NATIONAL ACADEMY OF SCIENCES

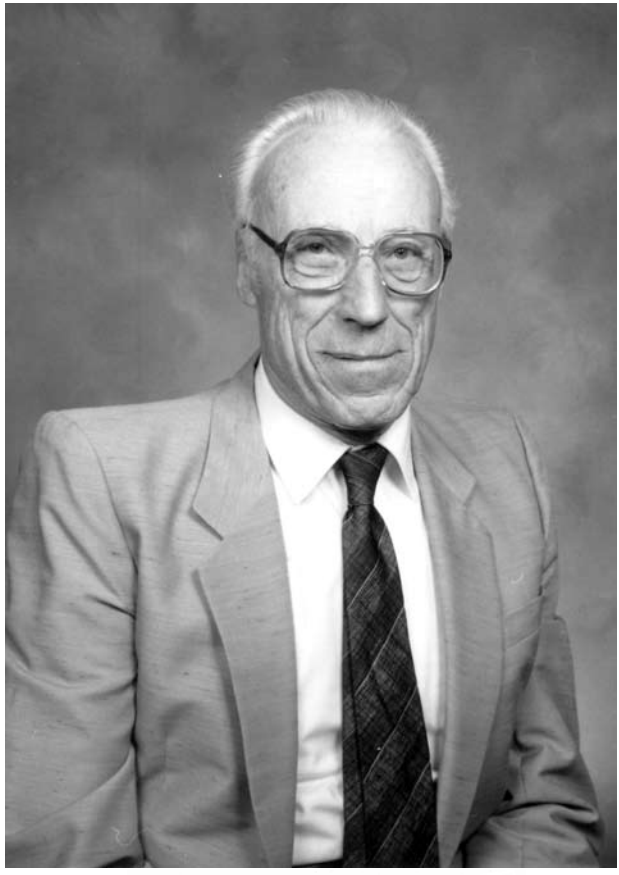
ESMOND EMERSON SNELL
1914—2003

A Biographical Memoir by

MARVIN L. HACKERT, EDITH W. MILES
AND LESTER J. REED

Any opinions expressed in this memoir are those of the authors and do not necessarily reflect the views of the National Academy of Sciences.

Biographical Memoir



Esmond E. Sull

ESMOND EMERSON SNELL

September 22, 1914–December 9, 2003

BY MARVIN L. HACKERT, EDITH W. MILES,
AND LESTER J. REED

ESMOND SNELL WAS ONE OF THE OUTSTANDING biochemists of the 20th century, well recognized nationally and internationally for his pioneering research on vitamins and the chemistry of their actions. Especially noteworthy was his development of microbiological assays for the identification and isolation of vitamin factors essential for animal nutrition, the discovery of two new forms of vitamin B6, pyridoxal and pyridoxamine, and the elucidation of the general basis for catalysis by vitamin-B6-dependent enzymes.

Esmond attended Brigham Young University, receiving his B.A. degree in chemistry in 1935. He earned an M.A. in biochemistry in 1936 and a Ph.D. in biochemistry in 1938 from the University of Wisconsin in Madison. In 1939 he was a postdoctoral research associate with Professor Roger J. Williams at the University of Texas at Austin. He was appointed assistant professor of chemistry at the University of Texas at Austin in 1941 and associate professor in 1943. He returned to the University of Wisconsin in 1945 as associate professor of biochemistry. Subsequently, he served as professor of biochemistry at the University of Wisconsin (1947-1951), professor of chemistry at the University of Texas at Austin (1951-1956), and professor of biochemistry at the University of California, Berkeley (1956-1976), including chairmanship

of the Department of Biochemistry (1956-1962). He returned to the University of Texas at Austin in 1976 as professor of both microbiology and chemistry and was chairman of the Department of Microbiology from 1976 to 1980. He became an Ashbel Smith Professor of Chemistry in 1980 and professor emeritus in 1990.

PERSONAL HISTORY

Esmond was born September 22, 1914, in Salt Lake City to Hedwig Elaine Ludwig and Huber Cyrus Snell. His parents met while serving as Mormon missionaries, married in 1905, and had five children. Esmond and his siblings attended Provo High School. Esmond attributed his interests in science to his high school chemistry teacher, a Mr. Hatch, and decided to major in chemistry at Brigham Young University. His shift into biochemistry was partly "accidental," as a result of receiving a $400 scholarship from the Wisconsin Alumni Research Fund from the University of Wisconsin and finding a vacancy in the laboratory of Professor W. H. Peterson, a biochemist who also happened to be the chair of the Fellowship Committee. After receiving his Ph.D. from the University of Wisconsin in 1938 and moving to the University of Texas at Austin, Esmond married Mary Caroline Terrill, a senior chemistry major, on March 15, 1941. Mary and Esmond were married for over 62 years and had four children: Richard, Allan, Margaret, and Esmond Jr., who was killed in action in Vietnam in 1968. Esmond Sr. died in Boulder, Colorado, on December 9, 2003, of prostate cancer and congestive heart failure, just six days after the death of his wife, Mary.

During his long and productive academic career starting as an assistant professor at the University of Texas at Austin in 1941, he trained more than 30 Ph.D. students, had more than 40 postdoctoral fellows and senior associates, and published about 400 scientific papers and reviews. He

received numerous awards, including the Eli Lilly Award in Bacteriology and Immunology from the Society of American Bacteriologists (1945), the Mead-Johnson Vitamin B Complex Award from the American Institute of Nutrition (1946), the Osborne-Mendel award from the American Institute of Nutrition (1951), the Kenneth A. Spencer award from the American Chemical Society (1974), and the William C. Rose award from the American Society of Biological Chemists (1985). He was elected to the National Academy of Sciences in 1955 and the American Academy of Arts and Sciences in 1962. He received an honorary doctor of science from the University of Wisconsin in 1982. He was a fellow of the American Association for the Advancement of Science and the American Institute of Nutrition, a former chair of the Division of Biological Chemistry of the American Chemical Society, and former president of the American Society of Biological Chemists. He served on many national and international committees and editorial and advisory boards. From 1963 to 1965 he served as chair of the U.S. National Committee for the International Union of Biochemistry. From 1968 to 1983 he served as editor of the *Annual Review of Biochemistry*, and from 1970 to 1985 as editor of *Biochemical and Biophysical Research Communications*.

DEVELOPMENT OF MICROBIOLOGICAL ASSAYS

At the University of Wisconsin, Esmond worked in Professor W. H. Peterson's laboratory, where he began his interests in microbial metabolism. Esmond's Ph.D. thesis project was to identify factors or supplements that were necessary for the growth of lactic acid bacteria. At that time the only B vitamins known were riboflavin and thiamine. Esmond's first publication, with F. M. Strong, in 1937 reported that a potato extract was the source of an unknown growth factor. Much later, using the bacterial assay provided for this growth fac-

tor, Lester Reed and his coworkers isolated crystalline lipoic acid (Table 1), which proved to be an essential cofactor for pyruvate dehydrogenase. Esmond's later work resulted in the discovery of several additional vitamins and related substances (Table 2) and in the development of the microbiological assay for following the purification of these substances and determining their concentration in nature (Table 1). He published in 1939 an assay for riboflavin (Table 1), which was the first widely used microbiological assay method for a vitamin. This assay served as a prototype for assays for each of the B vitamins. The method gave results comparable to those obtained by a much more lengthy, cumbersome, and expensive rat assay. The use of microbiological assays instead of animal assays for vitamins has resulted in untold savings in time and money. While at the University of Wisconsin, Esmond also published microbiological assays for pantothenic acid and nicotinic acid (Table 1).

TABLE 1 *Development and Use of Microbiological Assays*

Compound	Year
Pantothenic acid	1938
Nicotinic acid	1938
Riboflavin	1939
Biotin	1940
Pyridoxine	1940
Pyridoxamine	1942-1944
Pyridoxal	1942-1944
Essential amino acids	1947
Lipoic acid	1951

TABLE 2 *Summary of the Growth Factors Discovered or Studied by Esmond Snell*

Compound	Year
Avidin	1940
D-alanine	1940
Folic acid	1941
Pyridoxamine	1942-1944
Pyridoxal	1942-1944
Pyridoxamine phosphate	1947
Putrescine	1949
Spermidine	1949
Spermine	1949
Pantetheine	1950
Peptides	1953

Esmond left Madison in 1939 for his first job as a postdoctoral research associate with Professor Roger J. Williams at the University of Texas at Austin, where he became assistant professor in 1941. He developed a microbiological assay for the recently discovered vitamin biotin (Table 1). He used this assay for the first purification and characterization of avidin, a protein in egg white that binds biotin very tightly (Table 2). Next, using *Streptococcus lactis* R as a test organism, Esmond Snell, along with Hershel Mitchell and Roger Williams, purified a growth factor from four tons of spinach that was named folic acid (Table 2). The report of this work has been called "A Nutrition Classic." This microbiological assay is still used for the determination of folates in the blood.

In the course of investigations of microbiological assays for pyridoxine with different microorganisms, Esmond discovered two new forms of vitamin B6. He observed that an extraordinarily high amount of pyridoxine was required for growth of *S. faecalis* when filter-sterilized pyridoxine was added

to the medium, but that much lower amounts were needed when pyridoxine was heat sterilized with the medium. The growth effect of pyridoxine for the yeast *Saccharomyces carlsbergensis* was the same under both conditions. The finding that pyridoxine also became much more active for *S. faecalis* after ammonia treatment or mild oxidation suggested that pyridoxine was converted to aldehyde and amine forms. The structures of these compounds were determined by synthesis in a collaboration with Karl Folkers and his group at Merck & Co. and were named pyridoxal and pyridoxamine, respectively (Table 2). Esmond then developed differential assays for the three forms of vitamin B6 in natural materials, using three microorganisms that had different nutritional requirements for the different forms. After returning to the University of Wisconsin in 1945, he and his student Jesse Rabinowitz discovered pyridoxamine phosphate (Table 2).

Esmond's research at the University of Wisconsin also resulted in the discovery that D-alanine is a growth factor for *S. faecalis* in the absence of vitamin B6 (Table 2) and that bacterial cell walls contain most of the cellular D-alanine. He also discovered that an amide of cysteamine with pantothenic acid was a growth factor for *Lactobaccilus bulgaricus.* He named the disulfide form pantethine and the reduced thiol pantetheine (Table 2). He also found that putrescine, spermine, and spermidine were growth factors for *Hemophilus parainfluenzae* (Table 2). Once all of the vitamins required by the lactic acid bacteria were identified and commercially available, the vitamins could be added to the basal medium along with a complete assortment of amino acids. Individual amino acids required for growth could be determined by single omissions, and then organisms that required an amino acid could be used for quantitative assay of that amino acid. These assays were widely used until the automatic ion ex-

change procedures were developed and many are still used for some purposes today.

Esmond and his colleagues also clarified the roles of peptides as growth factors for lactic acid bacteria (Table 2). They established that peptides of a given amino acid may be more effective than the free amino acid, especially if the cell is unable to transport the amino acid, the free amino acid is readily degraded, or the uptake of the amino acid is blocked by antagonistic amino acids. Strepogenin, a peptide growth factor, was identified and its activity explained.

GENERAL MECHANISM OF CATALYSIS BY VITAMIN B6

For many, Esmond's name is associated with vitamin-B6-dependent enzymes. While investigating the natural forms of vitamin B6, Esmond found that pyridoxal and pyridoxamine were readily interconverted by a fully reversible nonenzymatic transamination reaction with glutamate and α-ketoglutarate (Eq. 1). The reaction also occurred with other α-amino acids and α-ketoacids, including aspartate and oxaloacetate (Eq. 2). Coupling of two such reactions (e.g., Eq. 1 and 2) would give a fully reversible nonenzymatic transamination reaction (Eq. 3) in which pyridoxal and pyridoxamine would act only as catalysts. In 1944 Esmond proposed that these compounds might play a similar role in enzymatic transamination.

(1) Glutamate + pyridoxal ⇔ Pyridoxamine + α-ketoglutarate
(2) Oxaloacetate + pyridoxamine ⇔ Pyridoxal + aspartate
(3) Glutamate + oxaloacetate ⇔ α–ketoglutarate + aspartate

Esmond and his colleagues next undertook a detailed investigation of the mode of action of vitamin B6. They found that pyridoxal catalyzed a series of nonenzymatic reactions of amino acids that simulated closely the corresponding reactions catalyzed in living organisms by pyridoxal-phos-

phate-dependent reactions. David Metzler initiated detailed studies of these model nonenzymatic reactions for his thesis work at the University of Wisconsin. He recalls that Esmond walked in with a bottle of a new compound Versene (EDTA) and asked what effect it would have on the nonenzymatic reactions. David's finding that EDTA was strongly inhibitory led to the discovery that the nonenzymatic reactions were metal ion dependent and established conditions for obtaining reproducible kinetic results. These studies led to the proposal in 1954 of a general mechanism for the action of vitamin-B6-dependent enzymes. This mechanism, which was similar to one proposed independently by A. E. Braunstein, helped to explain the multiple roles played by vitamin B6 in living organisms. For example, pyridoxal-phosphate-dependent enzymes catalyze a wide variety of reactions, including transamination, αß-elimination, ß-replacement, and decarboxylation, racemization, and the aldol cleavage reactions.

Esmond next turned to studies of vitamin-B6-dependent enzymes in order to test some aspects of the proposed general mechanism. The characterization of a pyridoxamine-pyruvate transaminase in 1962 demonstrated that the phosphate group of pyridoxal phosphate does not contribute to catalysis. Demonstration of the enzymatic cleavage of α–methylserine to D-alanine and formaldehyde in 1962 showed that this reaction does not require labilization of an α-H. The results of extensive studies of tryptophanase supported the proposal that ß-elimination and ß-replacement reactions proceed through an α-aminoacrylate intermediate.

DISCOVERY OF PYRUVOYL ENZYMES

Esmond and his coworkers also investigated several other pyridoxal-phosphate-dependent enzymes, including D-serine dehydratase, arginine decarboxylase, and histidine decarboxylase. Interestingly, there are two types of histidine decarbo-

xylases. One type, which is pyridoxal phosphate dependent, is found in Gram-negative bacteria and in mammals. The second type, discovered by Esmond, is found in Gram-positive organisms (such as *Lactobacillus* 30a) and contains an essential, covalently bound pyruvoyl prosthetic group that participates as a Schiff base in the catalysis of decarboxylation. Esmond and colleagues proved that the pyruvoyl group arises from a specific serine residue in the proenzyme by a previously unobserved, unique intrachain nonhydrolytic cleavage reaction. Peptide chain cleavage is coupled to an αß-elimination reaction to form an active enzyme that contains two chains, one of which has an N-terminal pyruvoyl residue.

Several other enzymes have been found subsequently to have pyruvoyl prosthetic groups: S-adenosylmethionine decarboxylase, L-aspartate-α-decarboxylase, phosphatidylserine decarboxylase, arginine decarboxylase, and proline and glycine reductases. In the mid-1980s Esmond, working with Marvin Hackert and Beth Parks and later Travis Gallagher, solved the three-dimensional structure of the pyruvoyl-dependent histidine decarboxylase from *Lactobacillus* 30a. The structure permitted identification of the active site and the structural basis for the subsequent analysis of its catalysis. The postulated roles of many of these residues are consistent with the results of site-directed mutagenesis studies carried out in both Esmond's laboratory and that of Jon Robertus, also at the University of Texas at Austin.

ROLE AS MENTOR AND HIS PHILOSOPHY OF SCIENCE

In addition to his scientific achievements, Esmond played a central role as a good citizen of the biological community and was widely regarded as a gentle, unassuming person. Esmond was also highly regarded as a teacher and mentor. During his work at the University of Texas at Austin in the early 1940s, Esmond worked actively in the laboratory, often

alone. When he moved to the University of Wisconsin in Madison in 1945 and had many more students and heavier teaching responsibilities, Esmond found that he needed to spend most of his time in his office. His students at the University of Wisconsin included J. C. Rabinowitz, L. M. Henderson, H. P. Broquist, D. E. Metzler, and G. M. Brown, all of whom have had outstanding careers of their own in academia and industry. David Metzler remembers that

> everybody was expected to be behind the bench at 8 a.m., six days a week. Sitting at a desk didn't count as real work. Esmond was very well read and could point us in new directions. We were always amazed at how many hours he spent writing in his office behind closed doors. He only came out for quick daily tours around the laboratory. We also saw him on a rigid weekly schedule in his office. He treated us all with respect, even when work was not going well. We all liked Es.

Gene Brown remembers:

> Esmond took me under his wing and taught me how to be a professional scientist. Without question, he has been the one person who has made the most impact on me and my life as a person and a scientist. He and Mary became my surrogate parents during my years as a graduate student.

The late Beverly Guirard was one of Esmond's earliest and longest associates. At the University of Texas in the early 1940s, she participated in the identification of the natural forms of vitamin B6. Beverly joined Esmond's group when he returned to the University of Texas in 1951. She remained with the group through the years in Berkeley (1956-1976) and the return to Austin in 1976 until her retirement in the 1990s. Beverly provided continuity to the laboratory and was very helpful to everyone. During Esmond's early days at Berkeley, his laboratory included several members who had come from the University of Texas, new graduate students (Charles Goodhue, Edith Wilson [née Miles], and Austin Newton) and postdoctoral fellows and senior research as-

sociates (H. Kihara, M. Ikawa, D. B . McCormick, J. Mora, T. K. Sundaram, W. B. Dempsey, and others). On Saturday mornings Beverly would prepare French-roasted coffee in the style of her native Louisiana, and Esmond would come out of his office to join those who were working. By that time, Saturday work was no longer required but was still appreciated. The Snell group enjoyed occasional lunch breaks at the botanical gardens in Strawberry Canyon, a natural beauty spot in the hills above the Berkeley campus.

Esmond also influenced many other graduate students at the University of California at Berkeley through his teaching the Introduction to Biochemistry course. The arrival of Hiroshi Wada in 1961 marked the beginning of important contributions by a number of Japanese scientists to the laboratory. Wada was followed by a series of other Japanese scientists, including Y. Morino, H. Kagamiyama, and H. Hayashi. All of these scientists later became professors in Japan. In 1971 Esmond enjoyed a sabbatical at Osaka University. One day after his lecture, a student asked him to write on a fancy paperboard a message for the students. He wrote,

> Hard work on an interesting problem is enjoyable and preferable to aimless wasting of leisure time. It may also lead to unexpected findings that give insights into important related problems. Such unexpected findings—sometimes called "luck"—frequently happen to the active researcher, but only rarely to those who prefer talk to study and work. So one should study and work hard, on interesting problems of any nature, with the purpose of explaining nature and helping others.

Many Japanese scientists have copies of this statement, which summarizes Esmond's philosophy of science.

Many international investigators regard Esmond Snell and Alexander Braunstein as the fathers of vitamin B6. The last 60 years have seen tremendous progress in studies of nonenzymatic and enzymatic catalysis, using modern techniques

of protein purification, recombinant genetics, and structural biology. Much of this progress has been reported in a series of international symposia on pyridoxal catalysis, which later included other carbonyl compounds as cofactors: see Nagoya (1967), Leningrad (1974), Toronto (1979), Athens (1983), Turku (1987), Osaka (1990), Capri (1994), Santa Fe (1999), and Southampton (2002). The meeting in Turku memorialized the death of Alexander E. Braunstein (1902-1986). The B6 meeting in Santa Fe in 1999 was dedicated, as a special honor, to Esmond Snell. Esmond retired from his laboratory at the University of Texas at Austin in 1990. He and Mary enjoyed many interesting travels in their later years as well as contacts with family and former colleagues.

THE AUTHORS GRATEFULLY ACKNOWLEDGE contributions of materials and comments from the memoriam posted on the University of Texas Web site and previous review articles, especially the review by Edith Miles and David Metzler that appeared in the *Journal of Nutrition* (vol. 134, pp. 2907-2910, 2004).

SELECTED BIBLIOGRAPHY

1939

With F. M. Strong. A microbiological assay for riboflavin. *Ind. Eng. Chem.—Anal. Ed.* 11:346-350.

1940

With R. E. Eakin and R. J. Williams. A constituent of raw egg white capable of inactivating biotin in vitro. *J. Biol. Chem.* 136:801-802.

With R. E. Eakin and R. J. Williams. A quantitative test for biotin and observations regarding its occurrence and properties. *J. Am. Chem. Soc.* 62:175-178.

1941

With L. D. Wright. A microbiological method for the determination of nicotinic acid. *J. Biol. Chem.* 139:675-686.

With H. K. Mitchell and R. J. Williams. The concentration of "folic acid." *J. Am. Chem. Soc.* 63:2284-2284.

1945

The nutritional requirements of the lactic acid bacteria and their application to biochemical research. *J. Bacteriol.* 50:373-382.

1948

With J. C. Rabinowitz. The vitamin B6 group, distribution of pyridoxal, pyridoxamine, and pyridoxine in some natural products. *J. Biol. Chem.* 176:1157-1167.

1954

With D. E. Metzler and M. Ikawa. A general mechanism for vitamin-B6-catalyzed reactions. *J. Am. Chem. Soc.* 76:648-652.

1957

With J. B. Longnecker and M. Ikawa. The cleavage of alpha-methylserine and alpha-methylolserine by pyridoxal and metal ions. *J. Biol. Chem.* 226:663-666.

1962

With H. Wada. Enzymatic transamination of pyridoxamine. I. With oxaloacetate and alpha-ketoglutarate. *J. Biol. Chem.* 237:127-132.

With E. M. Wilson. Metabolism of alpha-methylserine. I. Alpha-methylserine hydroxymethyltransferase. *J. Biol. Chem.* 237:3171-3179.

1965

With W. A. Newton and Y. Morino. Properties of crystalline tryptophanase. *J. Biol. Chem.* 240:1211-1218.

1968

With W. D. Riley. Histidine decarboxylase of Lactobacillus 30a—presence of covalently bound pyruvate as prosthetic group. *Biochemistry* 7:3520-3528.

1970

With P. A. Recsei. Histidine decarboxylase of Lactobacillus 30a. VI. Mechanism of action and kinetic properties. *Biochemistry* 9:1492-1497.

1973

With P. A. Recsei. Prohistidine decarboxylase from Lactobacillus 30a. A new type of zymogen. *Biochemistry* 12:365-371.

1975

Tryptophanase: Structure, catalytic activities, and mechanism of action. *Adv. Enzymol. Relat. Areas Mol. Biol.* 42:287-333.

1982

Some comparative aspects of pyridoxal phosphate and pyruvoyl-dependent amino acid decarboxylases. *Adv. Exp. Med. Biol.* 148:257-272.

1984

With B. M. Guirard and S. Tanase. Pyridoxal-P dependent bacterial histidine decarboxylase. *Prog. Clin. Biol. Res.* 144A:235-244.

1986

With G. L. Vaaler and M. A. Brasch. Pyridoxal 5'-phosphate-dependent histidine decarboxylase. Nucleotide sequence of the hdc gene and the corresponding amino acid sequence. J. Biol. Chem. 261:11010-11014.

With M. J. K. Nelson. Enzymes of vitamin B6 degradation. Purification and properties of 5-pyridoxic-acid oxygenase from *Arthrobacter sp. J. Biol. Chem.* 261:15115-15120.

1989

With T. Gallagher and M. L. Hackert. Pyruvoyl-dependent histidine decarboxylase—active site structure and mechanistic analysis. *J. Biol. Chem.* 264:12737-12743.

1990

With P. D. van Poelje. Pyruvoyl-dependent Enzymes. *Annu. Rev. Biochem.* 59:29-59.

1991

With A. V. Kamath and G. L. Vaaler. Pyridoxal phosphate-dependent histidine decarboxylases. Cloning, sequencing, and expression of genes from *Klebsiella planticola* and *Enterobacter aerogenes* and properties of the overexpressed enzymes. *J. Biol. Chem.* 266:9432-9437.

1993

From bacterial nutrition to enzyme structure—a personal odyssey. *Annu. Rev. Biochem.* 62:1-27.