Paul Talalay

1923–2019

BIOGRAPHICAL *Memoirs*

A Biographical Memoir by
Theresa Shapiro
and Philip Cole

NATIONAL ACADEMY OF SCIENCES

PAUL **TALALAY**

March 31, 1923–March 10, 2019

Elected to the NAS, 1987

Photograph courtesy of Tony Talalay

By Theresa Shapiro and Philip Cole

Paul Talalay was a leading biomedical scientist who made important contributions in two major areas, steroid enzymology and cancer chemoprotection—the identification or development of substances that help prevent the occurrence or reoccurrence of cancer. Talalay's discovery and analysis of ketosteroid isomerase have led to important paradigms in our understanding of enzyme mechanisms. His identification and characterization of sulforaphane as an inducer of protective enzymes helped launch the development of the field of dietary chemoprotection. He was an exceptional educator, mentor, and scientific program builder who has had a dramatic influence on generations of scientists.

Talalay earned his undergraduate degree in biophysics at MIT in 1944 before starting medical school at the University of Chicago; he transferred to Yale and received his M.D. degree in 1948. He completed his training with a urology-focused residency in surgery at the Massachusetts General Hospital before joining the University of Chicago faculty in 1951. He moved to the Johns Hopkins School of Medicine in 1963 to become director of its department of pharmacology and remained on the Hopkins faculty for the rest of his life.

Paul Talalay was the youngest of the four sons of Joseph and Sophie Talalay. His father and eventually his older brothers were accomplished inventors and engineers, holding numerous patents for making foam rubber, including some for procedures still in use. As related in a touching online biography written with Paul's assistance by his then ten-year-old granddaughter, after the 1917 Russian revolution the family emigrated west, eventually to Berlin, where Paul was born in 1923 [1]. He was schooled at home to avoid trouble from growing antisemitism, and one of his chores was to wash the dinner dishes. Memorably, on his tenth birthday in 1933, just months after Hitler became leader of Nazi Germany; Joseph excused Paul from this task, saying he would never see

those dishes again. That night, and by means of a Haitian passport presciently obtained by Joseph several years earlier, the family escaped from Germany, eventually settling in England before coming to the United States in 1940. In 1952, he returned to England for a year to study bacterial metabolism with E. G. Gale at Cambridge University, where he met and two weeks later decided to marry Ph.D. candidate Pamela Samuels. She assented.

Enzymes and Steroids

The period from the 1940s to the 1960s was a fertile time in the field of enzymology. It became increasingly common during this era to link an *in vivo* chemical reaction to a purified protein. Kinetic and chemical mechanisms of enzymes were being intensively investigated. The development of clever enzymatic activity assays involving spectroscopic changes was a key component of the progress during this time. After gaining a baccalaureate in biophysics at MIT in 1944, Paul made his entrance into the world of biomedical research as a first-year medical student at the University of Chicago. The legendary bioorganic chemist Frank Westheimer was at the university at that time. The future Nobel Laureate Konrad Bloch, who would become known for his work in cholesterol biosynthesis, would arrive three years later. Paul's most important and enduring mentor, however, was the surgeon Charles Huggins. Huggins, who would go on to receive a Nobel Prize in Medicine in 1966, had a decade earlier shown that testosterone and related androgens were important determinants in the growth of prostate cancer. This finding led Huggins to gain an interest in the enzymes involved in steroid hormone biosynthesis.

Paul's first crack at enzymology was to develop a facile kinetic assay to monitor the activity of serum phosphatases. It was long known that prostate cancer could invade bone as a frequent site of metastasis. A biomarker of prostate cancer metastasis was projected to be blood levels of phosphatase activity released upon bone breakdown. Paul synthesized a novel phosphatase substrate (a phosphate monoester of the pH indicator dye phenolphthalein) that upon enzymatic hydrolysis led to a marked color change. The resulting colorimetric assay, coauthored by Huggins and Paul and published in the *Journal of Biological Chemistry* in 1945, was an early example of the transformative power of a simple method to measure enzymatic activity [2].

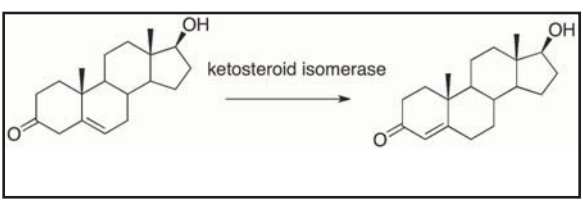
His father's declining health brought Paul to New Haven in 1946, where he completed medical school at Yale. He subsequently did a surgical residency at the Massachusetts General Hospital and in 1950 returned to the University of Chicago and the mentorship

of Charles Huggins, working in the Ben May Cancer Center as a junior faculty member. In this phase of his academic career, Paul focused on studying enzymes involved in steroid hormone transformations. He investigated the NAD (nicotinamide adenine dinucleotide)-dependent dehydrogenase enzymes that could reversibly convert the 3- or 17-hydroxy groups in steroids to their corresponding ketones.

At the time, working with the nearly intractable membrane-bound mixtures of steroid-transforming enzymes in mammalian systems was a major impediment to biochemical characterization. Paul therefore elected to focus on enzymes derived from bacteria rather than from mammalian tissues because of their high rate of activity and their greater amenability to purification and analysis. In short order, his new lab showed robust steroid hormone oxidoreductase activity that was dependent on NAD/NADH ratios [3]. He and his colleagues determined the substrate selectivities, catalytic efficiencies, equilibrium constants, and hydride-transfer mechanisms of these enzymes.

In collaboration with his colleague Guy Williams-Ashman, Paul proposed the provocative idea that cellular sensing of estradiol in target tissues might be achieved by enzyme-mediated alterations of NAD/NADH ratios in the conversion of estradiol to estrone [4]. This controversial proposal predated the discovery of estrogen hormone receptors several years later by Paul's Ben May Cancer Center colleague Ellwood Jensen. While it is fair to say that the concept of redox sensing by estradiol has been supplanted by the receptor model of estrogen action, it is notable that related proposed models have reemerged over the past decade in connecting NAD/NADH status, epigenetics, and gene expression regulation via the histone deacetylating sirtuin enzymes.

Paul's most sustained contribution to the field of steroid enzymes and the larger field of enzymology stemmed from his 1955 discovery of a double-bond isomerase in the bacterium Pseudomonas *testeroni* [5]. He recounted that he selected this Pseudomonas isolate from soil that had been regularly exposed to male dog urine, reasoning that bacteria in this environment would be enriched in testosterone-catabolizing enzymes. The double-bond-isomerizing enzyme, ultimately known as ketosteroid isomerase or KSI, converts delta-5-3-ketone steroids to delta-4-3-ketone steroids with high catalytic efficiency.



Highly proficient ketosteroid isomerase catalyzes the reversible isomerization of a double bond, here shown for testosterone.

In 1958 at the University of Chicago, shortly after receiving a lifetime award from the American Cancer Society to support his studies linking sex hormones with certain forms of cancer. (Photo provided by Tony Talalay.)

Unlike mammalian systems, in which delta-5 steroid isomerase activity is mediated by the same polypeptides that oxidize the 3-hydroxy group to the ketone (characterized years after KSI was discovered), Pseudomonas has separate enzymes for the redox and isomerase chemical reactions.

At the time of the discovery of KSI, acid- and base-catalyzed conjugation of keto-olfeins from the beta,gamma- to the alpha,beta-carbon positions was well-established in organic chemistry. Based on the chemical mechanism of these non-enzymatic reactions, it is commonly observed that deuterium from heavy-isotope water, D_2O, is incorporated into the organic compound at the gamma-methylenecarbon position upon double-bond isomerization. Such deuterium incorporation at the time could be determined by mass spectrometry and was deduced to result from solvent exchange by acid/base-mediated proton transfer to the gamma-carbon.

In Paul's initial characterization of KSI he showed that the enzymatic activity was distinct from the related non-enzymatic isomerization, since the hydrogen atoms of the steroid substrate were not substantially exchanged with the buffered aqueous solution [5]. This finding implied that a putative active site base in KSI is sufficiently shielded from bulk water to conserve the hydrogen at the 4-position (alpha-carbon) and move it to the 6-position (gamma-carbon) of the steroid.

Following the discovery of KSI, Paul's team was able to purify the enzyme by crystallization, which led to extremely homogeneous material that facilitated its detailed biochemical characterization [6]. Years before the availability of molecular cloning and routine DNA sequencing, Paul's group in 1971 was able to determine the complete 125 amino acid sequence of KSI by classical means, including Edman-degradation methods [7]. In addition, KSI was shown to be a homodimeric protein. It would be almost four decades after its crystallization that the high-resolution structure of KSI was determined both by NMR (nuclear magnetic resonance) and X-ray diffraction methods.

In 1963, however, Paul's lab performed a landmark experiment that provided unprecedented mechanistic insights into KSI catalysis. This experiment involved a UV-based

spectroscopic analysis of the binding of a series of steroid analogs to KSI. At the time of this work, KSI had been shown to lack a cofactor and was determined to be devoid of tryptophan residues. KSI was also known to have approximately ten tyrosine residues that could be monitored spectroscopically. The phenolic steroids estradiol and the horse estrogen dihydroequilenin (DHE) were known to be relatively potent competitive inhibitors of KSI, with K_i values in the 5-10 μM range. Such K_i values are considerably lower than the substrate androst-5-ene-3,17-dione K_m of 320 μM, suggesting that they might be akin to transition-state analogs, given their planar nature and enolic content.

Upon titrating estradiol or DHE with KSI, Paul saw that there was a dramatic bathochromic shift of absorbances. The shift was similar to that following the addition of sodium hydroxide to estradiol or DHE in the absence of KSI. Paul therefore attributed the spectroscopic behavior of the estradiol or DHE mixed with KSI to a phenolic moiety becoming deprotonated, forming a phenolate that was complexed to the enzyme active site [8]. Visualizing such an analog of a catalytic intermediate was a scientific breakthrough in the field of enzyme mechanisms. Paul presented this work during a visit to the Johns Hopkins University School of Medicine in 1962, where he was being considered for Director of the Pharmacology Department. The head of the search committee, Albert Lehninger, was apparently so excited about the experiment and molecular insights gleaned that he quickly moved to offer Paul the job, which Paul accepted.

In subsequent years extending into the 1990s, Paul continued interrogating the detailed enzyme mechanism of KSI via fruitful collaborations with fellow Johns Hopkins faculty members Cecil Robinson and Al Mildvan. With Robinson, he investigated a mechanism-based inactivator of KSI in the form of a secosteroid containing a beta, gamma-acetylenic ketone, which covalently labeled the KSI active site [9]. With Mildvan, Paul performed site-directed mutagenesis, which defined the active site base (aspartate-38) and general acid (tyrosine-14) that protonate the substrate carbonyl oxygen [10]. These elegant studies provided an incredibly deep understanding of the nature of KSI catalysis and established Paul as one of the preeminent enzymologists of the era.

Pharmacology

Paul was recruited to become Director of Pharmacology at Johns Hopkins in 1963. He took the position despite being strongly advised against it by peers and with little background in the field. He inherited a Department that was small and at an ebb scientifically but that had an impressive history. The Department of Pharmacology and Exper-

imental Therapeutics at Johns Hopkins was initiated by John Jacob Abel in 1893, widely considered the father of modern pharmacology in the United States. Among his accomplishments, Abel isolated and purified epinephrine and insulin. Abel was succeeded by E. K. Marshall, who in turn was followed by Gilbert Mudge. Paul would be just the fourth Pharmacology Director.

Paul was a self-made pharmacologist. When he came to Johns Hopkins to direct the department, his background included pharmacology as a component of his medical education at Yale, rigorous training in enzymology with Huggins, and clinical expertise as a surgeon. As Ward Haas, vice president for research and development at Warner-Lambert Pharmaceutical Company, noted in a 1970 letter to one of Paul's colleagues, "He's not exactly a classical pharmacologist…." Nevertheless, by the time Paul stepped down in 1975 he had revolutionized the medical student pharmacology course, established and obtained NIH funding for the first training program in pharmacology at Johns Hopkins, and appointed an unlikely but brilliant cadre of young assistant professors.

Within months of his arrival at Hopkins in 1963, a period shortly after the thalidomide tragedy and when the development and regulation of drugs in this country were under severe scrutiny (including by half a dozen congressional committees), the president and trustees of the university agreed that Johns Hopkins should host an elite conference to consider the complex problem of making safe and effective drugs available in our society. Paul was asked to organize this high-profile event, and it was the start of his self-education in the broad spectrum of pharmacology. His several-page proposal for the conference revealed a masterful command of the issues, with a thoughtful list of suggested world-class discussants, whose expertise included the expected academic and pharmaceutical aspects, but also included suggestions for the history of therapeutics, economics and advertising issues, and legal/ethical considerations. Results of the conference were later published in a well-received collection of essays, *Drugs in our Society* in 1964 [11].

Paul's strong and abiding belief in the importance of teaching was reflected in the med-student pharmacology course, which was the basis for his personal education in the particulars of pharmacology. He started from scratch with a newly designed curriculum and did most of the lecturing himself. Transcripts of his lectures, particularly those focusing on pharmacokinetics, reveal a novel approach to the subject, not evident (or available) in any of the contemporary pharmacology texts, and reflecting his

own methods for mastering this material. He himself attended every teaching exercise, without exception, and he required his faculty to attend all lectures. He considered the students to be partners in this activity, and the pharmacology course became, and for years remained, the most popular in the preclinical curriculum. For decades after relinquishing primary responsibility, Paul remained involved and supportive of med-student teaching.

Having established a high-quality medical pharmacology teaching program, and with a growing young faculty, Paul turned his attention to the training of pharmacologists. Initial efforts focused on postdoctoral fellows, supported from 1963 by an NIH training grant. In 1968 he obtained authority for the department to bestow Ph.D. degrees. Decidedly unusual for the time, the initial six graduate students included three M.D./Ph.D. candidates and four women.

In his 1971 description of the department he had built, Paul said that he had come to a deep appreciation of the science of pharmacology, writing, "The conceptual framework of modern pharmacology spans an extraordinarily wide range of problems and equally diversified approaches to their solution. Yet pharmacology owes its existence as an independent discipline to the fact that it is basically a medical subject born from the need for better understanding of the effects of chemical agents on man, irrespective of whether such interactions are relevant to therapeutics, to toxicology, or to the major social problems generated by the prolific use of addictive and psychedelic agents." This view was expanded in his 1976 chairman's summary of an invited review of another pharmacology department, "...academic pharmacology is a scientific continuum ranging from molecular processes to bedside problems. A simple division into molecular and cellular pharmacology *versus* clinical and physiological pharmacology is neither desirable, nor in the long run likely to be successful. The weaving of the basic scientific effort into a broader medical fabric is likely to yield important intellectual dividends for pharmacologists at both ends of the spectrum. It is in achieving this peculiar blend that pharmacology presents both its greatest challenges and opportunities."

During his years of service from 1963-1975, Paul proved to be a remarkable and visionary leader. Critical to this success were the faculty appointments that occurred under his directorship. This group of sterling but unorthodox faculty recruits included Cecil Robinson, Donald Coffey, Solomon Snyder, Catherine Fenselau, and Pedro Cuatrecasas. Paul had an uncanny ability to recognize and nurture talented people from unconventional backgrounds. Among the challenges he faced, Johns Hopkins has traditionally

been conservative in making available large financial start-up packages. Paul indicated that the resources to recruit faculty were particularly meager but that he would recycle funds after each appointment to somehow pull things off. He was quite a hands-on mentor for his young colleagues, to the point where if a pH meter broke, Paul would be the one to repair it.

Before joining Hopkins, Cecil Robinson was a medicinal chemist at Schering-Plough in New Jersey. He was a card-carrying organic chemist with a Ph.D. from Derek Barton at Imperial College, London. Organic chemistry was not considered a mainstream area in pharmacology departments in the early 1960s, but from Paul's perspective, Robinson's chemical rigor and creativity were major assets to the department and the medical school community. Robinson went on to become a leader in the bioorganic chemistry of steroids and steroidal enzymes. His pioneering work on aromatase and aromatase inhibitors laid the foundation for today's commonly prescribed and lifesaving anti-breast cancer agents.

Donald Coffey joined the Department of Pharmacology after completing his Ph.D. with Leslie Hellerman in the Hopkins Physiological Chemistry Department. Although his background was in flavin-dependent enzyme mechanisms, Coffey was passionate about understanding and treating cancer. He became an inspirational figure at Hopkins in promoting the study of cancer biology and is perhaps most responsible for the rise of the institution as an oncology mecca. His pioneering work in the area of the cell's nuclear matrix has had a profound impact in our understanding of chromatin structure. Coffey in turn helped mentor Hopkins luminaries Bert Vogelstein, Drew Pardoll, Bill Nelson, Alan Partin, and many other current leaders in cancer research.

What Coffey was to cancer, Solomon Snyder has been to the field of neuroscience. Snyder completed medical training at Georgetown and San Francisco and was a research fellow with Julius Axelrod at the NIH. He then moved to Johns Hopkins for a psychiatry residency but was interested in developing a neuropharmacology research program. Despite the unusual nature of the appointment, Paul recognized Snyder's awesome potential and invited him to be a faculty member in the Pharmacology Department while simultaneously completing his psychiatry training. In short order Snyder discovered several neurotransmitters as well as the existence of the opiate receptor, and has gone on to make major contributions to our understanding of neuronal signaling. He also became the founding chair of the Neuroscience Department at Johns Hopkins, which is now among the top such centers in the world.

Paul had the uncommon intuition in the 1960s to appreciate that mass spectrometry could revolutionize biomedical research. At that time, mass spectrometry was a technique confined to chemistry departments and primarily in the domain of hardcore gas-ion physical chemists. Paul sought to recruit a suitable young scholar with an interest in applying mass spectrometry to biological research. He identified Catherine Fenselau, who had received a Ph.D. from the lab of Carl Djerassi at Stanford, and enticed her to come to Hopkins Pharmacology, despite not yet having a mass spectrometer on campus. Paul and Fenselau reached out to a variety of philanthropic sources, government agencies, and companies to raise money for the purchase of a mass spectrometer. This perseverance paid off. At the time Fenselau was one of the few female faculty members in the basic sciences at Hopkins and only the second one to become a full professor (the first being Florence Sabin in 1917). Fenselau has made major contributions at Hopkins and later at the University of Maryland to the technology and application of mass spectrometry, including identifying the chemical mechanism of the major anti-cancer drug cyclophosphamide. Moreover, worldwide, mass spectrometry has become one of the most important modalities in modern biomedical research, validating Paul's instincts.

Pedro Cuatrecasas was recruited to lead clinical pharmacology, a shared division between the Departments of Medicine and Pharmacology at Hopkins. Cuatrecasas had the unusual background of having been a clinical medicine house officer at Johns Hopkins as well as being a research fellow with protein biochemist Chris Anfinsen, in whose lab he helped develop affinity chromatography. Cuatrecasas was at Hopkins for a relatively brief period, but while there, he carried out pioneering studies on the isolation and identification of the insulin receptor. He also mentored medical student and future Nobel laureate Peter Agre. Cuatrecasas went on to a high impact career in the pharmaceutical industry and helped develop dozens of commonly used drugs in the clinic.

Paul's assembly of this eclectic and influential group of scholars has left a local and global legacy in the biomedical sciences. It can readily be argued that he has been one of the most important program builders in the history of modern medicine.

Chemoprotection

Freed from the administrative demands of running the Pharmacology Department after stepping down in 1975, Paul wrote a foundational review article that provided a blueprint for the next 38 years of his research career [12]. He sought to determine what the chemical basis for chemoprotection was and how to optimize the human diet to prevent cancer and other diseases.

Paul's entrance into the field of chemoprotection had a component of serendipity. In 1977, he and collaborators made the incidental discovery that the double-bond isomerization of delta-5-3-one steroids by rat liver homogenates was mediated by the detoxifying enzyme glutathione S-transferase [13]. Glutathione and related transferases were well-established detoxification enzymes and had been recognized for their ability to derail carcinogens from causing mutations. Motivated by an appreciation that advanced cancer might always be difficult if not impossible to cure, Paul became fascinated by the possibility that it might be feasible to prevent the onset of cancer by adjusting one's diet. As Paul famously said about the early days of cancer prevention, "No room was small enough to accommodate those who were interested in the topic." Today the American Association for Cancer Research holds a meeting on cancer prevention every year, bringing together several thousand participants.

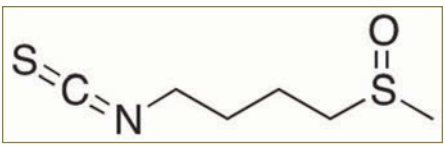
Paul was influenced by the work of cancer scientist Lee Wattenberg, at the University of Minnesota. Wattenberg, who came to be known as the "father of chemoprevention," reported that chemical substances, including phenothiazine, could induce metabolic enzymes that might disrupt the actions of environmental carcinogens. The general theory was that carcinogens that chemically react with DNA to produce mutations leading to cancer could be metabolically cleared by the body's own enzymes before they gained sufficient time and concentration to alter DNA structure. In Wattenberg's early work, his lab found that the antioxidant food additive butylated hydroxy toluene (BHT) or cruciferous vegetables—such as cauliflower, cabbage, kale, garden cress, bok choy, broccoli, brussels sprouts, and similar green leaf foods—containing compounds such as indole-3-carbinol, could induce the activity of cytochrome P450 enzymes. These enzymes could in turn detoxify carcinogens.

Paul worked closely in the beginning with Johns Hopkins colleague Ernest Bueding, who had independently discovered that the butylated hydroxyanisole (BHA) food additive could stimulate production of glutathione S-transferase and reduce mutagenicity of carcinogens. Several years later Paul and his team identified quinone reductase as a key enzymatic activity induced by BHA, and linked this enzyme (with which he was closely familiar from his work with Huggins) to potential metabolic detoxification [14].

Once again, the power of a robust quantitative high-throughput colorimetric-enzyme assay was crucial for the subsequent studies. Such assay was developed by Hans Prochaska, an M.D./Ph.D. student in Paul's lab, and became the basis for the search for compounds that could protect against cancer [15]. Based on a structure-activity rela-

tionship analysis of various inducers, Paul suggested specific electrophilic properties that seemed to be necessary for inducing phase II chemoprotective enzymes versus phase I aryl-hydrocarbon-receptor targeting [16]. Inspection of the chemical structures of the quinone-reductase inducer compounds led to the seminal observation that many were Michael addition acceptors containing α,β-unsaturated carbonyl moieties, and the prediction of the existence of a protein endowed with highly reactive cysteine residue(s) to serve as the intracellular inducer sensor [17]. This was the era of "ligand-receptor" interactions, and the concept of chemical sensing of inducers was not readily embraced.

As Paul had identified broccoli extracts as a particularly rich source of chemoprotective enzyme inducers, he worked in collaboration with Hopkins chemistry professor Gary Posner to isolate the principal compound endowed with this activity. Their studies led, after an extensive effort, to the identification in 1992 of a natural product called sulforaphane [18]. Sulforaphane was a previously described but poorly studied compound. It has a simple structure comprising a short-chain alkyl isothiocyanate linked to a methyl sulfoxide group.



Sulforaphane, a chemically active isothiocyanate, is released from glucoraphanin, its inert storage form in plant materials by enzyme myrosinase.

Paul and his colleagues showed that sulforaphane is a highly potent and efficacious inducer of chemoprotective enzymes. His team later found that glucoraphanin, the biosynthetic precursor form of sulforaphane and itself a relatively stable glucosinolate, predominates in broccoli.

Such dietary glucosinolates are enzymatically processed to the functional isothiocyanates upon plant tissue injury, such as occurs during homogenization or chewing. Thus, the discovery that sulforaphane is the principal inducer in broccoli extracts of chemoprotective enzymes was a fortuitous artifact of homogenization. It was followed by an exponential increase in scientific interest and research on sulforaphane, which continues today. In the early 1990s, with the generous philanthropy of Lewis and Dorothy Cullman, Paul inaugurated the Brassica Chemoprotection Laboratory at Johns Hopkins, dedicated to growing and optimizing broccoli as a source of chemoprotective compounds. This led to the recruitment of Jed Fahey, who brought extensive experience in plant biology. In 1997, Fahey and Paul showed that broccoli seeds and young sprouts were most rich in sulforaphane precursors, and this became the basis for many clinical studies involving broccoli sprouts [19].

Until the late 1990s, the molecular mechanisms that underpinned the stimulated expression of chemoprotective enzymes by sulforaphane and other Michael acceptors were unknown. The work of Masayuki Yamamoto and his colleagues in Japan led to key discoveries that catapulted forward our understanding of these processes. Yamamoto's team showed that the transcription factor Nrf2 is upregulated by cellular exposure to inducer compounds, in turn driving the expression of genes encoding a large network of cytoprotective proteins. Even more centrally, they reported in 1999 that a protein called Keap1 represses Nrf2, preventing it from entering the nucleus and turning on transcription [20]. They further demonstrated that inducer compounds added to cells could relieve the Keap1-mediated inhibition of Nrf2.

Hiking the Talalay trail on land he and Pamela donated to the Nature Conservancy, adjacent to his beloved home and summer retreat in rural Maine. (Photo provided by Tony Talalay.)

In 2002, Paul and Hopkins colleague Albena Dinkova-Kostova went on to show that the cysteine-rich Keap1 protein was the direct sensor of the small-molecule electrophiles such as sulforaphane [21]. With this finding, the molecular pathway principally responsible for cytoprotective enzyme induction was defined. A longstanding collaboration between Paul and Thomas Kensler led to an array of insights into the Keap1-Nrf2 signaling axis. In contrast to its clear potential to protect against cancer development, in more recent years Nrf2 has been identified as a proto-oncogene, which suggests that it can be a double-edged sword and its activity should be tightly controlled to ensure optimal function.

Building on these mechanistic studies, Paul's team and many other labs have investigated sulforaphane and related compounds in animal and human studies to determine their potential to protect against cancer and other diseases. It has been reported in numerous studies that such compounds may reduce the onset of cancer in animals exposed to a variety of carcinogens. In humans, sulforaphane can protect against inflammatory damage to the skin that can occur with ultraviolet radiation exposure. Most provocatively, the clinical features of autism in young men can be partially mitigated by broccoli sprout extracts [22]. Sulforaphane-containing broccoli-sprout extracts are currently being developed as frugal—low-cost—medicines to reduce the health risks associated with unavoidable exposures to environmental pollutants. The field of chemoprotection

Circa 2010 at the much-used blackboard in his laboratory at Johns Hopkins. (Photo provided by Tony Talalay.)

continues to grow in both basic discovery and clinical application and promises to play a major part in the future of medicine.

A second serendipitous offshoot of Paul's enzymology research was the collaborative development, with his then postdoc Ting-Chao (David) Chou, of a framework for analyzing the effects of two concurrently administered inhibitors. Initially devised to evaluate the impact of multiple simultaneous inhibitors against methionine adenosyltransferase (an enzyme inhibited by steroid hormone degradation products), they quickly realized the method could be generalized to a wide variety of biological systems, including most importantly the case of multiple drug combinations. The simple and elegant Chou-Talalay method allows facile assignment of a drug combination (applied in vitro or in vivo) as synergistic, additive, or antagonistic [23]. The method computes a combination index parameter by assessing the pharmacological effects of a range of fixed concentration ratios of two different drugs. A combination index of 1 indicates additivity, less than 1 synergy, and greater than 1 antagonism. The method is still in common use today, and citation numbers for the paper describing it are among the highest in the scientific literature [24].

Service to the Scientific Community

Paul was an outstanding citizen of the university. He served on a prodigious number of committees, including several that guided the institution through difficult decisions in the financially and socially troubled 60s and 70s. His warm correspondence over decades with university presidents and School of Medicine deans attests to their high regard for his thoughtful and wise analyses and recommendations. Opening paragraphs in his always beautifully written letters inevitably set the stage for the entire note, as in a 1971 letter to university president Milton Eisenhower: "I confess to a sense of unease..." regarding an incentive system designed to increase patient-care income. This was then followed by an insightful analysis of the possible impacts of an incentive system on multiple aspects of academic life, most not patently obvious and all reflecting great familiarity with both clinical and basic science missions. He often wrote colleagues to acknowledge a job well done, in 1968 to President Lincoln Gordon:

> *I do not write this letter with the purpose of communicating the reservations that I still hold..., but to convey my appreciation to you for developing the framework for discussion of what must surely be the most important decision which has faced the Medical Institutions since they have been in existence.*

Paul was sought nationally and internationally for advice, including, for example, for the independent review of other academic pharmacology departments. He provided similar help to numerous NCI task forces, the editorial board of the *Journal of Biological Chemistry*, and, in 1968, he suggested to the National Advisory Cancer Council that there should be a program on chemical carcinogenesis (now well-established). In appreciation for his service on an NIH study section, in 1967 the staff wrote an ode succinctly capturing the essence of the man, as in this first of six verses:

> *We're losing a man of great personal attraction–*
>
> *Paul Talalay, a charmer of exotic extraction.*
>
> *His manners are polished–his speech, continental*
>
> *And his knowledge of steroids is just monumental,*
>
> *For*
>
> *He knows each of the metabolites of cortisone*
>
> *And the estrones, epis, andros and progesterone.*

PERSONAL NOTES

Paul Talalay was first and foremost an imaginative and meticulous scientist, who tapped both his basic and clinical training to make discoveries important in their own right, but almost always with a medical dividend. He was also a warm, supportive, and ever-optimistic natural leader, with a keen eye for talent and a great deal of wisdom and wit. Many can recount his memorable quips ("Too complicated to be interesting; Given the current state of medical science it's premature to be practicing medicine"). Yet, he had the perfect doctor's ability always to make his family, friends, and colleagues feel better. He could reliably defuse the most difficult situations with a perfectly chosen and timed humorous comment. In an interview with the Johns Hopkins Magazine in April 2008, he said, "My greatest fear is that on my tombstone, they'll say, 'He made broccoli famous.'" He was a staunch supporter of his faculty, trainees, and staff, seeing to their personal as well as professional wellbeing. Paul was a determined and accomplished problem solver and there was great joy and compassion in his approach. In his later years, he cheerfully encouraged middle-aged colleagues by assuring them that their very best discoveries lay ahead of them, as he felt had been true for himself.

Paul died on March 10, 2019, three weeks short of his 96th birthday. He is survived by Pamela, his wife and best friend of 66 years, by their four accomplished children—Tony, Susan, Rachel, and Sarah and their spouses—and by four grandchildren.

ACKNOWLEDGMENTS

We thank Albena Dinkova-Kostova, Jed Fahey, and Tony Talalay for helpful comments on the manuscript. We thank the NIH for financial support.

REFERENCES

1. Talalay, M. J. 2015. Passport to freedom. Available at www.slideshare.net/mtalalay/passport-to-freedom-44608539. Accessed December 17, 2019.

2. Huggins, C., and P. Talalay. 1945. Sodium phenolphthalein phosphate as a substrate for phosphatase tests. *J. Biol. Chem.* 159:399-410.

3. Talalay, P., and M. M. Dobson. 1953. Purification and properties of a beta-hydroxysteroid dehydrogenase. *J. Biol. Chem.* 205:823-837.

4. Talalay, P., B. Hurlock, and H. G. Williams-Ashman. 1958. On a coenzymatic function of estradiol-17beta. *Proc. Natl. Acad. Sci. U.S.A*. 44:862-884.

5. Talalay, P., and V. S. Wang. 1955. Enzymic isomerization of delta5-3-ketosteroids. *Biochim. Biophys. Acta*. 18:300-301.

6. Kawahara F. S., and P. Talalay. 1960. Crystalline delta 5-3-ketosteroid isomerase. *J. Biol. Chem.* 235:PC1-2.

7. Benson A. M., R. Jarabak, and P. Talalay. 1971. The amino acid sequence of 5-3-ketosteroid isomerase of Pseudomonas *testosteroni. J. Biol. Chem*. 246:7514-25.

8. Wang, S. F., F. S. Kawahara, and P. Talalay. 1963. The mechanism of the delta 5-3-ketosteroid isomerase reaction: absorption and fluorescence spectra of enzyme-steroid complexes. *J. Biol. Chem*. 238:576-585.

9. Batzold, F. H., A. M. Benson, D.F. Covey, C.H. Robinson, and P. Talalay. 1977. Irreversible inhibitors of delta5-3-ketosteroid isomerase: acetylenic and allenic 3-oxo-5,10-secosteroids. *Methods Enzymol.* 46:461-468.

10. Kuliopulos, A., A. S. Mildvan, D. Shortle, and P. Talalay. 1989. Kinetic and ultraviolet spectroscopic studies of active-site mutants of delta5-3-ketosteroid isomerase. *Biochemistry* 28:149-159.

11. *Drugs in our Society.* 1964. Edited by Paul Talalay. Baltimore, MD: The Johns Hopkins Press.

12. Talalay, P., R. P. Batzinger, A. M. Benson, E. Bueding, and Y. N. Cha. 1978. Biochemical studies on the mechanisms by which dietary antioxidants suppress mutagenic activity. *Adv. Enzyme Regul.* 17:23-36.

13. Benson, A. M., P. Talalay, J. H. Keen, and W. B. Jakoby. 1977. Relationship between the soluble glutathione-dependent delta 5-3-ketosteroid isomerase and the glutathione S-transferases of the liver. *Proc. Natl. Acad. Sci. U.S.A.* 74:158-162.

14. Benson, A. M., M. J. Hunkeler, and P. Talalay. 1980. Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. *Proc. Natl. Acad. Sci. U.S A.* 77:5216-5220.

15. Prochaska, H. J., A. B. Santamaria, and P. Talalay. 1992. Rapid detection of inducers of enzymes that protect against carcinogens. *Proc. Natl. Acad. Sci. U.S.A*. 89:2394-2398.

16. Prochaska, H. J., and P. Talalay 1988. Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. *Cancer Res*. 48:4776-4782.

17. Talalay, P., M. J. De Long, and H. J. Prochaska. 1988. Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 85:8261-8265.

18. Zhang, Y., P. Talalay, C. G. Cho, and G. H. Posner. 1992. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A.* 89:2399-2403.

19. Fahey, J. W., Y. Zhang, and P. Talalay. 1997. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. U.S.A.* 94:10367-10372.

20. Itoh, K., N. Wakabayashi, Y. Katoh, T. Ishii, K. Igarashi, J. D. Engel, and M. Yamamoto. 1999. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev*. 13:76-86.

21. Dinkova-Kostova, A. T., W. D. Holtzclaw, R. N. Cole, K. Itoh, N. Wakabayashi, Y. Katoh, M. Yamamoto, and P. Talalay. 2002. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc. Natl. Acad. Sci. U.S.A.* 99:11908-11913.

22. Singh, K., S. L. Connors, E. A. Macklin, K. D. Smith, J. W. Fahey, P. Talalay, and A. W. Zimmerman. 2014. Sulforaphane treatment of autism spectrum disorder (ASD). *Proc. Natl. Acad. Sci. U.S.A.* 111:15550-15555.

23. Chou, T. C., P. Talalay. 1984. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul*. 22:27-55.

24. Cited over 6400 times (Google Scholar, accessed December 2019).

SELECTED BIBLIOGRAPHY

1945 With C. Huggins. Sodium phenolphthalein phosphate as a substrate for phosphatase tests. *J. Biol. Chem*. 159:399-410.

1953 With M. M. Dobson. Purification and properties of a beta-hydroxysteroid dehydrogenase. *J. Biol. Chem*. 205:823-837.

1955 With V. S. Wang. Enzymic isomerization of delta 5-3-ketosteroids. *Biochem. Biophys. Acta* 15:300-301.

1958 With B. Hurlock and H. G. Williams-Ashman. On a coenzymatic function of estradiool-17 beta. *Proc. Natl. Acad. Sci. U.S.A*. 44:862-884.

1963 With S. F. Wang and F. S. Kawahara. The mechanism of the delta-5-3-ketosteroid isomerase reaction: absorption and fluorescence spectra of enzyme-steroid complexes. *J. Biol. Chem.* 238:576-585.

1977 With A. M. Benson, J. H. Keen, and W. B. Jakoby. Relationship between the soluble glutathione-dependent delta5-3-ketosteroid isomerase and the glutathione S-transferases of the liver. *Proc. Natl. Acad. Sci. U.S.A*. 74:158-162.

1979 With R. P. Batzinger, A. M. Benson, E. Bueding, and Y.-N. Cha. Biochemical studies on the mechanisms by which dietary antioxidants suppress mutagenic activity. *Advances in Enzyme Regulation* 17:23-36.

1984 With T. C. Chou. Quantitative analysis of dose-response relationships: the combined effects of multiple drugs or enzyme inhibitors. *Advances in Enzyme Regulation* 22:27-55.

1992 With Y. Zhang, C. G. Cho, and G. H. Posner. A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A*. 89:2399-2403.

2002 With A. T. Dinkova-Kostova, W. D. Holtzclaw, R. N. Cole, K. Itoh, N. Wakabayashi, Y. Katoh, and M. Yamamoto. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc. Natl. Acad. Sci. U.S.A*. 99:11908-11921.