BIOGRAPHICAL MEMOIRS

Sidney Paul Colowick

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A Biographical Memoir by Robert J. Fletterick

Figure 1 Sidney Paul Colowick. *Photo courtesy of the Office of NIH History, National Institutes of Health.*

Sidney P. Colowick was an American-born biochemist who is recognized for his many contributions to metabolic biochemistry, especially glycogen and glucose metabolism in mammals, studies of hexokinase (a pivotal enzyme for glucose capture and glycolysis), and elucidation of the correct structure of NADH. Among his early accolades, he was the recipient of the 1947 Eli Lilly Award in Biological Chemistry, a prize for investigators under thirty-eight years of age administered by the Division of Biological Chemistry of the American Chemical Society. In 1955, he was the founding editor, with Nathan O. Kaplan, of *Methods in Enzymology*, an ongoing series that has been of immense practical value to generations of biochemists.

Sidney Colowick in the Cori Laboratory

Energy conversion in cells has always been a central focus of biochemistry. First among biological fuel molecules is the carbohydrate glucose ($C_6H_{12}O_6$). Its metabolism by cells, breaking chemical bonds, gives energy for life and leads to the terminal oxidized molecules carbon dioxide and water. Enzymes working in the temperature range compatible with life catalyze this critical chemistry. The burning of glucose is used to produce the molecular currencies of cellular energy, ATP and NADH, the latter of which can be used to make more ATP.

Studies of glucose metabolism were active at the Washington University School of Medicine in St. Louis, Missouri, starting from the 1920s. Prominent scientists at work at this great university included Carl F. Cori and Gerty T. Cori, who both had medical degrees and had met as medical students in Prague. This husband-and-wife team was remarkable. They joined the faculty at Washington University School of Medicine in 1931 and in 1947 were awarded the Nobel Prize in Physiology or Medicine for their discovery of the details of the catalytic conversion of the animal starch glycogen, a large polymer composed of glucose, to free glucose fuel molecules. The exceptionally productive Cori lab attracted many superb scientists, but no graduate students. They chose to mentor Sidney Colowick as their first Ph.D. student.

As recounted by Nathan O. Kaplan, a forty-year friend and colleague of Colowick's, in a revealing profile written in 1985 (and source of much of the personal information

in this biography), Sidney was born and raised in St. Louis and attended Washington University, earning his bachelor of science in chemical engineering in 1936, when he was just twenty. After graduation, however, finding an appealing first position thereafter proved to be challenging. He applied for and was offered a job working with Carl and Gerty Cori, serving first as a technical assistant.[1]

In his first year at the lab (1937), at age twenty-one, he coauthored a basic research paper on glucose 1-phosphate and showed how to purify it from muscle tissue.[2] Glucose 1-phosphate derives from the common glucose storage molecule glycogen, a high molecular weight polymer of glucose. The production of glucose 1-phosphate that they isolated required glycogen from muscle tissue, phosphate from the buffering solution, and adenosine monophosphate, AMP, an activator of the enzyme performing the catalytic severing of glucose from glycogen. Glycogen phosphorylase, the enzyme responsible for this production and characterized in 1943 by the Coris, is responsible for generating glucose 1-phosphate to feed the glycolytic pathway enzymes, comprising ten enzyme catalysts that process glucose to release its chemical bond energy.

In 1938, Colowick and the Coris published three more papers.[3,4,5] Sidney was a richly appreciated and talented scientist, and with these publications he was recognized as an unusually promising young biochemist. Indeed, the Coris had great confidence in Sidney, and that same year, he published—without the Coris co-authorship—a technical paper on synthesis of other simple biologically important sugars, mannose 1-phosphate and galactose 1-phosphate, using chemistry developed in his first paper.[6] Shortly thereafter, and based on these accomplishments, Colowick was formally accepted as a graduate student in the Cori lab. Sidney earned his Ph.D. in 1942 and was appointed as an instructor and then assistant professor there, but still ensconced in the Cori lab. In toto, he spent a decade with the Coris. From his experience there at that time, we learn something of Sidney's sense of humor. As related by Nathan Kaplan, when Sidney reflected on the listing of authors in those early papers, Colowick would refer to himself as the "meat in the Cori sandwich."

Colowick thrived in this scientific environment. At the time, Washington University attracted many talented biochemists, and the Cori lab was nourishing, welcoming as colleagues many great scientists, including eventual Nobel laureates Arthur Kornberg, Severo Ochoa, Luis F. Leloir, and Christian de Duve. Others included Arda A. Green, who purified glycogen phosphorylase and discovered the neurotransmitter serotonin; Gerhard Schmidt, an innovator in studying nucleic acid metabolism; and Herman M. Kalckar, who studied oxidative phosphorylation and nucleotide metabolism. Sidney would keep these exceptional scientists as lifelong friends.

Glucose metabolism requires numerous other macromolecules and associated pathways, and these greatly interested Colowick. In 1943, Kalckar and Colowick announced the discovery of adenylate kinase, then called "myokinase."[7] In response to changing nucleotide phosphate levels within the cell, it promotes interconversion of ATP, ADP, and AMP by catalyzing the reaction ATP + AMP ↔ 2 ADP. The discovery of adenylate kinase rationalized these phosphorylation reactions in both yeast and animal cells.

Sidney's interests next were to become focused on understanding hexokinase biochemistry because of its importance as a regulated decision point in deriving energy from glucose metabolism. Moreover, based on work in 1923 by Nobel laureate Otto H. Warburg, it was noted that most cancer cells relied on energy derived through a high rate of metabolism of glucose that leads to increased generation of metabolites that in turn encourages cancer cell proliferation.

While Sidney was a graduate student, Earl W. Sutherland Jr., then a medical student at Washington University, joined the Cori laboratory. Earl and Sidney became social and academic friends. Together they sought to know more about resident glucose, reserve glucose held in all animal and yeast cells. Chemical considerations argued that this "stored" glucose would not exist freely as low molecular weight molecules, but rather as a polymer. Thus, Earl and Sidney were principally interested in how glucose was converted into glycogen and published a classic paper in 1941 on the formation of glycogen from glucose-utilizing purified enzymes.[8] This discovery by Earl, Sidney, and Carl led to defining the principal enzymes and small molecules in the glycogen phosphorylase and glycogen synthase pathways. Sutherland later moved to the Western Reserve University School of Medicine in Cleveland, where in 1958 he discovered that a key molecule in this elaborate biochemical network was cyclic-3', 5'-AMP, a signaling molecule (so-called "second messenger") whose synthesis is evoked when cells are stimulated with certain hormones. For this seminal discovery, Sutherland was the sole recipient of the 1971 Nobel Prize in Physiology or Medicine. Sidney too was recognized for the discoveries he made at Washington University. He received the 1947 Eli Lilly Award in Biological Chemistry, an honor only bestowed on outstanding scientists under the age of thirty-eight.

Out on His Own

In 1946, after ten years in the Cori laboratory, Sidney accepted a position at the Public Health Research Institute of the City of New York, replacing Herman Kalckar, who returned to a position in Denmark. Sidney remained at the Institute until 1948, but after he failed to reproduce a

colleague's results on insulin and hexokinase, he resigned and accepted an associate professor position in the Department of Biochemistry at the University of Illinois Medical School in Chicago. He attracted Nathan O. Kaplan, whom he had met four years earlier in St. Louis, to join him. The emphasis of their positions at the medical school was on teaching biochemistry, rather than on conducting biochemical research, however. Finding themselves an inappropriate fit with that department, both Colowick and Kaplan decamped less than eighteen months after their initial appointments.

By 1950, both Colowick and Kaplan had accepted professorial positions in the McCollum-Pratt Institute of Johns Hopkins University in Baltimore, where they established independent labs but often collaborated on the study of pyridine nucleotides and cellular metabolism. Shortly thereafter, they co-authored a major review on the state of the field of carbohydrate metabolism.[9] A few years later, in a significant discovery, Maynard E. Pullman and Anthony G. San Pietro worked with Colowick to prove the correct chemical structure of NADH.[10] In 2005, this paper was selected as a *Journal of Biological Chemistry* classic article and features an insightful synopsis of Sidney's career and the impact of his work.[11] In that 1954 paper, they showed that the critical active hydrogen was at the *para* position of the pyridine ring of the nicotinamide moiety of NADH, and not in the *ortho* position that Otto Warburg had postulated.

Colowick's lab in Baltimore also fostered productive interactions with great biochemists then located nearby in the stellar Section on Enzymes of the Laboratory of Physiology at the National Institutes of Health in Bethesda, Maryland, including Arthur Kornberg and another eventual Nobel laureate, Christian B. Anfinsen, as well as Bernard L. Horecker, Alton Meister, Herbert Tabor, and Thressa C. and Earl R. Stadtman.

Sidney and Nathan Kaplan began working together in earnest in 1951 and jointly published nineteen research papers. They discovered and studied an enzyme that processes the key energy nucleotides NADH and NADPH. When studying a $NADP^+$-dependent isocitrate dehydrogenase (IDH) in extracts of the Gram-negative bacterium *Pseudomonas fluorescens* (whose products are a-ketoglutarate, CO_2 and NADPH), they detected an activity, which they dubbed a transhydrogenase, that could catalyze the generation of large amounts of NADH at the expense of the NADPH generated in the IDH reaction, that is, the following interconversion: $NADPH + NAD^+ \rightleftharpoons NADP^+ + NADH$.

The Colowick-Kaplan consortium showed how to assay and purify the newly discovered enzyme.[12,13] They then proved that this pyridine nucleotide transhydrogenase catalyzes the indicated oxidation-reduction chemistry via moving an H atom from the donor to the acceptor using an elaborate analysis exploiting a ^{14}C-nicotinamide-labeled NAD^+ and unlabeled NADPH. They found a strict reciprocal and stoichiometric relationship—as the unlabeled NADPH went from reduced to oxidized, the radioactively labeled NAD^+ went from oxidized to reduced.

Colowick and Kaplan also worked together outside of their own laboratories to promote biochemical discovery, and aid and expedite research experimentation generally, by deciding to establish what amounted to a series of practical laboratory manuals by noted experts on various biochemical procedures that would keep pace with advances in biochemical techniques. So in 1955, Kaplan and Colowick established *Methods in Enzymology* under the aegis of Academic Press and served as its founding editors-in-chief. Sidney continued his editorship for the next thirty years until his passing. Volumes of *Methods in Enzymology* continue to be published up to the present day.

Hexokinase

In 1959, Colowick moved yet again, this time to the Vanderbilt University School of Medicine in Nashville, as the American Cancer Society–Charles Hayden Foundation Professor of Microbiology, and remained until his death in 1985. There, he was known for his work on hexokinase and the regulation of hexose transport in cultured animal cells. In particular, Colowick is widely appreciated for his purification, crystallization, and biochemical discoveries on hexokinase isolated from yeast.[14] At the time of this research, enzymes were mysterious powerful catalysts. Lacking structure and amino acid sequence data made them challenging targets for studies of chemical mechanism. There was wide interest in hexokinase in many laboratories in the 1960s and 1970s; three prominent labs were focused on learning the molecular basis of catalysis by and regulation of this important allosteric enzyme. The main labs were directed by Sidney Colowick at Vanderbilt, Eric Barnard at the University of Cambridge in the United Kingdom, and eventual Nobel laureate Irwin A. "Ernie" Rose at the Institute for Cancer Research of the Fox Chase Cancer Center in Philadelphia. The Rose lab performed careful kinetic analysis to work out the chemical mechanism of hexokinase, and the Colowick lab continued to study the basic biochemical properties of hexokinase. These three labs interacted collegially. The Colowick lab provided purified and characterized hexokinase to the Rose lab. The detailed contributions to the biochemistry of hexokinase provided by each lab are nicely presented in a comprehensive review authored by Colowick in volume nine (1973) of *The Enzymes*, a series of monographs on enzymes published by Elsevier.[15]

Research on hexokinase was a major focus throughout Colowick's time at Vanderbilt. From 1961 to 1979, Sidney's lab published thirteen papers on yeast hexokinase,

discovering its basic biochemistry and stimulating interest in pursuing its three-dimensional structure. Colowick characterized multiple forms of hexokinase, surprisingly including protease-cleaved products that retained full activity. In the early days of biochemistry, it was generally assumed that a crystalline protein was ultra-pure and that such a crystal contained a single protein entity. Colowick showed, however, that protease attack could occur in crystalline enzyme preparations even at zero degrees. Colowick and others ultimately proved that, in yeast, there are only two physiological forms of intact hexokinase, called PI and PII. We know now that these two isoforms are the products of distinct genes: PI / HK-A (485 residues) encoded by HXK1, and PII / HK-B (486 residues) encoded by HXK2.[16] Hxk2 is the major isoform expressed when cells are grown on glucose (~160,000 molecules per cell for Hxk2 versus ~60,000 molecules per cell for Hxk1). Although Hxk1 and Hxk2 share a 77-percent amino acid identity and, if standard conservative substitutions are considered, an 89-percent amino acid similarity, Hxk2 exhibits a V_{max} that is four times higher than that of Hxk1. Also, Hxk2 forms dimers at neutral pH that are dissociated when glucose or phosphate are present.

In 1961, with then-graduate student Kenneth A. Trayser, Colowick purified, crystallized, and studied the catalytic and physical properties of yeast hexokinase (most likely Hxk2). The enzyme generates glucose 6-phosphate from glucose and ATP with Mg^{2+}. There was an interesting aberrant non-biological reaction that Colowick analyzed. They showed that with ATP and Mg^{2+} and large amounts of crystalline hexokinase (but no glucose), ADP and inorganic phosphate are formed in a wasteful hydrolysis reaction. The enzyme is designed to undergo a conformational closure around glucose and Mg^{2+}-ATP to initiate catalysis, but it cannot do so around H_2O and Mg^{2+}-ATP. This off-path ATP hydrolysis activity is one hundred times slower than phosphate transfer from Mg^{2+}-ATP to glucose.

Colowick showed that the weak but readily detectable ATPase activity described above resided in the highly homogeneous enzyme obtained through six recrystallizations and that ATPase activity also could be observed in all forms of yeast hexokinase. But there was additional confusion about the role of ATP in hexokinase catalysis. Studies by Colowick and others suggested that its activity depended on the presence of other metabolites, frequently the hallmark of a regulatory enzyme responding to allosteric effectors, and on a monomer-dimer transition. Colowick and long-time research associate Frances C. Womack showed hexokinase in the low activity state could be activated by metabolites, including citrate, 3-phosphoglycerate, and malate. But in a brilliant piece of biochemical detective work,[17] they showed, first, that the aluminum ion is frequently a contaminant in certain commercial preparations of ATP and causes strong inhibition of the PII yeast hexokinase activity at pH 7.0 or below and, second, that the small-molecule agents just listed, and described as activators of hexokinase, only do so by virtue of their ability to coordinate the Al^{3+} and prevent it from poisoning the assay system.

Evidence for structural changes in the catalytic mechanism of hexokinase was first revealed in Colowick's lab. He showed, with postdoctoral associate Irene T. Schulze, that glucose binding protects hexokinase from degradation caused by added protease that would otherwise sever flexible segments of the polypeptide chain.[18] This observation was consistent with the closing down of the two domains of hexokinase upon its binding of glucose and Mg^{2+}-ATP found by structural studies in the laboratory of eventual Nobel laureate Thomas A. Steitz at Yale University.

A Fateful Summer at Cold Spring Harbor Laboratory

Cold Spring Harbor Laboratory (CSHL) on Long Island in New York is a superb environment to study genetics and biology at the molecular level. Maryda Swanstrom was a graduate student at New York University whose Ph.D. supervisor was microbiologist Mark Adams. Maryda was Adams's teaching assistant in the legendary phage course when Sidney attended a scientific conference at CSHL in the summer of 1948. They met then and married three years later in Baltimore. In their thirty-three years together, they conducted scientific research and raised three daughters: Ann, Susan, and Nancy. In 1959, the family moved to Nashville, where Maryda continued to work with Sidney at Vanderbilt University.

The Vanderbilt Years

Nathan Kaplan, Sidney's colleague, collaborator, and friend for forty years, remarked in his *Methods of Enzymology* eulogy for Sidney,[19] "It is my strong belief that Sidney was the foremost representative of American-born biochemists who made modern biochemistry an American discipline." On a personal level, Kaplan wrote, "Sidney Colowick was an unusual individual. Not only was he a brilliant and creative scientist, but he was also a compassionate and warm human being. His leadership qualities were admired and respected at Vanderbilt."

Sidney was a mentor and contributor to his colleagues' academic research programs. He helped to develop junior scientists and strengthen the fabric of science at Vanderbilt. Sidney is honored at Vanderbilt University with the biannual Sidney P. Colowick Ph.D. Award, given for "research that serves as a platform for discovery in diverse areas."

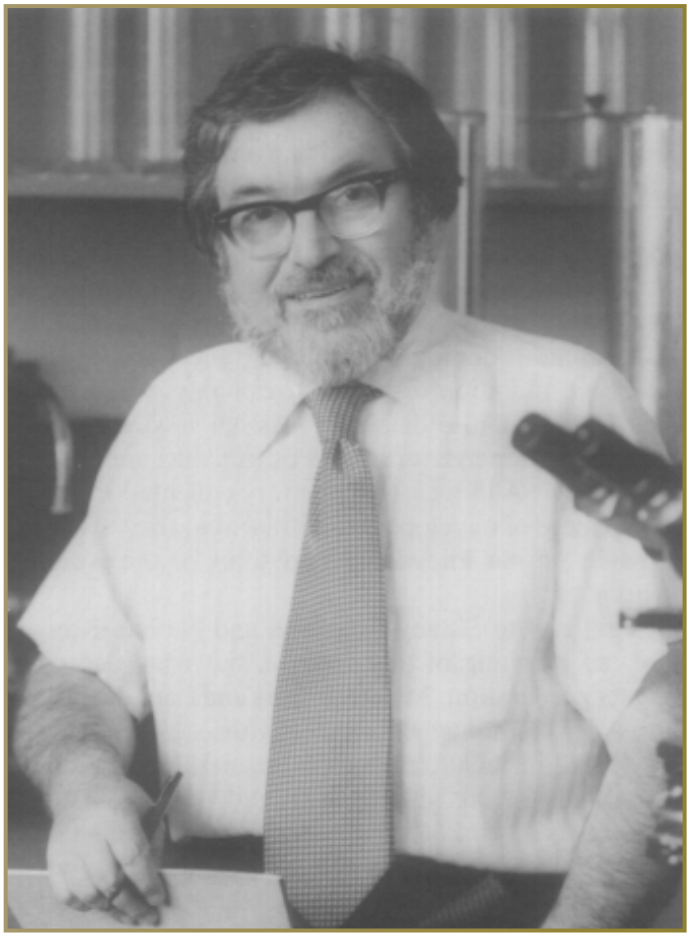

Figure 2 Colowick at Vanderbilt (circa 1980s).

Sidney and his colleague and then-department chair Charles Rawlinson "Rollo" Park, a pioneering diabetes researcher at Vanderbilt who had also been a postdoctoral fellow in the Cori lab from 1946 to 1954, worked energetically to transform Vanderbilt into a major center of excellence in biological research.

Sidney passed away on January 9, 1985, at sixty-eight years old. George Alexander Heard, who had been chancellor of Vanderbilt University from 1963 to 1982, spoke at the memorial service for Sidney that year and said:

> A university's distinction is found first of all and most of all in the intellectual merit of the members of its faculty. In our century, in our country, the American university has become a many splendored instrument of our culture, called upon and reaching out to serve humankind through ancient and novel means, toward ancient and novel ends. The heart of the university in western civilization is its duty to inquire and discover, and to interpret and communicate a useful harvest. In these central missions of the most influential institution of the twentieth century, the university, Sidney Paul Colowick excelled. He was a person of science, of the intellect, of the university, of the eternal human search to know and understand. He helped create Vanderbilt by doing most what a university is created to do.

Note

From 1971 to 1974, I was a postdoctoral fellow in the laboratory of Tom Steitz at Yale University. I helped determine the structure of yeast hexokinase (Hxk2) at atomic resolution using X-ray crystallography.[20–24] Colowick's path-finding biochemical work on yeast hexokinase helped guide our crystallizations and structural studies and, in turn, our structural insights help to interpret the underlying mechanisms behind the biochemical behavior observed by Colowick in his analysis of the properties of yeast hexokinase.

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