

Methods in Enzymology

Volume 113

GLUTAMATE, GLUTAMINE, GLUTATHIONE, AND
RELATED COMPOUNDS

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ACADEMIC PRESS, INC
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC (LONDON) LTD
24-28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER 54 9110

ISBN 0 12-182013-0

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88

9 8 7 6 5 4 3 2 1

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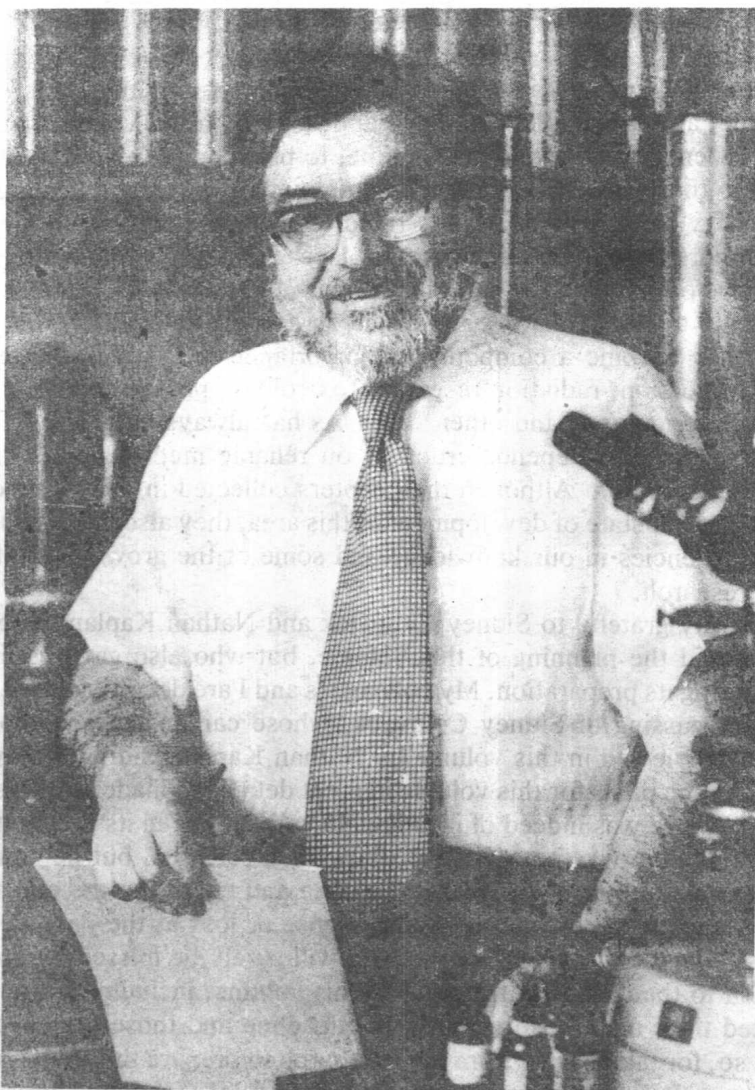
Preface

Although chapters on glutamate, glutamine, glutathione, aspartate, asparagine, and α -aminoadipate have appeared in earlier volumes of this series, it seems appropriate, at this time, to bring out a separate volume devoted to these structurally related compounds. Progress in this area of biochemistry has been remarkably rapid, and the metabolic and physiological importance of these compounds places them well beyond the traditional areas of enzymology and biochemistry. For example, glutamate is now known to be of major significance as a neurotransmitter, and glutathione has become a compound of importance to those interested in cellular protection, radiation research, toxicology, pharmacology, endocrinology, agriculture, and other fields. As has always been true in biochemistry, progress depends crucially on reliable methods of isolation and characterization. Although the chapters collected in this volume reflect the present state of development of this area, they also indicate some of the deficiencies in our knowledge and some of the growing points of current research.

I am truly grateful to Sidney Colowick and Nathan Kaplan, who not only initiated the planning of this volume, but who also gave essential advice during its preparation. My colleagues and I are deeply saddened by the recent passing of Sidney Colowick, whose career and accomplishments are reviewed in this volume by Nathan Kaplan. Sidney Colowick scrutinized the plans for this volume in great detail and made many useful suggestions. He was indeed of inestimable value to me in its preparation. Sidney Colowick was not only a distinguished scientist, but he contributed freely and extensively of his own time and energy to the efforts of others. I wish to record my own deep sense of loss at the passing of a generous friend and colleague, one who will surely be missed by many.

I wish to thank the contributors to this volume, including those who submitted their manuscripts before the deadline and those who eventually did so, for taking time from their research to prepare this material for publication. It is a pleasure to thank the staff of Academic Press for excellent assistance during all stages of the publication process. I also wish to acknowledge the efforts of Mrs. Susan D. Truog and Mrs. Edith H. Perryman for invaluable assistance in the preparation and collection of the manuscripts.

ALTON MEISTER



SIDNEY P. COLOWICK
January 12, 1916–January 9, 1985

Author of *...*

Sidney P. Colowick

It is difficult to write about a dear colleague and friend of more than forty years. There is so much to say, but words cannot easily express one's feelings. As I write this memorial, I recognize that my objectivity is compromised by my emotions.

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. Sidney was born and raised in St. Louis; he attended Washington University and obtained a degree in chemical engineering in 1936 at the age of 20. Jobs were difficult to obtain in those depressed times. He took a Civil Service test and passed an examination which qualified him to be a shrimp inspector. He was offered a position as an inspector in New Orleans, but his family thought him too young to settle in such a far away city. He was told of a laboratory opening in a biochemistry laboratory at the Washington University School of Medicine. After an interview with Carl Cori, he was given the position on a four-week trial basis. He was then given the position permanently even though in his first week he dropped a desiccator containing valuable compounds just outside of Carl Cori's office. He was associated with Carl and Gerty Cori for approximately ten years. At the age of 21, he published his first paper as coauthor with the Coris on the classical studies of glucose 1-phosphate. I take the liberty of listing his first few papers to illustrate the great significance of his early work.¹⁻⁴

With his characteristic remarkable wit Sidney used to refer to himself as the "meat" in the "Cori Sandwich." The potential "shrimp inspector," who had known nothing about biochemistry or physiology, had within a few years established himself as one of the most promising young people in the emerging field of biochemistry. The Coris had great confidence in Sidney, and in 1938, at the age of 22, he published, as sole author, a paper in the *Journal of Biological Chemistry* on the synthesis of mannose 1-phosphate and galactose 1-phosphate.

Sidney's ability was appreciated by the Coris, and he became Carl's first graduate student. Gerty and Carl believed that the way in which the

¹ Cori, C. F., Colowick, S. P., and Cori, G. T. (1937). The Isolation and Synthesis of Glucose-1-Phosphoric Acid. *J. Biol. Chem.* 121, 465.

² Cori, G. T., Colowick, S. P., and Cori, C. F. (1938). The Formation of Glucose-1-Phosphoric Acid in Extracts of Mammalian Tissues and of Yeast. *J. Biol. Chem.* 123, 375.

³ Cori, G. T., Colowick, S. P., and Cori, C. F. (1938). The Action of Nucleotides in the Disruptive Phosphorylation of Glycogen. *J. Biol. Chem.* 123, 381.

⁴ Cori, G. T., Colowick, S. P., and Cori, C. F. (1938). The Enzymatic Conversion of Glucose-1-Phosphoric Ester to 6-Ester in Tissue Extracts. *J. Biol. Chem.* 124, 543.

remarkable advances in biochemical technology could be introduced into their laboratories was to have talented individuals who had been exposed to recent developments spend time in St. Louis. Hence, eminent scientists such as Arda Green, Gerhard Schmidt, Severo Ochoa, Herman Kalckar, and Luis Leloir contributed to the growth and importance of the Cori laboratory. Sidney was a beneficiary of these unusual circumstances. He was the only graduate student in a laboratory filled with brilliant investigators. His education was greatly enriched by this exposure, and his association with these unusual individuals promoted both his skills and creativity. They became lifelong friends of Sidney and his wife Maryda.

Herman Kalckar and Sidney became interested in hexokinase. Meyerhof had been researching this enzyme, but there were some mysterious characteristics of the enzyme which Meyerhof had difficulty understanding. Sidney and Herman initiated an investigation of this enzyme, and during the course of these studies they discovered myokinase, which is now known as adenyl kinase. The discovery of this enzyme proved to be a key factor in the understanding of transphosphorylation reactions in both yeast and animal cells.

Earl Sutherland who was a medical student at Washington University became interested in basic research and joined the Cori laboratory. He and Sidney became close friends and worked together on several problems. They were principally interested in the conversion of glucose to polysaccharides, and published a classical paper in 1942 on the formation of glycogen from glucose utilizing purified enzymes. This work which Sutherland was doing with the Coris and Sidney led to Earl dissecting the phosphorylase system which eventually led to the discovery of cyclic AMP. Sidney, along with Milton Slein and Lou Berger, carried out pioneering studies on yeast hexokinase. These led to crystallization of the enzyme in 1961 by Sidney in collaboration with K. A. Trayser. The crystalline yeast hexokinase was studied in great detail both with respect to its catalytic and physical properties.

The years in the Cori laboratory were most fruitful and pioneering in the "new" biochemistry. After ten years in the Cori laboratory, Sidney joined the staff of the Public Health Research Institute of the City of New York, replacing Herman Kalckar who was returning to Denmark. He remained at the Institute from 1946 to 1948. This was an unhappy period for Sidney, although it demonstrated Sidney's unusual character. He spent the years attempting to repeat the work of a colleague whose results were questionable. Sidney was not remorseful about the inability to reproduce the findings, and showed no open hostility to the individual. I have

never met anyone in my lifetime who almost destroyed his own career and health to show the best in another individual.

I first met Sidney in St. Louis in 1944, and from that time on our lives were intertwined. Sidney moved with Otto Bessey in 1948 to the Department of Biochemistry at the University of Illinois Medical School in Chicago. He persuaded Bessey to offer me a position. I arrived in Chicago six months after Sidney did. Giulio Cantoni was also a member of the Department. Sidney had already made arrangements for Earl Sutherland to join the faculty a few months after my arrival. When I arrived Sidney was in the hospital with an ulcer. He had been given the responsibility of preparing a new laboratory syllabus. He wrote the syllabus all by himself, keeping just one experiment ahead of the class of over 300 students. Bessey assigned me to the syllabus task after Sidney became ill. I must have worked twenty hours a day on it. In two weeks I was so exhausted that it was difficult for me to believe that Sidney was able to carry out this task for three months. Two weeks after my arrival at the University of Illinois, Sidney was released from the hospital, and we then worked together to complete the syllabus. It seemed to me that working with Sidney was a "piece of cake" compared to the experience that I had as the only author.

The time spent in Chicago was both unhappy and unproductive for Sidney and me. It became apparent shortly after my arrival that Sidney and I had to leave, and a year after my arrival we both left.

Sidney and I were indeed fortunate to obtain positions at Johns Hopkins in the new McCollum-Pratt Institute headed by Bill McElroy. Ironically we both had been approached separately to be Director of the Institute. The change from Chicago to Baltimore was delightful. McElroy was a very effective leader and constructed a climate which was favorable and stimulating for research. Sidney and I collaborated on problems of pyridine nucleotides and metabolism. Although we worked together on several problems, we did have projects which we carried out separately. Our graduate and postdoctoral students were very close colleagues. As a result, the period spent at Hopkins was productive, exciting, pleasant, and rewarding. There was a great deal of interaction at the McCollum-Pratt Institute with other scientists in Baltimore as well as with the group of young biochemists at the National Institutes of Health which included Arthur Kornberg, Bernie Horecker, Herb Tabor, Al Meister, Leon Hoppel, Chris Anfinsen, and Earl and Terry Stadtman. The groups were close, and Sidney played an important role as an intellectual leader.

During the period at Hopkins, Sidney made many noticeable contributions. Sidney, in collaboration with Maynard Pullman and Tony San Pietro, established the correct structure of NADH—that the hydrogen was in

the para position and not in the position that Warburg and Karrer had postulated almost twenty years previously. Sidney summarized a large part of the work at Hopkins in an article in a book which was dedicated to me.⁵

I left Hopkins in 1957. Sidney moved to Vanderbilt in 1959 as American Cancer Society Professor in the Department of Microbiology. At Vanderbilt, Sidney focused his work on hexokinase, and made many ingenious and notable contributions on hexose transport in cultured animal cells. His latest paper appeared in a March 1985 issue of *PNAS* in collaboration with his last student Wendelyn Inman.⁶ This paper clearly showed that growth factors are involved in the uptake of glucose. Sidney's interest in glucose metabolism began with his research in Cori's laboratory and remained a lifelong interest. Sadly, Sidney died roughly two months after his mentor, Carl Cori.

It is my strong belief that Sidney was the foremost representative of American-born biochemists who made modern biochemistry an "American discipline." His leadership qualities were admired and respected by all who knew him.

Sidney played an unusual role at Vanderbilt. His presence attracted Earl Sutherland. He gave much of himself to help develop the many talents that were at Vanderbilt so that this institution is now one of the world's leading institutions in the field of regulatory biology. There is no doubt that Sidney and Rollo Park were the major forces that transformed Vanderbilt into a major center of excellence. What Sidney meant to Vanderbilt and to the academic world was best expressed by these elegant words of Alexander Heard, Chancellor Emeritus of Vanderbilt, at a memorial service for Sidney on January 15, 1985.

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.

⁵ Colowick, S. P. (1982). Origins of Pyridine Nucleotide Research at the McCollum-Pratt Institute in the 50's. In "Pyridine Nucleotide Coenzymes" (J. Everse and K.-S. You, eds.), Academic Press, New York

⁶ Inman, W. H., and Colowick, S. P. (1985). *Proc. Natl. Acad. Sci. U.S.A.* 82, 1346-1349.

I know of no one who was as helpful and gave so freely of his time as Sidney. He would spend days rewriting someone's manuscript or document. I have known him to rewrite a whole chapter for which he was given no credit. Sidney did not worry about credits; he did these activities because he felt strongly about his university involvement. I believe that nearly every colleague at Hopkins and Vanderbilt had a paper reviewed by Sidney before submission for publication.

Sidney, although most helpful, could hold very firm to his views. An example of this was a story told to me by a distinguished professor who had submitted a paper to the *Archives of Biochemistry and Biophysics* which was sent to Sidney, who was on the Editorial Board, for review. Sidney felt that there was something intrinsically wrong with the results. Of course, the professor disagreed. After nine months of exchanges, the professor finally agreed that he was wrong and altered his views.

Sidney was an extraordinary human being. He rarely said anything derogatory about individuals, many of whom were deserving of being chastised. He could have scientific disagreements, but these never became personal. He certainly maintained close friendships with people with whom he had scientific disagreements.

Sidney received many honors and awards. He received the Eli Lilly Award in Biochemistry in 1947. He was elected to both the National Academy of Sciences and the American Academy of Arts and Sciences. In 1978, he was chosen as the Harvie Branscomb Distinguished Professor by the Vanderbilt Board of Trustees. He was also the Charles Hayden—American Cancer Society Professor of Microbiology. He was a member of many advisory committees, and served on the editorial boards of a large number of journals.

Herman Kalckar once said of Sidney and Maryda that their hospitality was legendary. Not only did they open their door to all, they showed compassion and understanding to individuals whose lives were besieged by tragedy and unhappiness. Their warmth gave new hope and encouragement to many.

Sidney loved music, and in recent years obtained much enjoyment in performing in chamber groups. But Sidney had a virtue unusual for scientists: not only was he involved in social events, he also spent much time working for a number of worthy humanitarian causes.

I would like to say a few words regarding *Methods in Enzymology*. We were approached in 1951 by the late Mr. Kurt Jacoby of Academic Press to do a series comparable to *Methoden der Ferment Forschung* which was edited by Baumann and Myrback, four volumes of which were published in German in 1940 and which was a pet project of Mr. Jacoby. Although we resisted taking on the enterprise, we were unable to with-

stand Mr. Jacoby's forceful and convincing arguments. The planning and publishing of the first two volumes were difficult and time consuming, but Mr. Jacoby used combined doses of encouragement and nagging. We somehow managed to finish the first two volumes. Actually, Mr. Jacoby took great pride in our accomplishments and praised us whenever possible. We did four more volumes, and then we thought that to proceed further would be difficult because of the rapid growth of biochemistry and that it was almost impossible to intimately know the many new fields that had come into existence. Again, Mr. Jacoby became a tremendous influence; he would not let us quit. He insisted that we had created a most useful and successful series, and that it was our duty to continue. From discussions with Mr. Jacoby and James Barsky evolved the new format for the volumes which has continued to the present. We wished to change the title of the series, but Mr. Jacoby insisted that *Methods in Enzymology* was already an established name and that its contents could cover nearly every field in the biochemical sciences. Sidney and I in later times agreed that Mr. Jacoby had a potent foresight which we ourselves did not realize.

Although we were separated physically, the *Methods* kept us together. We talked on the phone several times a week, and we had the opportunity to see each other a number of times each year. As time passed we became closer. These contacts certainly enriched my life and my perspective about many things. It was "fun" talking to Sidney.

Now I have lost my friend, colleague, and partner. Biochemistry has lost a creative and critical mind. The world has lost a humane individual whose compassion and warmth influenced the lives of many. To Maryda and his daughters, I extend my deepest sympathy. No wife was more devoted than Maryda was to Sidney. His daughters can be proud of their father, both for his accomplishments and as a human being.

I am indebted to all the editors and contributors to the volumes; they have made the *Methods* a successful venture. I would also like to thank James Barsky and Shirley Light of Academic Press for their support and cooperation, particularly during the past several months. I hope that the *Methods* will continue to be the timely, useful, and high quality series that Sidney had strived for, and that in the future we will be able to implement some new concepts to the volumes that Sidney and I had been discussing.

NATHAN O. KAPLAN

75-6710-1

METHODS IN ENZYMOLOGY

EDITED BY

Sidney P. Colowick and Nathan O. Kaplan

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