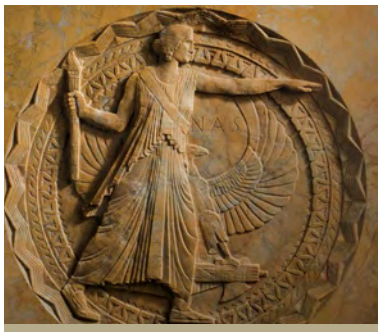
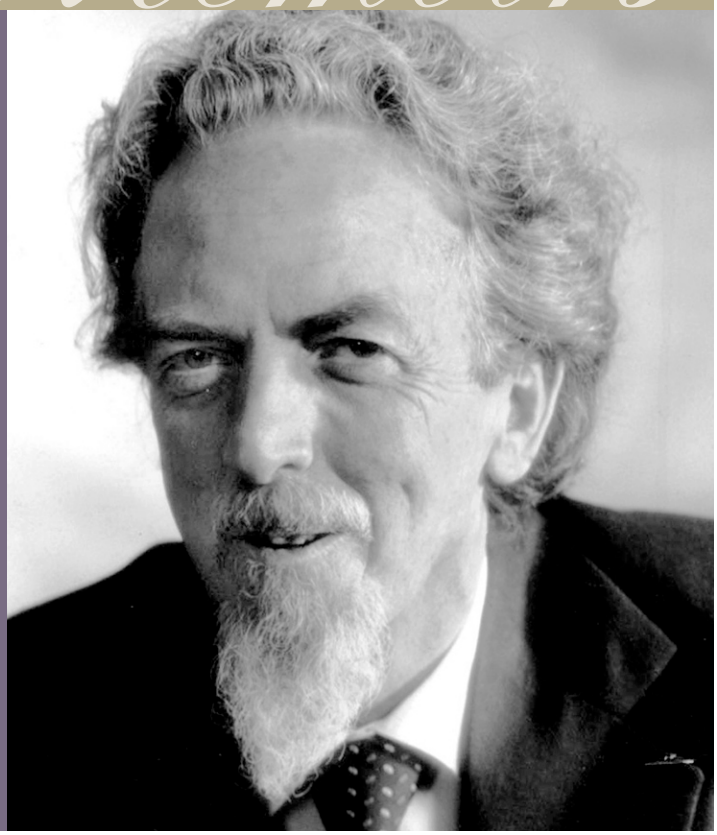


Vincent Massey

1926–2002

BIOGRAPHICAL *Memoirs*

A Biographical Memoir by
David P. Ballou and
Charles H. Williams, Jr.



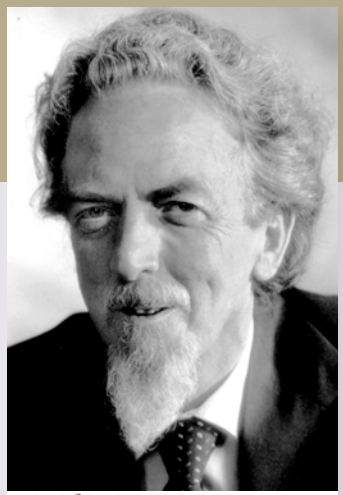
NATIONAL ACADEMY OF SCIENCES

VINCENT **MASSEY**

November 28, 1926–August 26, 2002

Elected to the NAS, 1995

Vincent Massey[1]—Vince, to all who knew him—lived life very fully. Carol Strickland, the wife of a former graduate student of Vince's, captured his vitality when she said: "The stop-flow might have been Vince's favorite research tool, but in life he was all flow without stop. I can still see Vince, his eyes twinkling, practically chomping the stem of his pipe in two as he guffawed at something that struck him as funny. What a bon vivant he was! Whatever he did, he did full-bore, 'sucking all the marrow out of life,' as Thoreau put it."



By David P. Ballou and Charles H. Williams, Jr.

Vince gained international distinction in physical biochemistry. His pioneering efforts to relate flavin chemistry to flavin enzymology resulted in a new understanding of flavin charge-transfer complexes, free radicals in flavoproteins, oxygen reactivity of flavins, interactions of the flavin ring structure with proteins, and the classification of flavoenzymes. His early discovery that Straub diaphorase is in fact lipoamide dehydrogenase and that it functions as a critical component in both the pyruvate and 2-ketoglutarate dehydrogenase complexes was a milestone in understanding metabolism.

Vince's development of totally innovative methodology for the determination of intermediates in enzyme catalysis through transient kinetics made flavoproteins one of the best-understood enzyme families. These techniques allowed him to define the mechanism by which the drug allopurinol inhibited xanthine oxidase: one of the first instances in which the effects of a drug on an enzyme were understood chemically.

Vince also had a great human impact on science by inspiring and training others and through his determination to maintain the integrity of the scientific method and to foster basic research.

1. This memoir originally appeared, in slightly different format, in *Biographical Memoirs of the Royal Society (2003)* 49:335-350 and is reprinted with permission.

He had many passions, and we will enjoy recalling them later, but Vince's preeminent passion was hard work—particularly on flavoproteins. One of his favorite stories was about Sir Hans Krebs, who had built an excellent biochemistry department at the University of Sheffield just before Vince arrived there. Some time after Krebs' move to Oxford, he returned to give a seminar in Sheffield. After the seminar, a student asked Sir Hans to what he owed the secret of his success. He commented, "it was luck," Vince recalled, but when the applause at his modesty ended, he became serious and said: "I had a certain amount of luck in my life, but I made a correlation—the harder I worked, the luckier I got." Surely this story appealed so much to Vince because it confirmed his own predilections.

Figure 1. Photo taken on the occasion of a lecture by Sir Hans Krebs in the 1960–1961 academic year at the University of Sheffield. Professor Minor J. Coon had given a lecture the day before and had stayed over to hear Sir Hans, at Vince's urging.

Faculty • Front row: Sir Hans Krebs, fourth from the left; his successor, Quentin Gibson, fourth from the right. Third row: Gregorio Weber, Theo Hofmann, and Vincent Massey, who were part of the Sheffield "brain drain" (see below), second, third, and fourth, respectively, from the left. Professor Gibson was also part of the "brain drain."

Students of Vince • Third row: Rod Bennett and Graham Palmer, on the right and fourth from the right, respectively. Fourth row: Phil Brumby, second from the left; Stephen Mayhew, on the right, was an undergraduate in biochemistry and later became a postdoc with Vince after his move to Ann Arbor. Back row: Ben Swoboda and Anne Wren, second and fourth from the left, respectively.

A remembrance of Vince has appeared in *Flavins and Flavoproteins 2002* and an obituary can be found in *TIBS*. Much of the material in the section below on Scientific Achievements has been taken from the *TIBS* article (with permission) and some of the personal tributes are from the remembrance. The present text is taken, with minor revisions, from our memoir published in *Biographical Memoirs of Fellows of the Royal Society.*

Family background and early Life

Born on November 28, 1926—in the countryside outside the tiny village of Berkeley, near the coast of New South Wales south of Sydney—Vince was the third and youngest child of Walter and Mary Ann Massey. There were about 150 people living in the village, most of them relatives. Vince's father was a fisherman, as were his uncles. They lived near a freshwater lake full of fish, which they caught and sold. They also fished along the seashore. Vince helped out on quite a few fishing endeavors and found them exciting, but when asked if he ever considered becoming a fisherman, he answered, "Not on your life!"

His epiphany came during his teenage years—Vince's world changed dramatically when he started high school. He had attended primary school in a oneroom, one-class school in his village, having one teacher and 20 students of different ages. High school was in the town of Wollongong, about 10 miles away. Perhaps the effort of getting to the high school enhanced its value, for he had to cycle five miles to the train station, catch a train to town, and then walk to the school. The whole trip took about two hours each way. The high school, with about 2000 students from all walks of life, was where the world of ideas opened up to him. He had classes in chemistry, physics, and math. He read voraciously and was tremendously excited by learning and discovery. It was at that point that he realized he did not want to fish and that he would be heading in a direction away from the sea. It was also at that time that he read a biography of Louis Pasteur, which stimulated his interest in becoming a scientist.

Fascinated by chemistry experiments, Vince set up his first laboratory in his bedroom. But the sulfuric acid he used to dissolve other chemicals also dissolved the carpet and his trousers, so he had to find another site. His father had bought a large building for processing the fish and prawns he caught and sold, so he helped Vince to build a lab in part of this "depot," as it was called. Vince spent his pocket money on setting up a workbench and apparatus, and his parents chipped in. He earned money for his experiments by collecting the mushrooms that grew in abundance in the surrounding countryside after a heavy rain, packing them in boxes and shipping them off to market.

Vince was the first person in his family to go to university. His parents thought he was a bit strange, but they were proud that "Vin was brainy"—that he had special talents that were out of the ordinary—and they helped him. Despite being poor, they penny-pinched to pay for his board at the University of Sydney, from which he earned a bachelor of science (honors) degree in 1947. Vince worked as a camp counselor and at an industrial lab to earn money during the vacations, but he studied full-time during the academic year. He majored in biochemistry, which interested him the most because it involved studying the chemistry of life. As a discipline, biochemistry had existed since the turn of the century, but it grew dramatically in the 1950s and he was in at the beginning of that expansion. He found it exciting then, and continued to find it exciting until the end of his life.

Vince's first professional position was as a research biochemist at a government animal-health laboratory in Sydney. His first five publications date from this short period (1947–50), in which he studied inhibition of the TCA cycle in nematodes by fluoroacetate (1949). It was during this time that he met and married his wife, Margot Grünewald, a German-Jewish refugee who had come to live with her aunt and uncle in Sydney and whose cousin was a college classmate of Vince. His employer, the Commonwealth Scientific and Industrial Research Organization (CSIRO), gave him a two-year fellowship to study for his doctorate in biochemistry at the University of Cambridge in England. This was at a time when higher degrees were not awarded by Australian universities. He and his bride set off for England in 1950, never to live in Australia again.

Postgraduate career

At Cambridge, Vince was a member of Emmanuel College. It was only a short bike ride from college to the Biochemistry Department on Tennis Court Road and only a slightly longer ride to the house he shared with Margot on Histon Road, just off the Chesterson Road. They were soon joined by their first child, Charlotte.

The following description by Vince of biochemistry at Cambridge in the '50s is taken in part from the memoir of Malcolm Dixon. Vince also presented it in his after-dinner speech at the 2002 International Symposium on Flavins and Flavoproteins in Cambridge, shortly before he died. It has been edited slightly:

> *By coincidence, it is exactly 50 years ago that I got my Ph.D. degree, here at the University of Cambridge, so I thought it might be interesting for many of you to know what Cambridge and biochemistry were like in the 1950s.*

I did my thesis work with Malcolm Dixon, who at that time ran the dynamic Unit of Enzymology, and who, along with Edwin Webb, was writing the classical book called simply Enzymes*, which was for many years the standard reference work on enzymology.In Malcolm's lab I did not work on a flavoprotein, rather on a colorless but very fascinating enzyme, fumarase. However, there were several people in the lab who were working on flavins and flavoproteins, so I did get a lot of exposure to these fascinating molecules, and Malcolm himself was of course the first person ever to purify and study xanthine oxidase.*

Figure 2. Left to right in this photograph are Jean Ballou, Vincent Massey, Richard Perham, and Charles Williams. The picture was taken by Dave Ballou outside the chapel of St. John's College, Cambridge, at the garden party preceding the banquet of the 2002 International Symposium on Flavins and Flavoproteins.

In the lab at that time was Gordon Whitby, who did his thesis work on the isolation and characterization of FAD. He was the first to isolate it in pure form and determine its extinction coefficient. Also there, and a great influence to me throughout my whole life, was Gregorio Weber, already an expert in fluorescence, who was at that time developing theoretical and practical aspects of fluorescence polarization. It was Gregorio who demonstrated that the tenfold lower fluorescence intensity of FAD compared with riboflavin or FMN was due to an internal complex between the flavin and adenine ring systems in FAD. Also in the lab was a young South African woman, Nerina Savage, whose project was the further purification and characterization of Straub diaphorase. Although

I did not know it at the time, her work also had great relevance to later work of mine. Bob Morton was working on yeast lactate dehydrogenase.

It is difficult to believe now, but at that time there were only a dozen or so known flavoproteins, and many of the ones that were known had no known physiological function. Let me list them: The old yellow enzyme of Warburg and Theorell, the new yellow enzyme of Haas, D-amino acid oxidase, xanthine oxidase, Straub diaphorase, L-amino acid oxidase from snake venom, the three acylCoA dehydrogenases characterized by Helmut Beinert, NADH-cytochrome c reductase, succinate dehydrogenase, lactate dehydrogenase, glycolate oxidase, and glucose oxidase.

In 1952 Cambridge was undoubtedly the world center of biochemistry. Biochemistry as a discipline had been largely developed at Cambridge under the leadership of Fredrick Gowland Hopkins, who died shortly before I arrived. The first International Congress of Biochemistry had been held there in 1949.

While I was in Cambridge, 1950–1955, there was a constant stream of visitors from overseas coming to spend sabbatical leaves, including Frank Putnam, Al Lehninger, Chris Anfinsen, Emanuel Margoliash, and, in the flavin field, Tom Singer and Edna Kearney.

The intellectual atmosphere in Cambridge at the time was as exciting as you could ever expect to experience. John Kendrew and Max Perutz, just down the road in the Cavendish Laboratory, were the first people to determine the structure of proteins, and not to forget Jim Watson and Francis Crick. Fred Sanger was using the newly developed methodology of paper chromatography and his FDNB labeling technique to determine for the first time the amino acid sequence of a protein. I saw this on a daily basis, since he did most of it on a bench directly across from mine. Then there was Peter Mitchell, a flamboyant character with long hair, already laying the foundations of the chemiosmotic hypothesis. In the Molteno Institute, less than 100 yards away, were David Keilin and Bill Slater, who with the aid of Cees Veeger organized the very first Flavin Symposium in Amsterdam in 1965. Keilin and Malcolm Dixon were old friends, and when I succeeded in crystallizing fumarase, Malcolm was excited enough that he wanted to show the crystals off to Keilin.

In Australia I had been working for three years in the CSIRO, on the metabolism of nematode parasites. In the course of this I had found that fluoroacetate was a potent inhibitor, and had tracked down its mode of action to blocking citrate utilization. I thought that this would be a good starting point for a Ph.D. project, but Malcolm, because of his friendship with Sir Rudolph Peters, and Peters' continued active interest in fluoroacetate toxicity, ruled that out as a suitable topic. But typically he did introduce me to Sir Rudolph at the earliest opportunity. Peters, of course, showed a very gracious interest in my work; indeed this interest was maintained through the rest of his life, and he was often of much help to me, supplying rare chemicals and being generally supportive.

So instead of working on fluoroacetate, Malcolm set me to work on fumarase. This enzyme had been partly purified from pig heart the previous year as a Part II Class exercise, and Malcolm thought that it would be an interesting enzyme to study. How right he was! Within six weeks of arrival I had pure crystals, and Malcolm was quite delighted. I suspect that he was somewhat amazed by these young Australians in the department (Bob Morton, Ted Thompson, Frank Hurd, and I) who used to work both night and day, a most unusual thing in Cambridge at that time! Anyway, he quickly arranged a trip to Oxford, where Sandy Ogston and Rupert Cecil determined both sedimentation and diffusion coefficients of the enzyme, and showed its homogeneity. In [my] spare time, [I] carried out a research project in collaboration with a fellow graduate student, Brian Hartley, on active-site labeling of chymotrypsin.

We cannot resist one more bit of history in Vince's own words on the occasion of his presenting the American Society of Biochemistry and Molecular Biology's Fritz Lipmann award to Helmut Beinert:

Lipmann was one of the great pioneers in the field of intermediary metabolism, starting his career in Germany with Otto Meyerhof on the reactions and intermediates of glycolysis, and thus an early recognizer of the importance of energy-rich phosphate linkages and the potential importance of protein-phosphorylation. At the time that I was getting my B.S. degree in Australia in 1947, [Lipmann] had succeeded in the isolation of coenzyme A, and by the time I had gotten my Ph.D. in England in 1953, he was awarded the Nobel Prize together with Hans Krebs.

After completing his doctorate, Vince went to the United States and spent a summer in the Department of Chemistry at the University of Wisconsin-Madison, continuing his work on fumarase with Bob Alberty. The 1954 paper of Alberty and Massey is a classic of enzymology, perhaps the first thorough steady-state kinetic study of an enzyme as a function of pH. Tom Singer and his wife Edna Kearney, who Vince had met in Cambridge, were by this time working on flavoproteins at the nearby Enzyme Institute. They had discovered that the FAD of succinic dehydrogenase was covalently bound to the protein. Shortly afterward, Singer was hired to head the Edsel B. Ford Institute for Medical Research at Henry Ford Hospital in Detroit, and he recruited Vince as part of his initial group. Vince worked with Tom on succinic dehydrogenase, and this initiated his career in flavins and flavoproteins, an area in which Vince was to become recognized as the leading figure. Most biochemists become specialized in one area and Vince was no exception. "There are no renaissance men in biochemistry," he once said.

Academic career

Vince returned to England in 1957 to accept the position of lecturer at the University of Sheffield, where he established his first independent research laboratory as part of the Biochemistry Department, which was headed by Quentin Gibson. Vince's close friend Gregorio Weber had moved there a few years earlier. Having been schooled in the British tradition, Vince took teaching very seriously, and not surprisingly the third year honors practical work revolved around flavoproteins. He was promoted to senior lecturer in 1961.

In 1963 Vince made a major career change by moving to Ann Arbor as professor of biological chemistry at the University of Michigan. Professor Minor J. Coon, although not yet department chair in Ann Arbor, had visited Vince several times in Sheffield and was instrumental in Vince's recruitment. Vince was, alas, part of the "brain drain" that so devastated biochemistry in Sheffield, as well as many other departments in British universities. He became very active at the University of Michigan in the affairs of the department, the medical school, and the graduate school. He was named the J. Lawrence Oncley Distinguished University Professor of Biological Chemistry in 1995.

Vince held the title of permanent guest professor at the University of Konstanz (Germany) from 1975. This involved a residency at the university for one term per year and a series of lectures. It has already been noted that he took teaching seriously and he did, occasionally to the point of going over the students' heads; allegedly, this was often the case in Konstanz. Nevertheless, this guest appointment provided ample opportunity for collaboration with his close associates Peter Hemmerich and Sandro Ghisla.

Vince was the recipient of many awards and honors, including the Alexander von Humboldt Award in 1973, election as fellow of the Royal Society in 1977, election as senior fellow of the Michigan Society of Fellows 1975–1980, and the University of Michigan Distinguished Faculty Achievement Award in 1983. Tokushima University presented him with an honorary doctor of science degree in 1994 in recognition of his many collaborations with Japanese scientists. He was awarded the Henry Russel Lectureship, the highest recognition given to faculty members at the University of Michigan, in 1995. Vince was also elected to membership of the National Academy of Sciences (USA) in 1995. He was chosen by the Biochemical Society of Great Britain for the Harden Medal and for the Jubilee Lecture in London in 1999.

Scientific achievements

Vince's first major independent discovery was that Straub diaphorase (a flavoprotein that had been known for more than a decade) was in reality dihydrolipoamide dehydrogenase, and further that this enzyme was a component of both of the pyruvate and 2-ketoglutarate dehydrogenase complexes (1960). His subsequent mechanistic studies on lipoamide dehydrogenase launched flavoprotein enzymology into its ascendancy. His meticulous notebooks from this era survive, and they make a fascinating record of the history of these discoveries. A number of letters were tucked into the notebooks revealing his correspondence with others in the field, including Lester Reed and I. C. Gunsalus. A letter from Vince to Paul Boyer explained, in rather colorful prose, David Green's misunderstanding of his work. Later, they made a bet that Vince won. One dollar of the $5 payoff (at the International Biochemistry Symposium in Moscow) was framed on Vince's office wall. In spite of all of this, Vince and David had profound respect for each other, so much so that David's daughter, Rowena Matthews, became Vince's graduate student; the paper by Matthews and Massey (1969) is now a classic in the enzyme charge-transfer field. Rowena has gone on to a highly impressive scientific career and is also a member of the National Academy of Sciences (USA).

By virtue of his own graduate education with Malcolm Dixon, Vince was skilled in the steady-state kinetics of enzymes that convert a single substrate to a single product. However, flavoproteins utilize two or more substrates and produce at least two products. Fortunately, the Biochemistry Department at the University of Sheffield in the early '60s was superbly qualified to guide Vince in the study of such systems. Among the faculty were two scientists who dramatically influenced his strategies in the study of flavoproteins: Keith Dalziel, one of the outstanding practitioners of steady-state kinetics of

enzymes with multiple substrates and products; and Quentin Gibson, who developed the stoppedflow technique to study rapid reactions.

In his early studies on dihydrolipoamide dehydrogenase, Vince showed how these two approaches could be used synergistically to define with remarkable precision the kinetic mechanism. He showed that the overall reaction could be separated into two half-reactions: first, dihydrolipoamide reduces the enzyme; and then the reduced enzyme reduces NAD to NADH, thus completing the cycle. This is a clear case of a ping-pong enzyme mechanism whereby one substrate reduces the enzyme and its product is released, while a second substrate is converted to product as the enzyme returns to the oxidized state. This mechanism applies to many flavoproteins, and because the flavin gives a clear spectroscopic signature of each catalytically important intermediate, it is possible to study the individual halfreactions in detail.

The development of kinetic techniques continued in Vince's studies of the flavoprotein hydroxylases, particularly 4-hydroxybenzoate hydroxylase (1976), leading to some of the most detailed mechanistic studies in any area of enzymology. He and his colleagues, especially Dave Ballou and Barrie Entsch, developed methods for resolving transient spectral intermediates by stopped-flow optical techniques. These strategies are now widely used and have become paradigms for the elucidation of flavoenzyme reaction mechanisms.

Vince exploited with considerable elegance the light-absorbing characteristics of flavins in their several redox states. In particular, a close collaboration with Graham Palmer demonstrated, by virtue of their characteristic colors, (a) the participation of the half-reduced flavin (semiquinone) and (b) the existence of charge-transfer complexes formed with exogenous reagents or with internal electron donors. They showed that the blue flavoprotein semiquinones were neutral and that the red semiquinones were anionic (1966). Figure 3 displays the spectra first established by Massey and Graham. This was the first demonstration that flavoproteins could be classified by their spectral properties: oxidases have red semiquinones and dehydrogenases have blue, except, as shown, the blue semiquinone could be induced by low pH in glucose *oxidase*; this led Vince to additional and more complex classifications (1969).

The study of flavin radicals led to the important realization that significant quantities of superoxide are produced when oxygen reacts with reduced flavins and flavoproteins. Vince and colleagues demonstrated unambiguously that the superoxide generated by reduced flavins reacts directly with cytochrome c and with erythrocuprein (1969), a protein that Irwin Fridovich and Joe McCord had demonstrated to have superoxide dismutase activity. The reactions of reduced flavins and flavoproteins with oxygen were always of great interest to Vince, such that his seminal studies led to an understanding of how the several classes of flavoproteins react with oxygen (Figure 4). His findings have evolved into many further studies.

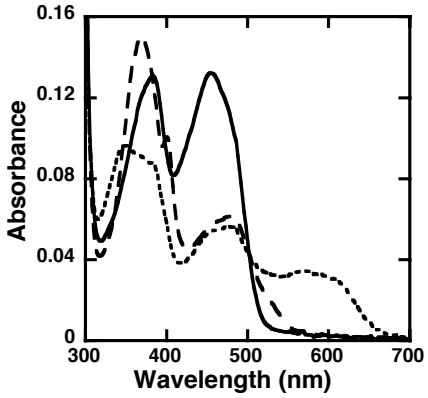


Figure 3. Spectra of glucose oxidase in the oxidized (solid line), neutral or blue (dotted line), and anion or red (dashed line) semiquinone states.

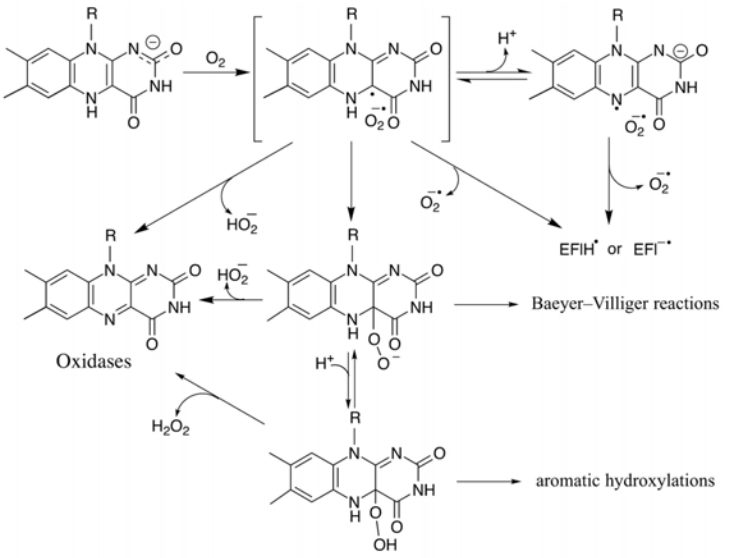


Figure 4. Reactivity of reduced flavin with oxygen.

Working with Peter Hemmerich (in Konstanz) and Graham Palmer (in Ann Arbor), Vince also found that flavins can react photochemically with EDTA and other electron donors to become reduced. He showed that 5-deazaflavin in such reactions provides a gentle universal reductant for most biological redox systems; this forms the basis for a now widely used method for reducing redox proteins (1977). Vince also pioneered the use of a wide variety of modified flavins to probe the active sites of flavoproteins, as well as to show the regiospecificity and stereospecificity of hydride transfer from pyridine nucleotides to flavins (Figure 5). These modified flavins (referred to affectionately as "funny flavins) were used to characterize flavin environments, intermediates in reactions, and accessibility of various regions of the flavin to solvent (1986). In his last 15 years, a large fraction of his research employed funny flavins, in part because they had such interesting spectra.

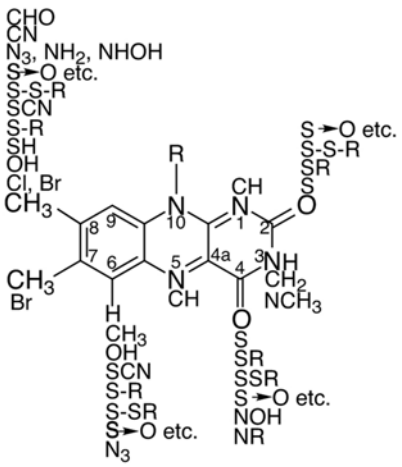


Figure 5: Modifications of the flavin ring.

Vince's flair for collaboration was important in all of his work on flavin chemistry, in which he often teamed with Peter Hemmerich, Franz Müller, and Sandro Ghisla. With Gertrude Elion and Graham Palmer he demonstrated the mechanism of inhibition of xanthine oxidase by the important pharmaceutical, allopurinol (1970). Later, Palmer, Dave Ballou, Dale Edmondson, John Olson, and Vince showed by optical and EPR kinetic methods that the mechanism of this multi-centered redox protein could be described by a rapid equilibrium model (1974). Electrons are believed to enter as hydride equivalents from xanthine to reduce molybdenum and very rapidly distribute statistically to the other redox centers (FAD and two [2Fe-2S]) of the enzyme according to their redox potentials. The reaction with oxygen reverses this process by similar rapid equilibria.

Many studies of other multi-centered redox proteins have utilized these concepts. Vince also showed how xanthine oxidase could be used as a veritable working electrode to deliver electrons slowly to a wide variety of biological systems via a low-potential dye such as methyl viologen. In the presence of an indicator redox dye, the continuously varying

spectra associated with the simultaneous slow reduction of the dye and the protein prosthetic group permitted calculation of the redox potential of the prosthetic group.

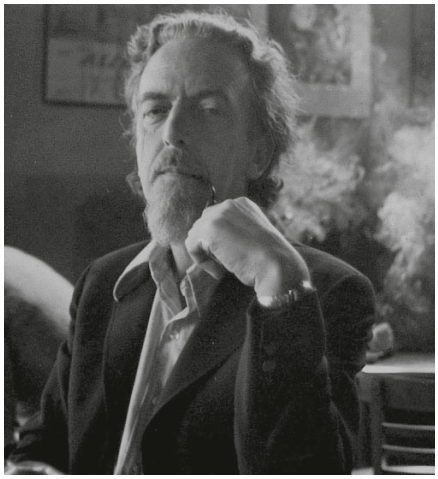
Vince had an abiding faith in basic research—study whatever turns you on and what you think is important. This concept may currently be out of favor, but when Vince applied in 2002 for renewal of his National Institutes of Health grant, for years 40–43, one of us (CW) urged him to put in something trendy. "Why?" "Well, because pure basic research is not in favor." "So what!" The application was scored in the 96th percentile and was of course funded.

All of Vince's colleagues knew that he would die in harness, and so he did. During his last afternoon in the lab, while one of us (CW) was making overheads to back up a PowerPoint presentation, Vince was, as usual, doing an experiment—this time on old yellow enzyme. It is ironic that we still do not know the function of this enzyme—a problem that Vince worked hard to correct for a very long time. He made significant progress but the final solution eluded him. That Friday afternoon, Vince's experiment was going so well he called CW over to look at the computer screen. There was a beautiful array of spectra that CW duly admired.

Our laboratories and several others held a joint research meeting weekly, continuing a practice that Vince had started in Sheffield; no paper ever emerged from our labs until it had passed muster in Chatters (or The Chats), where we all talked about our research without any time constraints. In the early days in Ann Arbor, we met on Monday evenings and often went past 10 pm. Every Friday Vince would prowl around seeing who had fresh data. Because he knew what everyone was doing, there was no escaping him; anyone caught had the weekend to get it together. There was always beer and soda pop until the university decided that alcohol should be banned. But Chatters went on anyway—beer flowed later at a local bar—and maintained its lively and high-level critical-but-constructive analysis of research. Problems were usually solved by the presenter going to another lab within the group to carry out new experiments. When Vince got interested in the new prime-time TV show *Laugh In*, Chatters was rescheduled to Tuesday evening; there was never any question of having it in the daytime, lest it interfere with work.

It is not possible to understand Vince's approach to research without knowing his predilection for collaboration. He knew that if you wanted to ask truly hard questions, you would probably need help in answering them. Although both of this memoir's authors had our own laboratories and did most of our work independently, our joint research

with him was indeed crucial to our success. In the case of one of us (DB), the collaboration with Vince extended over almost 40 years and involved a large number of publications.



Photograph by Charlotte Massey.

Vince's first major collaboration was with Cees Veeger, who as a graduate student with Bill Slater in Amsterdam came to Sheffield to collaborate with Vince and Quentin Gibson in 1960. Cees thus recruited Vince to his thesis committee. Their collaboration worked out the mechanism of lipoamide dehydrogenase. Perhaps Vince's most intense collaboration was with the preeminent flavin chemist Peter Hemmerich. After Peter's untimely death in 1981, the work continued with Peter's student Sandro Ghisla. In his contribution to the Massey remembrance in *Flavins and Flavoproteins* 2002, Sandro captured the essence of the Hemmerich collaboration:

> *Peter described Vince, with his typical exaggeration, and with poorly concealed admiration, as an Australian playboy, continuously smoking a pipe while driving red sports cars and reading Playboy magazine....Vince and Peter grew personally closer, while their scientific diatribes became more and more emphatic. Peter was telling Vince he did not know anything about chemistry, while Vince was responding—more diplomatically, but firmly—that the same held [true] for Hemmerich and biochemistry. Nevertheless, they did complement each other; they were both smart enough to give in when a case was hopeless. This led to a most fruitful interaction that lasted for a decade. Each was forcing the other to do the right experiment in order to prove the counterpart wrong. Obviously, Vince was at an advantage in this respect, due to his ability to design and carry out the right experiment in no time.*

We know that Vince loved England and Australia dearly, but we think his favorite countries were Italy and Japan. Thus it is not surprising that he cultivated extensive long-term research partnerships in both countries. The Italian connection began with the late Bruno Curti's sabbatical year in Sheffield and continued, involving a host of Bruno's colleagues, for the rest of Vince's life. In Bruno's remembrance, he described the connection this way:

> *The establishment in Ann Arbor of an 'Italian colony,' as Vince used to call it, derived from an appreciation of the good work made by the Italian researchers; but it was also due to the personality of Vince, who born in Australia, educated in England, resided in the United States, [but] remained in his heart a man with a great love for Europe, Shakespeare, the Italian Renaissance, and the music of Vivaldi, Bach, and Mozart. In Ann Arbor, the Italians [in Vince's laboratory] included Giuliana Zanetti, Severino Ronchi, Umberto Branzoli, Armando Negri, Gabriella Tedeschi, Loredano Pollegioni, and the late Luigi Casola; and in the laboratory of Rowena Matthews [there was] Maria Antonietta Vanoni. They all felt at home with Vince, even living on the other side of the ocean.*

We should add that Vince's wife Margot was fluent in several languages, and she enhanced the feeling of home by speaking to them in Italian.

The Japanese connection began as a research rivalry between Vince and Kunio Yagi; they had sharply differing views about the mechanism of D-amino acid oxidase. Yagi called the relationship one of "friendly enemies." Vince later took a half-year sabbatical in Yagi's laboratory. At meetings in Japan, Vince got to know many younger Japanese—in particular Takeshi Nishino and Tomoko Nishino in Yokohama and Tokyo, Kazuko Yorita in Tokushima, and Youichi Niimura in Hokkaido and Tokyo. In his remembrance, Takeshi described an all-too-believable incident:

> *Vincent visited our laboratory at Yokohama City University Medical School near Tokyo several times for discussions about experimental results or to give lectures to the medical students. He was very nervous every time he gave a lecture and spent a long time in preparation of the materials. He paid no attention to the fact that some parts of his lectures were too advanced for the medical school students to understand; still, he imparted a lot of the spirit of science. He spent three months as the first-guest professor from a foreign country at the Yokohama City University Medical School in 1988.*

During Vince's stay, he and Takeshi performed a number of experiments together and published papers amounting to 46 pages in the *Journal of Biological Chemistry*. Once Takeshi and Vince drove to the countryside in Takeshi's sports car. Vince complained to Takeshi about his fast driving, saying, "you drive like you were on the Autobahn." But

when Vince drove the car in Japan even faster than Takeshi, he got a speeding ticket of 10,000 yen.

Collaborations with two crystallographers were crucial to Vince's work: Martha Ludwig in Ann Arbor and Andrew Karplus, first at Cornell and later at Oregon State University. Martha and Vince studied several structures of a clostridial flavodoxin, including one of a flavodoxin reconstituted with 1-deaza-FMN replacing the FMN; he also worked with Martha and Dave Ballou to characterize the so-called "wavin flavin" in 4-hydroxybenzoate hydroxylase (1969, 1994). This elucidation of the wavin flavin in this protein led to a much better understanding both of its mechanism and the importance of dynamics in enzymes. Vince also studied the structure of old yellow enzyme with Andy (1995).

Beginning in 1965, Vince was a key organizer of all 14 of the International Symposia on Flavins and Flavoproteins during his lifetime, including the one he hosted with Charles Williams in Ann Arbor in 1981 and the one he attended in 2002 in Cambridge, England, where he was the keynote speaker. The 15th symposium in 2005 was organized by Takashi Nishino and was dedicated to Vince's memory.

Vince was one of the outstanding biochemists of his generation. His colleagues and students found inspiration in his enthusiasm for basic research, admired his unusual skill at experimentation, and valued his investigations as models of originality and precision. His mentorship at the bench distinguished his research style from that of most other biochemists. Most of the more than 340 refereed papers that he authored included many or all of the experiments performed with his own hands. He established Ann Arbor as the Mecca for flavin research.

Private life and personality

We said at the beginning that Vince was a man of many passions. Having dealt with his major passion for hard work on flavoproteins, we can now take up the personal ones: cooking and consuming good food and wine, music and art, gardening, sailing, and, above all, family and friends.

It seemed remarkable that Vince, married to a superb cook like Margot, would himself enjoy cooking, except that many chemists do. After Margot's severe stroke in 2001, Vince took over the cooking totally. It was at least a month before he relented and even allowed his children to make contributions toward a meal. The level of hospitality in the Massey household was awesome. Guests were often put up for a month at a time; that way they

could discuss research around the clock. Those of us who have experienced such hospitality consider it one of the rare privileges of our lives. Massey hospitality even extended to a dinner for CW upon his arrival in Sheffield as a new postdoc in 1961. It was a special occasion for more than one reason: after coffee, Margot remarked, "Vince will take you home now because I am going to have a baby." Rachel was born later that night.

Vince's taste in music was somewhat specialized but his taste in art was catholic. The 18th century, encompassing Bach, Handel, Mozart, and Haydn, was his musical focus, in that order; after that, he was highly selective, with very little beyond Beethoven, Schubert, Brahms, Dvorak, and Mahler catching his fancy. The Massey home was filled with books about art from a wide variety of artists.

Vince was an avid gardener. It is undoubtedly hyperbole, but it is said that if he looked sternly at a plant one day, it bloomed the next. He was always keen to guide his friends through his gardens, pointing out the roses and the wide variety of lilies. Vince was a bit competitive about his gardening. His night-blooming cereus was huge. When he observed that a bloom was coming around 8 or 9 o'clock in the evening, he would call a cadre of close friends to come and watch—and, while watching, to imbibe a few bottles of excellent wine, which undoubtedly enhanced the appearance of the blooms.

Atypically, Vince was not competitive about sailing, at least later in life. He started sailing in Sydney Harbor, where sharks were not unheard of. Margot hated sailing, in part because Vince had capsized with her on their first outing. Later, in Michigan, he liked to sail with CW in his International 470 (an Olympic class racing boat) around Whitmore Lake and discuss research or the philosophy of life. The only time we capsized was when we were too engrossed in chat; all sailors should know that the wind does not like to be ignored.

While science was always foremost in Vince's life, he delighted in the role of father and grandfather. Well do CW and his then wife-to-be Angela, arriving to babysit, remember finding Vince giving the children their baths, as he did every night. Thirty years later, his youngest grandson Owen, who lived in Ann Arbor played a very big part in Vince and Margot's life. Then in recent years, both daughter Charlotte and son Andrew and their families moved back to Michigan (sadly, only a few months before Vince's death), making possible a good number of grand get-togethers of the four grandchildren and their parents at the Massey house.

Vince died quietly in his sleep of an apparent heart attack at the age of 75 after a particularly happy weekend with family and close friends. A memorial to celebrate his life took place in Ann Arbor a few days later. About 200 people from as far away as Japan gathered to say goodbye to their good friend and teacher. Several of J. S. Bach's "Goldberg Variations" were interspersed through the service, and as the audience departed to the reception that followed, Percy Grainger's "Country Gardens" cheered us on. Vince's ashes were interred on a beautiful hillside in the Forest Hill Cemetery, Ann Arbor. With so many guests in town, it seemed inappropriate to part at four in the afternoon, so, in time-honored Vincent Massey tradition, the group reconvened for a party that lasted into the wee hours.

We end as we began with a quote from Carol Strickland: "They say life's not supposed to be a rose garden. But in a way—with the beauty, the thorns, and finally the petals dropping—it is. I think the rose garden Vince planted will bloom in us for a long time."

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