to examine the specific PKC isoforms activated by various agonists in fibroblasts. Surprisingly, Kwon-Soo Ha, an energetic postdoc from Korea, found that α-thrombin and PDGF caused a differential translocation/activation of PKC isoforms in
these cells. Thrombin caused a rapid membrane association of the α- and ε-isozymes, which correlated with rapid increases in IP₃/Ca²⁺ and DAG due to PIP₂ breakdown. On the other hand, PDGF did not cause a translocation of PKCα, but caused a delayed translocation of PKCε that correlated with an increase in DAG due to PC breakdown (38). Our general interest in PKC led us to a study of the atypical PKCζ isozyme, about which very little was known. This first had to be isolated and purified before its regulation could be studied. Hiroyuki Nakanishi, a postdoc from Kobe, examined the effects of various lipids and found the enzyme to be the first known target of phosphatidylinositol 3,4,5-trisphosphate (39), a lipid now known to be generated by insulin and other growth factors and an important second messenger.

We next devoted a lot of effort to the purification, characterization, and cloning of mammalian PLD. However, the cloning of the two mammalian (human) PLD1 and PLD2 isozymes was first accomplished by the group of Michael Frohman at the State University of New York at Stony Brook based on their earlier cloning of yeast PLD. Seung-Kiel Park from Korea, in association with Joe Provost, a colorful ex-Army man, cloned the rat liver enzymes by an analogous approach (40), and this initiated an extensive program to characterize their structure, kinetics, and regulation (41). Much of what we found paralleled the findings of the Frohman group, and in contrast to my earlier research, no controversies arose between us! The PLD1 isozyme was of particular interest because of its complex regulation by PIP₂, PKC, ARF, and members of the Rho family of small G proteins (Rho, Rac, and Cdc42). Furthermore, PKC and the Rho proteins were shown to mediate the actions of certain agonists on the enzyme in intact cells. Interestingly, in our early efforts to purify PLD from rat brain, we inadvertently purified phospholipase A₁ to homogeneity, and because little was known about this enzyme, I encouraged the postdoc to characterize it. This was Matthew Pete, an ex-Marine who tackled the project like it was Iwo Jima!

PLD isozymes from most sources are characterized by the presence of two highly conserved motifs, termed HKD, which are required for catalytic activity. In PLD1, they are separately located in the N and C termini. Zie (Julie) Xie, a gifted postdoc, ably assisted by Wan-Ting (Tina) Ho, found that expression of one-half of the enzyme containing one HKD motif produced no catalytic activity, whereas expression of both halves did (42, 43). The conclusion that both HKD motifs were required to form a dimeric catalytic center was borne out by subsequent structural studies. Julie and Tina later found that a short C-terminal sequence in mammalian PLDs was absolutely required for activity. The reason for this remains unknown. Seunghyi Kook and Do Sik Min, two postdocs from Korea, localized the binding site of PKC on PLD1 to certain sequences in the N terminus (44) while Songmin Cai from China identified the binding site for Rho in the C terminus (45). We also did the reciprocal experiments. Thus, Tianhui Hu identified a residue in PKCα that was critical for PLD1 activation, and Chang Dae Bae from Korea identified residues in the activation loop of RhoA that were specifically involved in activating the enzyme. Chang was a brilliant postdoc, but was concerned about his health. He asked me to move a freezer nearer to his bench because the distance, not far, was straining his leg muscles! An interesting aspect of the regulation of PLD1 is the striking synergism between certain of its activators. In an analysis of this in collaboration with Alex Brown and Lee Henage, a brilliant graduate student, it was found that the synergism occurred only if a catalytic activator such as ARF was combined with a binding activator such as one of the Rho proteins or a mixed activator such as PKC (46).

PLD is a ubiquitous enzyme that is a member of a superfamily that has been conserved through evolution. This implies that it subserves some important functions. We and others have identified a variety of cellular functions of the PLD1 and PLD2 isozymes, but no underlying themes have emerged, suggesting that the fundamental importance of PLD has yet to be defined. Another area of importance in PLD research is to determine the crystal structure of the mammalian enzymes. The structures of the enzyme from Streptomyces antibioticus and of some low molecular mass members of the PLD superfamily have been defined and have provided very useful information about the catalytic center and the mechanism of catalysis. However, the reasons why certain isozymes are critically dependent on PIP₂ for activity and the molecular mechanisms by which PKC, ARF, and Rho activate PLD1 remain unknown. Thus, much more work needs to be done to define the structure, regulation, and functions of these enzymes, the distribution of which is so widespread.

Address correspondence to: john.exton@vanderbilt.edu.

REFERENCES

1. Batt, R. D., and Exton, J. H. (1956) The catabolism of dihydropyrimidines by rat tissue preparations. Arch. Biochem. Biophys. 63, 368–375
2. Exton, J. H. (1964) Metabolism of rat-liver cell suspensions. 1. General properties of isolated cells and occurrence of the citric acid cycle. Biochem. J. 92, 457–467
3. Exton, J. H. (1964) Metabolism of rat-liver cell suspensions. 2. Fatty acid oxidation and ketone bodies. Biochem. J. 92, 467–472
4. Exton, J. H., and Park, C. R. (1965) Control of gluconeogenesis in the perfused liver of normal adrenalectomized rats. J. Biol. Chem. 240, PC955–PC957
