LESTER J. REED

January 3, 1925–January 14, 2015
Elected to the NAS, 1973

A Biographical Memoir by Marvin L. Hackert

LESTER J. REED, Ashbel Smith Professor Emeritus of Chemistry and Biochemistry at The University of Texas at Austin (UT), was recognized nationally and internationally for his pioneering research on the isolation of lipoic acid and his contributions to our understanding of the mechanisms of action and structures of multi-enzyme complexes and their regulations via phosphorylation/dephosphorylation. He began his distinguished career at UT as an assistant professor in 1948 (at the young age of twenty-three!) and was promoted to associate professor in 1955 and to professor by 1958. He conducted a productive research program for fifty-three years until his retirement in 2001.

EARLY LIFE AND EDUCATION

Lester J. Reed was born on January 3, 1925, in New Orleans, Louisiana, the son of John T. and Sophie Pastor Reed. Inspired by a Gilbert chemistry set given to him by his sister Julia, Lester demonstrated an early aptitude for scientific inquiry and discovery. His initial experiments were performed on a small table in a back bedroom. When the house filled with the rotten egg odor of hydrogen sulfide, however, his "lab" was banished to a shed under the raised house. Eventually, his need for a proper laboratory was recognized, and soon a sink and running water were added and the space more formally enclosed.

Lester Reed attended Tulane University in New Orleans, where he worked in the laboratory of William Shive as an undergraduate student. He received a bachelor of science in chemistry in 1943 and then entered graduate school at the

Figure 1 Lester J. Reed.

University of Illinois Urbana-Champaign, earning a Ph.D. in organic chemistry in 1945—in just two years and at the young age of twenty! From there, he continued his training as a postdoctoral fellow in biochemistry with Vincent Du Vigneaud at Cornell University Medical College in New York City. Du Vigneaud was recognized with the Nobel Prize in Chemistry in 1955 for his work on biochemically important sulfur compounds and especially for the isolation and synthesis of the pituitary hormones vasopressin, which acts on the muscles of the blood vessels, and oxytocin, the principal agent causing contractions of the uterus and secretion of milk.

NATIONAL ACADEMY OF SCIENCES

During Lester's time at Cornell, two life-changing events occurred: He was introduced to the growing field of biochemistry, and he met and married the love of his life, Janet Gruschow. Lester and Janet spent long hours together in the cold room purifying proteins and fell in love. They were married on August 7, 1948. That year, Lester was recruited to the Department of Chemistry and the Clayton Foundation Biochemical Institute at UT by William Shive, who had since moved from Tulane to UT. Lester and Janet confessed later that Lester did not want to go to Austin alone, and for Janet, who was born and raised in Rochester and had never been south of New York City nor west of Buffalo, the marriage provided an opportunity for adventure and to see more of the country. At UT, Reed joined a team led by Roger J. Williams, William Shive, Robert Eakin, and others discovering and studying B vitamins. This led to a trail of research that spanned over fifty years, from the initial isolation and characterization of a microbial growth factor (lipoic acid) to the elucidation of the structure, function, and regulation of α-keto acid dehydrogenase complexes and a broader understanding of protein complexes and regulation of metabolic pathways.

THE MULTI-ENZYME COMPLEXES JOURNEY BEGINS— ISOLATION OF LIPOIC ACID

These discoveries began in the spring of 1949 and have been recounted in Lester's own words in a 2001 Reflections article in the *Journal of Biochemistry*.[1] About six months after Lester joined the faculty in the Department of Chemistry at UT, he started working on the isolation of a factor that replaced acetate in the growth medium for certain lactic acid bacteria. Research on the "acetate-replacing factor" had been initiated by Esmond Snell and associates at the University of Wisconsin and then at UT. They had established that this factor was widely distributed in animals, plants, and microbial cells, and that liver was a rich source. The factor was tightly bound to liver protein(s) and was released by proteolysis or by acid hydrolysis. At that time, pharmaceutical companies were processing large amounts of porcine and bovine liver to obtain extracts suitable for possible treatment of pernicious anemia. The active ingredient in liver was shown later to be vitamin B_{12}. Its deficiency or the inability to absorb B_{12} is the cause of pernicious anemia. Fresh liver was extracted with warm water to remove the water-soluble factors, such as B_{12}, but the residual liver proteins and fatty material that had little biochemical value and were dried and sold as an animal feed supplement. Lester made arrangements with Eli Lilly and Co. to obtain this liver residue, and his lab developed procedures for extracting and purifying the acetate-replacing factor, processing about six pounds of liver residue at a time and achieving purification at factors of 16,000X to 50,000X. By the fall of 1950, the Lilly Research Laboratories had adapted and scaled up isolation procedures, so instead of processing six-pound batches of liver residue at a time, Lilly was able to process 250-pound batches of liver tissue. Concentrates of the acetate-replacing factor that were less than 1 percent pure were sent to Lester's laboratory at UT for further purification. In March 1951, he obtained the first pale yellow crystals of the acetate factor. It was given the name α-lipoic acid.[2] The isolation involved a 300,000X purification. In total, approximately 30 milligrams of crystalline lipoic acid were isolated from an estimated 10 tons (or ~9 trillion milligrams) of liver residue!

Wanting to know more about the functional role of this simple but unique organic compound, Reed and his colleagues went on to discover that lipoic acid was bound to a protein involved in the CoA- and NAD+-linked oxidative decarboxylation of pyruvate and α-ketoglutarate. Thus began a trail of five decades of research to study the mechanism of oxidative decarboxylation of α-keto acids.

The discovery of how the lipoic acid moiety was bound to protein was elucidated with the help of radioisotopes—a common tool in the 1950s to map metabolic pathways. When *E. coli* (Crookes strain) was grown aerobically in the presence of lipoic acid labelled with ^{35}S, the radioactive substance was incorporated into the pyruvate and α-ketoglutarate dehydrogenation systems. This led to isolation of highly purified complexes permitting rapid progress in the late 1950s in identifying the moiety to which lipoic acid was bound. The protein-bound radioactive lipoyl moiety was oxidized with performic acid, and the protein was partially hydrolyzed with hydrochloric acid. From the hydrolysates, a ninhydrin-positive, radioactive conjugate was isolated and identified as ε-*N*-(6,8-disulfooctanoyl)-L-lysine by degradation and synthesis. The lipoyl moiety in the two complexes was therefore bound in a flexible amide linkage to the ε-amino group of a lysyl residue of a protein.[3]
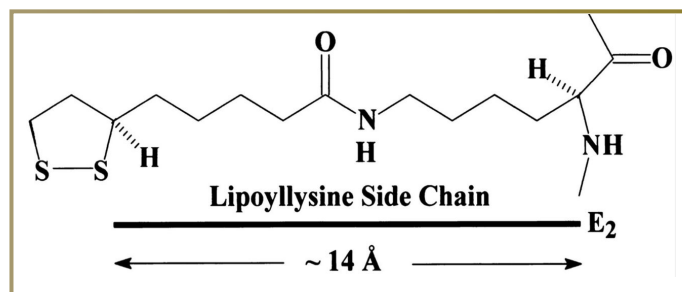

Figure 2 **Functional form of lipoic acid:** The carboxyl group of lipoic acid is bound in amide linkage to the ε-amino group of a lysine residue in the acyltransferase component (E_2) of the α-keto acid dehydrogenase complexes. This flexible linkage provides a "swinging arm" that facilitates communication between active sites. *Image from L. J. Reed, 2001.*

FROM LIPOIC ACID TO LARGE MULTI-ENZYME COMPLEXES

The next milestone was to identify, purify, and reconstitute the proteins containing the lipoyl moiety. Prior to 1950, pyruvate and α-ketoglutarate oxidation preparations were not suitable for detailed analysis. Reed's laboratory developed procedures for purification of both the pyruvate and α-ketoglutarate oxidation systems from *E. coli*. In collaboration with William Carroll at the National Institutes of Health, analytical ultracentrifugation (another important research instrument of that time) was used to demonstrate that they had obtained a major symmetrical peak for each of the two highly purified preparations and that the boundary of the yellow color of the flavoprotein was associated with the main peak. The molecular weights of these multienzyme units were determined to be 4.8 and 2.4 million, respectively. Reed and colleagues were able to dissect the pyruvate and α-ketoglutarate dehydrogenase complexes into their component enzymes, characterize them, and reassemble the large functional units from the isolated enzymes. They demonstrated that each of these functional units was composed of multiple copies of three enzymes, a pyruvate or an α-ketoglutarate decarboxylase-dehydrogenase (E_1), a dihydrolipoamide acetyltransferase or a succinyltransferase (E_2), and a flavoprotein, dihydrolipoamide dehydrogenase (E_3). These three enzymes, acting in sequence, catalyze the reactions shown below. E_1 catalyzes both the decarboxylation of the α-keto acid (Reaction 1) and the subsequent reductive acylation of the lipoyl moiety, which is covalently bound to E_2 (Reaction 2). E_2 catalyzes the acyl transfer to CoA (Reaction 3), and E_3 catalyzes the reoxidation of the dihydrolipoyl moiety with NAD^+ as the ultimate electron acceptor (Reactions 4 and 5). This process is demonstrated in Figure 3 from Reed's 1974 article on multienzyme complexes.[4]

Reed's laboratory determined that the lipoic acid was covalently attached to the E_2 component and that E_2 had both catalytic and structural roles, binding the other components.
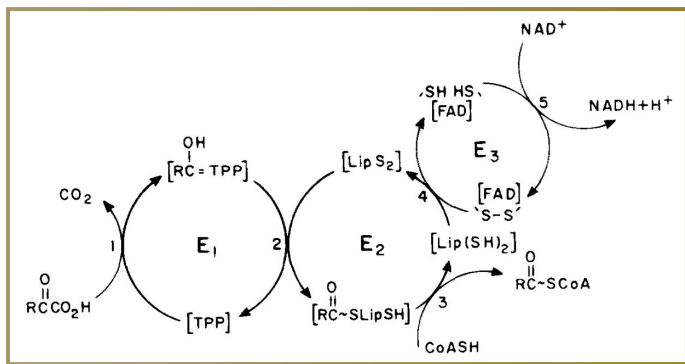

Figure 3 **Reaction sequence in α-keto acid oxidation:** TPP, thiamin diphosphate; $LipS_2$ and $Lip(SH)_2$, lipoyl moiety, and its reduced form. *Image from L. J. Reed, 2001.*
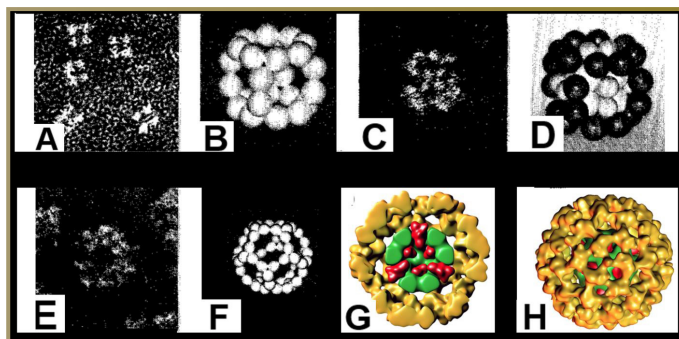

Figure 4 Electron micrograph images and interpretative models of images of the pyruvate dehydrogenase complexes and E_2 cores (*E. coli* A D) and the bovine kidney pyruvate dehydrogenase complex (E–H). *Images A–F from L. J. Reed, 1974; images G–H from Z. H. Zhou et al. 2001.*

His laboratory later used electron microscopy (another relatively new tool at the time) to discover that there were two different architectural forms of these large complexes (the cube with twenty-four E_2 subunits, and the pentagonal dodecahedron with sixty E_2 subunits), as shown in Figure 4. He went on to propose a novel "swinging-arm" active-site coupling mechanism to explain how multiple copies of these three enzymes were able to efficiently catalyze successive reactions using five vitamin-related cofactors.

This concept was confirmed and extended by electron microscopy studies conducted by his long-time associate Robert Oliver. Electron micrographs of the *E. coli* PDH complex and its component enzymes negatively stained with phosphotungstate revealed that the complex had a polyhedral structure with a diameter of about 300 Å, that the acetyltransferase (E_2) formed the center polyhedron, and that the molecules of E_1 and E_3 were distributed on its surface. The shape of the acetyltransferase indicated that it had a cubelike structure. The shape of the succinyltransferase component of the *E. coli* KGDH complex was very similar. These results, together with biochemical data, demonstrated that both E_2s consist of twenty-four apparently identical polypeptide chains arranged as eight trimers (morphological subunits) at the vertices of a cube. This proposed structure was confirmed later by x-ray diffraction analyses carried out by collaborators David DeRosier and Marvin Hackert demonstrating that both acyltransferases possess 432 molecular symmetry.

REGULATION VIA PHOSPHORYLATION/DEPHOSPHORYLATION

The next major discovery trail was that two additional components (a kinase and a phosphatase) were involved in the regulation of the activity of these complexes via phosphorylation /dephosphorylation, which today is recognized as a common regulatory control mechanism. In the late

1960s, their research effort was redirected toward the isolation and characterization of the mammalian PDH and KGDH complexes. New procedures were developed for preparation of mitochondria on a large scale from porcine and bovine kidney and heart to isolate the PDH and KGDH complexes. In the course of attempts to stabilize these complexes in crude extracts of bovine kidney mitochondria, it was observed that the PDH complex, but not the KGDH complex, underwent a time-dependent inactivation in the presence of ATP. A systematic investigation revealed that the bovine kidney and heart PDH complexes are regulated by a phosphorylation/dephosphorylation cycle.[5] Phosphorylation and concomitant inactivation of the complex is catalyzed by an ATP-dependent kinase tightly bound to the complex, and dephosphorylation and concomitant reactivation are catalyzed by a Mg^{2+}-dependent phosphatase, which is loosely attached to the complex. In the early 1960s, the three known examples of enzyme regulation by phosphorylation/dephosphorylation were phosphorylase, phosphorylase kinase, and glycogen synthase. Reed's results with the mammalian PDH complex indicated that this regulatory mechanism was far more general than had been recognized previously.

Further understanding of structure-function relationships in eukaryotic PDH complexes was initiated in the late 1980s using emerging molecular biology techniques and molecular genetics studies of the PDH complex in the yeast *Saccharomyces cerevisiae*. The genes encoding the five proteins comprising the complex ($E_1\alpha$, $E_1\beta$, E_2, BP, and E_3) were cloned, sequenced, expressed, and disrupted. Studies on E_3-binding protein (BP) confirmed and extended previous studies of Thomas Roche and Gordon Lindsay and their associates on the protein X component of the bovine PDH complex. In the 1990s, cryoelectron microscopy and three-dimensional image reconstruction, in collaboration with James Stoops and Timothy Baker and their associates, revealed a unique structural organization of the tE_2·BP-E_3 complex.[6] In 2001, more than fifty years after he began work on lipoic acid and in one of his last research papers, Reed and his colleagues published the three-dimensional reconstruction of the bovine kidney pyruvate dehydrogenase complex (Mr ~7.8×10^6). This complex is comprised of about twenty-two molecules of pyruvate dehydrogenase (E_1) and about six molecules of dihydrolipoamide dehydrogenase (E_3), with its binding protein associated with the sixty-subunit dihydrolipoamide acetyltransferase (E_2) core. This understanding provides considerable insight into the structural and functional organization of the largest multi-enzyme and important complexes known.[7]

HONORS AND AWARDS

Reflective of Reed's remarkable scientific career, he received the Eli Lilly Award in 1958, was elected to the National Academy of Sciences in 1973 and elected a Fellow of the American Academy of Arts and Sciences in 1981 and won the Merck Award from the American Society for Biochemistry and Molecular Biology in 1994. Reed was a member of Phi Beta Kappa, Sigma Xi, the American Chemical Society, the American Society for Biochemistry and Molecular Biology, the Protein Society, and the American Association for the Advancement of Science. He served on many advisory councils and editorial boards and was the recipient of several national honors. He was director of the Clayton Foundation Biochemical Institute from 1963 through 1996 and was appointed an Ashbel Smith Professor in 1984. He received an honorary doctorate of science degree from Tulane University in 1977. During his fifty-one years on the faculty at UT, the trio of Roger Williams, Lester Reed, and William Shive (along with Esmond Snell, Robert Eakin, and Karl Folkers) were responsible for the isolation, naming, and characterization of more vitamins than at any other place in the world.

A LIFE WELL LIVED

To many of his scientific colleagues, Lester Reed was a quiet, even shy person who often stayed out of the spotlight, but he had a gleam in his eyes that made you know that there was a lot of intensity in this man. Although Reed had a very competitive spirit when it came to his science, one had to be impressed by the high principles that characterized that science. Reed was always very careful and precise in his work. This was true in his writing, as evidenced in more than 200 publications, and in his speech—he expressed his thoughts clearly and concisely, and he always told you just what he thought. Reed had a very dry, but amazing sense of humor. Often he would crack a joke and then break into that familiar, broad smile. Outside the lab, Reed enjoyed boating and fishing, and he never missed the annual weekend outing of the "Central Texas Society of Applied Piscatology."

Lester and Janet Reed were a true team. They settled into the Austin community in 1948 and put down deep roots in the community. As his career progressed, Reed was invited to give seminars and lectures in many different countries, which gave him and Janet many opportunities to visit parts of the world they had always wanted to see. By 1960, they had welcomed their four children: Pam, Sharon, Richard, and Robert. Photos of a relaxed, young Lester playing with his kids, enjoying a boat ride on the lake with family and friends, or simply sipping a drink while enjoying their pool, reveal a loving husband, father, and later in life, a very proud grandparent. Lester and Janet were married for over sixty-six years. In later years, they traveled together extensively, visiting every continent, and experiencing life in many countries. In 1997, Janet created the Lester J. Reed Professorship in Biochemistry at The University of Texas at Austin to honor Lester and his

love of science. He taught at the university until 1999 and conducted a productive research program until 2001, when he retired after fifty-three years. He died peacefully in Austin, Texas, on January 14, 2015, at the age of ninety. He is still missed by his colleagues, friends, family and many former students and postdocs around the world. Those of us that were lucky enough to have known and worked with Lester Reed were richly blessed by our time shared with him.

NOTE

This memorial tribute is an expansion of two prior memorial tributes to Lester J. Reed,[8,9] his published obituary,[10] and information from Lester's own description of his research journey published in the *Journal of Biological Chemistry* in 2001.[11]

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