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LEON ALMA HEPPEL
1912–2010

A Biographical Memoir by
MAXINE SINGER

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Leon A. Heppel

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LEON ALMA HEPPEL

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BY MAXINE SINGER

LEON ALMA HEPPEL died on April 9, 2010, in Ithaca, New York. He was professor emeritus of biochemistry at Cornell University, where he had been since 1967. Before that, he was, from 1958, chief of the Laboratory of Biochemistry and Metabolism of the National Institute of Arthritis and Metabolic Diseases at the National Institutes of Health. Although he pursued several lines of research in his almost 60 years in the laboratory, he was best known for his extraordinary contributions to early biochemical work on nucleic acids and the enzymes active with nucleotides and nucleic acids.

As an undergraduate student at the University of California, Berkeley, Heppel was honored by election to membership in Phi Beta Kappa and Sigma Xi. He stayed on at Berkeley and was awarded the Ph.D. degree in biochemistry in 1937. In 1941 he received the M.D. degree from the University of Rochester, New York, and was elected to Alpha Omega Alpha. Heppel was a recipient of a Guggenheim fellowship in 1953. He was, at various times, on the editorial boards of the *Journal of Biological Chemistry* and *Archives of Biochemistry and Biophysics*. In 1959 Heppel received the Hillebrand Award of the Washington Section of the American Chemical Society. In 1970 he was elected to the National Academy of Sciences and the American Academy of Arts and Sciences.

Heppel was the first-born offspring of a couple who had emigrated from Germany to Utah, where they became Mormons. After his birth in 1912 in Granger, Utah, four more children were born. The family struggled with Utah farm life and moved on to San Francisco. As a high school student, his early interest and talent in chemistry and an ambitious mother landed him a position doing analytical work in a chemical company. This job supported Heppel through high school but midway through his undergraduate studies at Berkeley the position was terminated when the chemical company merged with another. Heppel failed to convince the new leadership to keep him on and he wrote in his own memoir that it was this cruel treatment that made him give up his study of chemical engineering. Instead, he completed a B.S. degree in chemistry in 1933 depending on scholarships and fellowships for this and for his subsequent graduate studies. When he enrolled for graduate studies at Berkeley he took up biochemistry, thinking it "would be a gentler profession" than chemical engineering (2004).

Heppel decided to continue his studies at Berkeley. Biochemistry professor C. L. A. Schmidt was interested in potassium metabolism, and Heppel decided to do his thesis on this topic under Schmidt's supervision. These studies demonstrated that dietary potassium is required for growth and survival of young rats. The work was excellent but when Heppel completed his Ph.D. degree in 1937, the hard fact was that jobs were scarce. In those years, as in 2010, staying in school was an alternative to the dismal job prospects. All of Heppel's years of study at Berkeley were in the shadow of the Great Depression. He lived at home in San Francisco and took a grueling commute to Berkeley every day—streetcar, ferry across the bay, and train from Oakland to Berkeley—and back home each evening. Frugal habits he developed then would stay with him all his life. Even when a laboratory chief

at the NIH, Heppel daily folded his used brown paper lunch bag and took it home to serve another day.

Some years before Heppel completed his doctoral degree, George Whipple left San Francisco to start the new medical school at Rochester, New York. Whipple told Schmidt that support would be available for a Ph.D. student of Schmidt's who wanted to attend the new medical school. Whipple was as good as his word and Heppel left for the east where he continued his work on potassium metabolism in young rats under the general guidance of W. O. Fenn. In an early application of radioactive isotopes, Heppel demonstrated that contrary to prior ideas, both potassium and sodium ions can enter animal cells through the cell membrane, thereby contributing significantly to the ongoing interest in electrolytes (1940). From Heppel's description it seems likely that Fenn was the model for two of Heppel's lifelong practices: working at the bench and allowing postdoctoral fellows to be sole author on papers describing their independent work.

Heppel had a choice of excellent residency positions when he completed his M.D. degree in 1941, but once again national issues, this time the entry into World War II, dictated otherwise. Along with his classmate and good friend Arthur Kornberg, Heppel enlisted in the U.S. Public Health Service. Kornberg served a year at sea before joining Heppel at the young National Institutes of Health in Bethesda, Maryland. At the navy's request Heppel was assigned toxicity studies on halogenated hydrocarbons, research he found tedious but that led, after wartime censorship ended, to 15 published papers between 1944 and 1949. Meanwhile, the most important developments in Heppel's scientific career were proceeding independently. He and Kornberg had made friends with Bernard Horecker, an enzymologist, and Horecker began to tutor Heppel, Kornberg, and Herbert Tabor in enzymology. All four of these men became eminent scientists and were

elected to the National Academy of Sciences. Heppel was then able to investigate the action of enzymes on the toxins he had been studying.

What started as tutoring became a serious course of self-education when the group began bringing brown bag lunches to a daily (except Christmas Day) meeting. Eventually, others including postdoctoral fellows were invited to join, and this lunch club went on for decades even as its founders dispersed. I started attending the lunch club in 1956 when I joined Heppel as a postdoctoral fellow; I stopped only when I went to join the National Cancer Institute in 1975.

By 1950 Horecker, Kornberg, and Heppel were all investigating different aspects of the biochemistry of phosphate-containing compounds. Heppel concentrated on the hydrolysis and phosphorolysis of ribonucleotides and RNA and their derivatives; much of this and future work was done in collaboration with Russell J. Hilmoe (1951). One of their main interests was a phosphodiesterase found in bovine spleen (1955). Although they purified the enzyme to some extent, they were unable to characterize satisfactorily the products of the digestion of polyribonucleotides.

Heppel decided to take a leave from the NIH and spend 1953 in Roy Markham's laboratory in Cambridge, England, where he hoped to solve the problem of the structure of the products of the spleen phosphodiesterase. He was counting on Markham's teaching him his innovative techniques for partition chromatography and electrophoresis on paper strips to identify and analyze nucleic acid components. With the support of a Guggenheim Fellowship and a travel grant from the American Cancer Society, Heppel, his wife, Adelaide, and their two sons (ages five and one) sailed across the Atlantic on the S.S. United States. It was a long way from the ferry across San Francisco Bay.

Markham, a few years younger than Heppel, was, with his associates, interested in determining the structure of the internucleotide bonds in RNA.[1] Did the phosphodiester link go between the 5′-hydroxyl on one nucleotide and the 2′-hydroxyl or the 3′-hydroxyl on its neighbor? Both acid and alkaline hydrolysis yielded 2′,3′-cyclic phosphodiester intermediates and a mixture of 2′- and 3′-mononucleotides as final products. Similarly, while RNase A yielded only 3′-mononucleotides it did so through a 2′,3′-cyclic phosphodiester intermediate. Neither of these results could establish unequivocally what kind of bond was in the RNA itself. Many people thought there was good reason to favor the 5′ to 3′ linkage in analogy with the internucleotide bond in DNA and because RNase A hydrolyzed pyrimidine 3′-benzyl but not 2′-benzyl phosphodiester. However, the standard of proof required by biochemists of the day demanded a more rigorous demonstration. Heppel and Markham and their colleagues settled the issue by the ingenious use of highly specific enzymes. In particular, the partly purified phosphodiesterase from bovine spleen that Heppel had brought to Cambridge yielded 3′-purine mononucleotides from oligonucleotides such as ApApU with no intermediary formation of cyclic nucleotides (1953, 1955). Heppel had not only accomplished the aim of his stay in England but also used that knowledge to solve a problem central to understanding RNA. Because of this and related work during that busy year in England, Heppel returned home as one of the few recognized U.S. experts on nucleic acids. He had worked as prodigiously in England as he did at home. And, as at home, he and his wife found time to enjoy London and the life around them.

Besides pinning down the internucleotide bond in RNA, Heppel that year also demonstrated that RNase A and the spleen phosphodiesterase could catalyze nucleotide transfer reactions; for example, incubation of 2′,3′-cyclic AMP, meth-

anol, and enzyme yielded adenosine-3´ methyl phosphodiester (1955). RNase A would even use a nucleoside or nucleotide as acceptor and catalyze the synthesis of polyribonucleotides (1955). In this paper the authors proposed a modification of the then current abbreviations for polynucleotides, and their terminology is used to this day. While it soon became clear that polyribonucleotides are not synthesized by transphosphorylation in cells, the work had several consequences. One consequence was personal for me. It was the work on transphosphorylation that attracted the interest of Joseph S. Fruton, my Ph.D. professor at Yale, and lead him to recommend that I apply to Heppel for postdoctoral training.

Most importantly, in 1955 when Marianne Grunberg-Manago and Severo Ochoa discovered polynucleotide phosphorylase (PNPase) in extracts of *Azotobacter vinelandii,* they turned to Heppel, now back at the NIH, to collaborate in the characterization of the polyribonucleotide products. This effort and Heppel's growing interest in the mechanism of polymerization and of the reverse reaction, phosphorolysis of polyribonucleotides occupied him for several years (1956, 1957). It also made his lab attractive to investigators interested in joining the now burgeoning field of nucleic acids. They came as visitors to learn techniques from him, primarily the use of specific enzymes and separation by paper chromatography. Among the many visitors in those years were H. Gobind Khorana, M. Grunberg-Manago, I. R. Lehman, and U. Littauer, all of whom made major contributions to the rapidly growing field of nucleic acid chemistry and biochemistry. These independent investigators came for a few weeks or months. Heppel always prepared bench space (however meager) and materials for them. Often, if they had told him what they wanted to learn, he also had a complete program worked out, a program that included evening work at the bench and sometimes a couple of hours to visit

one of Washington's splendid (and free) museums. Others came for longer training periods. Audrey Stevens came as a postdoctoral fellow and discovered (simultaneously with two others) RNA polymerase; her paper, like many of those of other Heppel postdoctoral fellows, is published under her name alone.[2] When, in 1962, the first Nucleic Acids Gordon Conference was held, Heppel was cochair.

In 1961 the polyribonucleotides Heppel had been making received worldwide recognition through an unexpected, major breakthrough. A newly independent investigator in a neighboring NIH lab, Marshall Nirenberg, established an active cell-free system for protein synthesis that was stimulated by the addition of RNA. Nirenberg realized that this could be the key to deciphering the genetic code. As most scientists know, this hunch was proved in the very first experiment when poly U supported the synthesis of polyphenylalanine but not other polypeptides. Heppel's freezer held many enzymatically synthesized polyribonucleotides, and Nirenberg and his postdoctoral fellow, Heinrich Mattaei, received samples of any they wished; others were made to order for the exciting work on the genetic code. The composition of the code words for all the protein amino acids other than phenylalanine followed quickly, many of them determined with polymers in Heppel's freezer.

Heppel began to decrease his efforts with polyribonucleotides early in the 1960s. His last paper on polyribonucleotides is the first rigorous demonstration of an important phenomenon that had only been hinted at previously: the inhibition of nucleic acid polymerizations by polymers that can form stable hydrogen bonds with the newly building molecule. In other words, he documented antisense inhibition of polymerization (1963). At this time Heppel initiated studies on the release of molecules from *E. coli* cells after osmotic shock and other treatments. Two excellent postdoctoral fellows, Harold

C. Neu and Nancy G. Nossal, joined him in this effort. The work succeeded quickly and four papers were published in 1964 and others in subsequent years (1964, 1965, and 1966) as well as an important review article in 1967.

It is interesting to ponder why, just as nucleic acids were moving to the center of biochemical research, this important figure in the field shifted focus so dramatically. He had made fundamental contributions to nucleic acid chemistry and enzymology, and had taught not only students but established investigators who were eager to move into nucleic acid work. Heppel was a master at manipulating the huge molecules enzymatically, which was the only viable approach in those days. Perhaps that dramatic change in research path was simply a piece of Heppel's highly individualistic personality. Another interpretation is that he decided to leave the nucleic acid work in the hands of those who had been postdoctoral fellows in his laboratory.

Heppel's customary caution in interpreting results could not mask the importance and novelty of demonstrating that a diverse group of hydrolytic enzymes are localized in the so-called "periplasmic space" between the outer and inner (cytoplasmic) cell membranes of gram-negative bacteria. Still, his conclusions received some criticism.

H. Ronald Kaback was at the time a postdoctoral fellow in Earl Stadtman's lab at the NIH (he is now a professor at UCLA and a member of the National Academy of Sciences). Heppel presented his work to that group in one of the many forums that then fostered communication between different groups and institutes at the NIH. Kaback, who characterizes his younger self as "brash," found Heppel's interpretations of his data unconvincing and said so. Kaback's reservations stemmed from published experiments he had participated in while still a medical student and work he and Stadtman were then doing. At the time, Heppel was working on the

review article (1967). He responded to Kaback by inviting him to write an addendum to the review article explaining his alternative views; the addendum appears at the end of the published paper. This story is likely to be unique and illustrates Heppel's extraordinary sense of the nature of science and the scientific endeavor.[3]

Heppel's NIH colleagues, who thought that he was firmly rooted in Bethesda, were surprised when Efraim Racker succeeded in recruiting him to Cornell University in Ithaca, New York. After the move, Heppel seemed to thrive in the combined tasks of teaching and doing research. At Cornell, Heppel's research focused initially on the periplasmic space and the role of bacterial membranes in the transport and function of key metabolites.

Work on periplasmic enzymes and their release was continually productive. Importantly, it evolved into a study of proteins concerned with active transport of molecules, especially amino acids into the bacterial cells. Heppel valued greatly the talented students and postdoctoral fellows who did this research with him. In his own memoir he mentions every one of them by name (2004). A paper by Weiner and Heppel (1971) on the active transport of glutamine by *E. coli* was designated a *Journal of Biological Chemistry* "Classic."[4] Fenn's example so many years earlier was not forgotten; often Heppel is not a named author on the papers that emerged from his Cornell lab. In later years, however, he gave up that practice because a reviewing editor thought he was motivated by a lack of interest in the work (2004).

Always ready for new challenges, Heppel went on sabbatical to London to learn more about working with animal cells. He made several trips to the laboratory of Henry Rozengurt in London in the mid-1970s, where he initiated his studies on the effect of ATP on the permeability of animal cells, a permeability that he and his colleagues showed was typical

of many transformed cells (1985). Molecules, including nucleotides that are normally unable to cross the plasma membrane were found to be taken up by the cells after ATP treatment. The effect is highly specific for ATP. Permeabilization was interpreted as the consequence of the formation of abnormal channels. Moreover, Heppel and his colleagues demonstrated that the ratio of external to internal cellular ATP concentrations is important in determining whether cells become permeable or not. Heppel must have been amused when his group discovered that an early effect of ATP was the influx of sodium ions into and efflux of potassium ions from the cells, thus connecting to the research he had done years before. Many of the papers on these topics were authored by his students, postdocs, and collaborators and do not carry Heppel's name; only the acknowledgements reveal that the experiments were carried out in his lab and with his guidance. When the group discovered that the ATP effect was modulated by Ca^{2+} and also by the Ca^{2+}-calmodulin complex, Heppel arranged to spend time working in Claude Klee's laboratory at the National Cancer Institute. His last published paper extended the studies on the mechanism by which external adenosine leads, indirectly, to regulation of intracellular levels of cyclic AMP (1997); it was 57 years since his first publication.

The visits to Bethesda for laboratory work also meant that Leon and his wife, Adelaide, could visit with Celia and Herbert Tabor, close friends and colleagues for so many years. On visits and in his home lab Heppel insisted on doing experiments himself. If he didn't wield the pipette, then his name wasn't on the manuscript. Heppel never wanted to have a large group of students and postdoctoral fellows. If the group was too big, it would leave little or no time for his own experiments. Heppel was proud of the fact that women scientists were part of his research group long

before they were widely accepted as equal colleagues in the larger community (2004). Marie Lipsett and I were in the lab in the late 1950s, a period when Heppel collaborated with Elizabeth S. Maxwell and Elizabeth P. Anderson, and Audrey Stevens and Nancy Nossal joined soon after.

Heppel was married for more than 60 years to Adelaide Keller Heppel, who predeceased him. They are survived by two sons, David and Alan. Whether at home or on sabbatical in London, Adelaide and Leon were careful and sensitive observers who could make a short walk or a single painting or symphony into a world of experience. He shared his observations in marvelous long letters to friends and colleagues. The letters from London written during his trips to Rozengurt's laboratory suggest that the Heppels valued their time at the National Gallery almost as much as the laboratory experience. Frequently the letters included challenging quizzes on a work of art or music he loved. Always they reflected his quirky sense of humor and habits. One investigator who, as a young postdoctoral fellow worked in Heppel's department, aptly characterized Heppel as "careful, meticulous, childish, and screwy, but always calculatedly so."[5]

Heppel's habitual disregard for mail meant that unattended letters piled up on his desk for months. Then, on one of the quarterly clean-up days, a junior staff member would be assigned the task of putting it all in order. One day in 1958, upon receiving a letter from Earl Sutherland, Heppel, not nearly as "screwy" as he sometimes seemed, excavated the pile for an otherwise forgotten letter from David Lipkin. Lipkin's letter held the chemical clue that allowed Sutherland to identify cyclic AMP as a second messenger. Without adding a word of his own Heppel mailed Sutherland's letter to Lipkin and Lipkin's to Sutherland thereby advancing understanding of a central biochemical molecule. This story,

typical of Heppel's devotion to science and his colleagues, has often been retold.[6,7]

Leon Heppel exemplified the highest standards of scientific research. Those standards and his dedication inspired all who knew him and especially those who had the privilege of working in his laboratory. He also contributed hugely to the recognition, worldwide, of the National Institutes of Health as a major and excellent research center for biomedical science.[8]

NOTES

1. P. R. Whitfeld and R. Markham. Natural configuration of the purine nucleotides in ribonucleic acids. *Nature* 171(1953):1151-1152.
2. A. Stevens. Incorporation of the adenine ribonucleotide into RNA by cell fractions from *E. coli*. *Biochem. Biophys. Res. Commun.* 3(1960):92-96.
3. Personal communication, email message, March 14, 2011, from H. R. Kaback.
4. N. Kresge, R. D. Simoni, and R. L. Hill. Polyribonucleotide synthesis and bacterial amino acid uptake: The work of Leon A. Heppel. *J. Biol. Chem.* 282(2007):e13-e15.
5. R. G. Martin. A revisionist view of the breaking of the genetic code. In *NIH: An Account of Research in Its Laboratories and Clinics,* eds. H. D. Stetten Jr. and W. T. Carrigan, pp. 281-296. New York: Academic Press, 1984.
6. I. Pastan. Cyclic AMP. *Sci. Am.* 227(1972):97-105.
7. J. Hurwitz. Retrospective: Leon A. Heppel (1912-2010). *ASBMB Today* Jul. (2010):12-13.
8. Substantial portions of this memoir were published previously in M. Singer. Leon Heppel and the early days of RNA biochemistry. *J. Biol. Chem.* 278(2003):47351-47356.

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