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PHILIP PACY COHEN

September 28, 1908–October 25, 1993

BY ROBERT H. BURRIS

PHILIP P. COHEN HAD a distinguished career in biochemical research that emphasized nitrogen metabolism in the animal body. He was a pioneer in studies of transamination reactions and in the investigation of urea production. His career in research and administration was spent predominantly at the University of Wisconsin, where he served as chairman of physiological chemistry for twenty-seven years and as acting dean of the medical school for two years. In addition he functioned on many national boards and had a substantial impact on a number of Asian, Mexican, and South American institutions through his collaborative associations.

Philip Pacy Cohen was born September 28, 1908, in Derry, New Hampshire. He died on October 25, 1993, in Portland, Oregon. He attended high school for a year in Everett, Massachusetts, and then attended Boston English High School for three years. He graduated in 1926. Cohen received his B.S. degree from Tufts in 1930. He attended graduate school at the University of Wisconsin from 1930 until he received his Ph.D. in 1937. His major was in the Department of Physiological Chemistry and his minor in physiology. He continued, and received an M.D. degree in 1938. Professor

E. J. Witzemann served as major professor for Cohen's Ph.D. work, and Professor Harold C. Bradley was on his examining committee. E. B. Fred, a member of the National Academy of Sciences, signed Cohen's thesis as dean of the graduate school. The thesis was entitled "Studies in Ketogenesis," and it covered preparation of tissue slices and breis, the synthesis of several hydroxy and alpha-keto acids, the determination of acetoacetic and beta-hydroxy butyric acids, and the oxidation of a variety of fatty acids, amino acids, and keto and hydroxy acids. The studies were published in the *Journal of Biological Chemistry* (1937, 1938).

In June 1935 Cohen married Rubye H. Tepper, who had been a student in the University of Wisconsin Medical School. They had four children: Philip T., David B., Julie A., and Milton T., all of whom were students in the Madison, Wisconsin, school system.

After completing his Ph.D. and M.D. degrees, Phil Cohen was awarded a National Research Council Fellowship, and he spent 1938-39 in the laboratory of Hans A. Krebs in Sheffield, England, before returning to Yale University to complete the fellowship. The Krebs laboratory was a very lively research center, and Krebs and Cohen published a paper in *Nature* (1939) on glutamic acid as a hydrogen carrier in animal tissues. In 1939 they also had a paper in the *Biochemical Journal* on the metabolism of α-ketoglutaric acid in animal tissues. Thus, Cohen's many papers on nitrogen metabolism in animal tissues were launched with a distinguished investigator (Krebs later received the Nobel Prize) in prestigious journals.

Cohen (1939) also was sole author of two papers in the *Biochemical Journal*. The first detailed a method for the microdetermination of glutamic acid, which involved the conversion of glutamic acid to succinic acid with chloramine T plus acid hydrolysis, followed by the manometric determi-

nation of succinic acid with succinoxidase. The second paper concerned transamination in pigeon breast muscle. He found the transamination reactions of aspartic acid and alanine with α-ketoglutaric acid to give glutamic acid were far more active than other transamination reactions.

During his stay in the Yale University Department of Physiological Chemistry, Cohen did his research in the laboratory of Cyril N. H. Long. During this period he published two papers in the *Journal of Biological Chemistry* on transamination (1940). If you read them now, many years later, you are impressed with the thoroughness of the papers and the insight of the author. The papers certainly were important in placing studies of transamination on a solid footing. Transamination studies were initiated by Aleksander E. Braunstein and M. G. Kritzman (*Enzymologia* 2:[1937]:129-46), but Cohen pointed out a number of problems with their earlier methods and interpretations. They considered transamination a very general reaction, whereas Cohen reported it was highly active with only a limited number of amino acids. It was particularly active in the reversible reactions of glutamic acid plus oxalacetic acid to α-ketoglutaric acid plus aspartic acid; glutamic acid plus pyruvic acid to α-ketoglutaric acid plus alanine; and aspartic acid plus pyruvic acid to oxalacetic acid and alanine. Cohen and G. Leverne Hekhuis (1941) examined transamination rates in a variety of animal tissues. Cohen (1940) stated, "Braunstein suggested the names of glutamic aminopherase for the former, aspartic aminopherase for the latter. Since the original term *Umaminierung* used by these workers has been accepted with the English (and French) equivalent of transamination, it is suggested here that the enzyme (or possibly enzymes) catalyzing the transfer of amino nitrogen be termed transaminase. The latter term is more euphonious and does not suffer from a mixed etymology."

With his return to the University of Wisconsin, Phil Cohen continued his research on transamination and branched into other aspects of nitrogen metabolism in animal tissues. Much of his work was with pigeon breast muscle, but he also used rats and guinea pigs as sources of liver, spleen, testis, kidney, brain, and intestinal tissues. As a plant biochemist, I am pleased to find, in reviewing Cohen's work, that he published a couple of papers on plant materials (oat seedlings in 1943 and Jack beans and soybeans in 1946). He was careful to establish reliable methods, and his work was well planned and analytical.

Cohen became interested in the changes in nitrogen metabolism that occur during metamorphosis, and he chose the conversion of the tadpole to the adult frog as his object of study. His investigations, which spread well over a dozen years, are nicely summarized in his Harvey Lecture (1966) and his review in *Science* (1970). As he pointed out, the metamorphosis of the frog in the postembryonic period is marked by extensive morphological, cytological, and chemical changes. During this period the tadpole adapts from an aquatic life to a terrestrial life, and its developmental biochemistry undergoes many interesting modifications. The physical changes are apparent, but what is happening to the liver and other organs? Apparently the liver does not undergo cell division during metamorphosis, but the liver cells change in their biochemistry. Tadpoles excrete ammonia before metamorphosis (ammonotelic), but after metamorphosis starts they shift toward urea production (ureotelic). Cohen and coworkers developed methods so that they could follow these changes and the alteration in enzymes that promoted these changes.

The rate-limiting enzymes for urea production appeared to be carbamyl phosphate synthetase and arginosuccinate synthetase, and their activity appeared to correlate directly

with urea excretion. Thyroxine can induce metamorphosis, but the changes in the urea-producing enzymes were the same whether metamorphosis was natural or thyroxine-induced. A marked increase in carbamyl phosphate synthetase preceded the morphological changes accompanying metamorphosis. The rate of formation of this enzyme was increased by added thyroxine and also was increased by raising the temperature of the tadpole's bath. At 15° C only about half as much carbamyl phosphate synthetase was formed as at 25° C. Cohen's group observed new synthesis of ribosomal and soluble RNA promptly after thyroxine treatment and before the induction of synthesis of carbamyl phosphate synthetase. Further studies suggested to them that thyroxine treatment modified chromatin in some way to make it a more effective template for RNA synthesis. They concluded that the effect of thyroxine on induction of carbamyl phosphate synthetase involves transcriptional as well as translational events.

Cohen and colleagues carried out a systematic study of the ultrastructural changes in the liver of tadpoles during metamorphosis with or without thyroxine induction, and they also followed changes in the adult liver. These changes observed by electron microscopy in liver sections from tadpoles exposed to thyroxine could be reproduced in vitro by adding thyroxine to pieces of liver taken from tadpoles before metamorphosis. They observed that the so-called cubed-liver preparations could survive under appropriate conditions for forty-eight hours with cellular integrity and capacity for biosynthesis.

Cohen's group also investigated glutamic acid dehydrogenase in the developing frog. The crystalline enzyme from frog liver and from tadpole liver exhibited different kinetics and substrate specificity and different molecular weights. This suggested that studies should be directed to differences

between enzymes from the fertilized ovum stage to the tadpole stage, as well as to the changes that had been observed between the tadpole and the adult frog.

Cohen continued his interest in urea synthesis and the interconversions of ornithine, citrulline and arginine in urea production. When Krebs and Kurt Henseleit *(Hoppe-Seyler's Zeitschrirft für Physiologische Chemie* 210[1932]:33-66) had first proposed their theory of urea synthesis, they described it only in intact liver cells, but Cohen and Mika Hayano (1946) were able to demonstrate the cycle in liver homogenates by adding glutamic acid, ATP, cytochrome C, and magnesium ions, and operating aerobically. They concluded that glutamic acid was an obligatory intermediate in the introduction of ammonia at the citrulline to arginine conversion step of the urea cycle. Others had reported no transamination in homogenates of kidney but had found transamination in kidney slices. Cohen and Hayano (1946) found such activity in a variety of preparations in decreasing order of activity: liver homogenates, kidney slices, kidney homogenates, and liver slices. The homogenized preparations required added ATP, glutamic acid, cytochrome C, Mg ions, and oxygen. They found no transamination in brain, testes, or heart homogenates. Cohen and Santiago Grisolia (1948) could not demonstrate the ornithine to citrulline conversion under anaerobic conditions.

Grisolia and Cohen (1948) employed $^{14}CO_2$ in exploring CO_2 fixation in the synthesis of citrulline. They demonstrated the incorporation of ^{14}C into the carbonyl group of citrulline and into urea, and they concluded that the reactions were vigorous enough to establish citrulline as an obligatory intermediate in the urea synthesis cycle, a role that had been questioned by some investigators. The conversion of ^{14}C-citrulline to the carbonyl of urea yielded urea with essentially the same ^{14}C specific activity as the ^{14}C-citrulline

supplied. Cohen and Grisolia (1950) concluded that in the synthesis of citrulline from ornithine, carbamyl-L-glutamic acid was an intermediate; it was not active in the absence of ammonium ion. The Cohen group and the Henry A. Lardy group (Patricia MacLeod, Grisolia, Cohen, and Lardy) (1949) joined forces to investigate the role of biotin in the synthesis of citrulline from ornithine. Biotin-deficient rat livers were only about half as active as livers from pair-fed controls. Injection of biotin twenty-four hours before testing the rats restored their livers to normal activity.

Earlier Cohen had questioned the conclusion of Braunstein and Kritzman that transamination was a general reaction among the amino acids, as he had found much higher activities among glutamic acid, aspartic acid, and alanine. P. S. Cammarata and Cohen (1950) reexamined the issue and reversed their opinion on the generality of transamination when they found that twenty-two amino acids in addition to alanine and aspartic and glutamic acids transaminated with aqueous extracts of pig heart, liver, and kidney. They stated, "Each transamination reaction appears to be due to a different transaminase." They had devised new methods for these tests and had found that pyridoxal phosphate accelerated the reactions.

Phil Cohen rather promptly established himself as an expert in the area of nitrogen metabolism in animal tissues, and in 1945 he contributed a chapter on proteins and amino acids to the *Annual Review of Biochemistry*. This review had very broad coverage of the topics of protein synthesis, plasma and tissue proteins, amino acid requirements, intermediary metabolism, deamination, transamination (a rather short treatment considering this was an area of special expertise for Cohen), and about a dozen other topics. Later he contributed a wider ranging review with Henry J. Sallach (1961) in a book entitled *Metabolic Pathways*. As they pointed out,

they were concerned primarily with enzymatic systems involved in the transformation or transfer of the amino, amine and amide nitrogen moiety of amino acids, amines, and amides, and they gave preference to studies that emphasized the enzymatic aspects of chemical transformations. With this definition of their interests they then covered the field rather thoroughly in a 78-page treatment supported by 511 references. They even brought the subject up to date with an addendum.

A good summary of his extensive work on the changes of nitrogen metabolism during metamorphosis of tadpoles to frogs is given by George W. Brown, Jr., and Cohen in a symposium presentation (1958). By 1958 Cohen's group had studied this intriguing transformation of the ammonia-excreting tadpole to the urea-excreting frog rather extensively. They had concluded that the Krebs-Henseleit ornithine-urea cycle was induced during metamorphosis. They pointed out that the nitrogen metabolism observed involved a highly integrated system consisting of nine enzymes operating in three subcycles. The net reaction at steady state kinetics converted two moles of ammonia and one mole of bicarbonate to urea at the expense of three moles of ATP. The investigators used tadpoles of the giant bullfrog, which often have livers that weigh over a gram. As the ratio of the hind limb/tail increased, the percentage of nitrogen recovered as urea increased dramatically. Brown and Cohen followed changes not only in the ammonia to urea ratio with development but the changes in a variety of other pertinent enzymes as well. They present an interesting discussion of the evolutionary development of the urea cycle.

Phil Cohen was a graduate student at Wisconsin during the 1930s and did his postdoctoral stints with Krebs in England and then at Yale. When he returned to Wisconsin it was an era of great research activity on respiratory enzymes.

Conrad A. Elvehjem and Perry W. Wilson had done postdoctoral work at Cambridge University, and they had their graduate students busily investigating respiratory processes with manometric methods. They organized a very active seminar group and as a product published a book in 1939 entitled *Respiratory Enzymes* with Elvehjem and Wilson as editors. They also staged a successful symposium concerned with respiratory enzymes on the Wisconsin campus in 1941 and published the proceedings as a book. Phil Cohen contributed to both volumes. There was good interaction between the biochemistry and physiological chemistry departments, and a number of joint papers came from research collaboration between the two departments. Later members of the group published a book on manometric techniques that went through five editions.

After returning to Wisconsin from his postdoctoral period in England and at Yale, Cohen moved rapidly through the ranks. During 1941-43 he was research associate in physiological chemistry, 1943-45 assistant professor, 1945-47 associate professor, and in 1947 professor. In 1968 he was appointed Harold C. Bradley professor of physiological chemistry. Cohen succeeded Bradley as chairman of physiological chemistry. Bradley had held the chairmanship for many years and was well known locally not only for his university functions but also for his support of skiing before it was a widely accepted sport, and for the accomplishments of his seven sons on the ski slopes and elsewhere. Cohen filled the transition between chairmen seamlessly and continued as chair for twenty-seven years; obviously, he was well accepted by his colleagues. Along the way there were some turbulent years in the medical school, and Cohen aided in smoothing out the problems by accepting the post as acting dean of the medical school for two years. He also served on and chaired the University Committee, the most influential and

demanding committee on the campus. He chaired the Wisconsin Section of the American Chemical Society and was president of the Wisconsin Section of Sigma Xi.

Cohen's skills were recognized far beyond his department. For the National Research Council he served on the Committee on Growth (chairman), the Executive Committee of the Division of Medical Sciences, and the Review Committee on Biomedical Research in the Veteran's Administration. He served as an advisor to the Commission on International Relations of the National Academy of Sciences. For the National Institutes of Health he chaired the Physiological Chemistry Study Section, was a member of the National Advisory Cancer Council, Advisory Committee to the director of NIH, and National Advisory Arthritis and Metabolic Disease Council. He functioned on the Research Advisory Council of the American Cancer Society and on the Board of Scientific Counselors of the National Cancer Institute. He was a member of the National Board of Medical Examiners, Biochemistry Test Committee, and served on the Advisory Committee on Biology and Medicine for the U.S. Atomic Energy Commission. He was on the President's Public Health Service Hospital Commission and dealt with the Commonwealth Fund Award for Oxford University. Cohen was a consultant in biochemistry for the U.S. Department of State to the University of Mexico and on the Advisory Committee on Medical Research to the Pan American Health Organization. He chaired the Battelle-Northwest Biomedical Advisory Committee and was on the Advisory Committee on Biology and Medicine for the Los Alamos National Laboratory. Cohen was consultant to the administrator of the U.S. Energy Research and Development Administration and was a member of the National Commission on Research. In 1978 he chaired an ad hoc Committee for Review of Basic

Research in the Life Sciences for the U.S. Department of Energy.

Phil Cohen also was active in a number of scientific societies: the American Chemical Society (Executive Committee, Division of Biological Chemists), American Society of Biological Chemists (treasurer and member of the council), American Association for the Advancement of Science (fellow), Biochemical Society, Sigma Xi (president, Wisconsin Chapter), and the National Academy of Sciences. He also was an honorary member of the Chiba Medical Society of Japan, the Harvey Society, the Medical School Faculty of the University of Chile, the National Academy of Medicine of Mexico and the Japanese Biochemical Society.

Cohen traveled rather extensively, not only to committee meetings in the United States, but also to Japan, Korea, and Taiwan. He had visitors and students in his laboratory from these countries. His many ties with Mexico and South America were very important to him. He traveled widely there and served on committees concerned with research problems south of our borders. A number of investigators from Mexico and South America did research in Cohen's laboratory. Best known of these was Santiago Grisolia, who spent an extended period with Cohen and published at least ten papers in various aspects of nitrogen metabolism from Cohen's laboratory. Guillermo Soberon was another distinguished associate who studied with Phil Cohen and published four papers with him during the period 1957 to 1963; these papers were concerned with the formation and metabolism of uric acid and its role in diabetes. Soberon later served as president of the University of Mexico, one of the largest universities in North and South America. Cohen's contributions in Mexico were recognized with an honorary doctorate, and he was made an honorary member of the medical school faculty of the University of Chile.

Phil and Rubye Cohen and their four children lived for many years in Shorewood Hills. This is a Madison, Wisconsin, suburb directly west of the university campus, and it always has been well populated with members from the university staff. It is an independent village except for its association with the Madison school system. Many married graduate students reside in the Eagle Heights housing units adjacent to Shorewood Hills, and their children attend the Shorewood Hills grade school. Currently these children represent twenty-six foreign countries, so the Cohen children were exposed to a broad cultural spectrum while attending the Shorewood Hills school.

Dr. Cohen was a dedicated member of the faculty who focused on research, teaching, and administration. However, he did enjoy his Friday evening poker sessions with an equally intense group of faculty members from diverse departments. He was also a trout fisherman. I cannot testify to his prowess, because my own trout fishing talent was hardly exemplary. At least I did learn to avoid the alder bushes (nitrogen-fixing nonlegumes) on the backcast, as alders were dominant along many Wisconsin trout streams.

Phil and Rubye Cohen had a happy marriage of fifty-seven years. Rubye predeceased Phil by about a year. When Phil's health started to fail, he moved to Portland, Oregon, where he stayed with his daughter Julie until his death on October 25, 1993. He had a full and very diverse career in research, teaching, and public service. His role in the university was exemplary and he left many admiring and firm friends when he passed on.

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