Reminiscences of Leon A. Heppel

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Leon A. Heppel
From the Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York 14853

My parents were converted Mormons who had emigrated from Germany to Utah planning to live on a farm. The oldest of five children, I was born in Granger, Utah, in 1912. Farm life proved difficult and after 10 years our family moved to San Francisco. There, the city encouraged interesting local activities particularly for poor people, and life was more pleasant than in Utah.

In school, I became interested in chemistry. While a high school student, my mother, who was ambitious on my behalf, persuaded John Stauffer, president of Stauffer Chemical Company, to give me a job doing analytical work at the American Cream Tartar Company in San Francisco. This supported me through high school and afterward when I enrolled at the University of California, Berkeley to major in chemistry and chemical engineering.

At Berkeley

Unhappily, my job at the American Cream Tartar Company and the support it provided did not last. In 1931, the Stauffer Chemical Company merged with the Schilling Spice Corporation and the combined company owned American Cream Tartar. A vice president of Schilling Spice undertook to effect economies, but the only economy he could find was getting rid of me. Shocked and urged by my mother to plead my case, I told the vice president how much I depended on the job. His cold reply was, “You need Schilling Spice Company but does Schilling Spice need you?” I never forgot those cruel words. Because of them, I abandoned my plan to be a chemical engineer turning instead to physiological biochemistry, which I thought would be a gentler profession. Fortunately I received a fellowship that allowed me to complete a B.S. degree in 1933. That same year I entered Berkeley’s graduate school as a biochemistry student.

Living in midtown San Francisco and commuting each day to Berkeley was a tiring chore. The Bay Bridges had not been built. In early morning, I took a streetcar to the Ferry Building where I boarded a boat for Oakland; on good days this took half an hour, but if the fog was intense, it was a much longer trip. From Oakland an electric train went to Berkeley and the university. In midafternoon, I returned across the Bay and spent a few hours working in one of the several Stauffer Chemical factories. Aside from the commute, however, life and science in Berkeley were exciting. During this period, Ernest O. Lawrence and others were doing great work and were anxious to talk about it. I made good friends among the chemists, one of whom discovered $^{14}$C (Martin D. Kamen in 1940).

Nutrition was a major subfield of biochemistry in the 1930s, and I decided to do my thesis in that subject under Professor C. L. A. Schmidt. Schmidt was harsh and domineering but helpful. In later years when he became dean of the College of Pharmacy at the University of California, San Francisco, he hired my mother to take charge of equipment and supplies.

For my thesis research, I decided to work on potassium ($K^+$) metabolism in white rats. The experiments showed that $K^+$ was essential for the growth and survival of young rats, and there was some evidence that sodium ($Na^+$) could partially replace $K^+$. Rubidium ($Rb^+$) supported...
good growth in K\(^+\)-free diets for a month, but thereafter the rats developed sudden tremors and died. My Ph.D. degree in biochemistry was awarded in 1937, a year when there were no jobs available for a biochemist. Luckily, Schmidt came to my rescue. He remembered a promise that George Whipple had made when he left Berkeley to start a new medical school in Rochester, New York. Whipple had told Schmidt that if he ever had a Ph.D. student who decided to come to medical school in Rochester, the student would receive partial support from the school. Right after receiving the Ph.D., I boarded a train for Rochester.

**At Rochester**

Good fortune in the shape of a mentor came my way in Rochester. My work at Berkeley had attracted the attention of W. O. Fenn, a brilliant young physiologist who was a very quiet person and unusually kind. Fenn spent much of the day doing experiments with the help of a cheerful but somewhat talkative young woman. He gave me a position and suggested that I continue to study K\(^+\) metabolism in young rats. My initial results replicated my earlier finding that the rats grew well for a while when Rb\(^+\) replaced dietary K\(^+\) but then quickly developed tremors and died. In the early phase, although the rats appeared to be healthy, 7.5% of their muscle K\(^+\) was replaced by rubidium. Other experiments demonstrated that Na\(^+\) could replace K\(^+\) to some extent, and studies with radioisotopes confirmed that K\(^+\) and Na\(^+\) were able to cross an animal cell membrane. This was an astonishing finding, as German physiologists believed that the lipid cell membrane prevented passage of hydrophilic metal ions. Thanks to the generous spirit of Fenn, I was the sole author on three papers describing this work (1–3).

**The War Years**

By 1942 when I completed the M.D. degree and internship at Rochester, my work there had drawn considerable attention, and I received three offers for assistant residency positions from schools where interest in electrolytes was great: Yale Medical School with John Peters, Columbia University with Robert Loeb, and San Francisco Medical School. However, the entry of the United States into World War II interrupted normal, peacetime activities. Arthur Kornberg, a close medical school friend, and I joined the United States Public Health Service. Kornberg received sea duty while I was assigned to the National Institutes of Health (NIH). At NIH under orders from the Navy, I carried out tedious studies on the toxicity of halogenated hydrocarbons. Most importantly, the future began to take shape when I made a new friend, the enzymologist Bernard Horecker. Also, I persuaded Rolla E. Dyer, Director of NIH, to bring Kornberg to Bethesda. Together with Kornberg and Herbert Tabor and with the help of Horecker, I began to learn enzymology. Kornberg then left to spend a year (1946) in the laboratory of Severo Ochoa in New York and another (1947) with Gerty and Carl Cori in St. Louis. When he returned to NIH, he started a new research section for the study of enzymes and invited Horecker and me to join.

**Enzymology at NIH**

Leaning on my background in toxicology, I began to examine the behavior of enzymes in toxic situations. Also, I investigated the metabolic reactions that convert inorganic nitrite to nitrate and nitroglycerines. I also purified inorganic pyrophosphatase and crystallized it with the help of Moses Kunitz (of the Rockefeller Institute (now University)) and purified 5\(^\prime\)-nucleotidase.

**A Sabbatical Year in England**

Then, in about 1951, my attention turned more generally to the phosphorylation and hydrolysis of purine ribonucleosides. This led, quite naturally, to an interest in enzymes that might hydrolyze RNA. Accordingly, my technician, Russell Hilmoe, and I purified from spleen an enzyme that partially solubilized RNA. The next step was to determine which linkages in RNA were split and which were resistant to the enzyme action. Roy Markham and J. D. Smith in Cambridge, England had demonstrated that fragments produced by RNA hydrolysis could be separated using paper chromatography and paper electrophoresis. Fortunately, I succeeded in obtaining a year’s leave of absence from NIH, one of the first sabbaticals to be offered there, and spent a profitable year abroad in the laboratory of Markham. My work in England included the demonstration that the natural configuration of purine nucleotides in RNA was 3’–5’ rather than the alternative 2’–5’ (4). Further evidence for this linkage was obtained from a study of the action of nucleases on mononucleotide esters carried out with Daniel Brown and
Lord Alexander Todd (5). Also, the early steps in the hydrolysis of RNA by pancreatic ribonuclease were worked out in a collaboration with Paul R. Whitfeld (6). This work lead to the isolation, by paper chromatography and paper electrophoresis, of cyclic terminal oligonucleotides. Whitfield, an Australian graduate student in the laboratory, was an excellent colleague in research and deserving of the credit he received when his name appeared on five of our publications (for example, Refs. 6–8).

Later on, I had an interesting interaction with Markham and Sutherland. Dr. Markham found that heating ATP with dilute alkali caused the formation of substantial quantities of a new compound whose properties puzzled him, as he related in a letter to me. At a later date, Dr. Sutherland wrote about a compound isolated from liver in minute quantities. It was biologically active. The two letters ended up in different parts of a pile of mail. However, one day I chanced to re-read both letters and I figured that these compounds were the same. This turned out to be so, and thus cyclic adenylic acid became readily available.

**Nucleic Acid Biochemistry at NIH**

I returned to NIH in January of 1954. Interesting and stimulating visitors began to come to the laboratory to learn techniques and collaborate. Henry Kaplan, a very distinguished Professor of Radiology at Stanford spent a sabbatical in the laboratory. Three joint papers were published with Horecker and Jerard Hurwitz, then a beginning researcher and now a distinguished biochemist. Jack Strominger was also a welcome visitor; the two of us, together with Elizabeth Maxwell, studied the phosphorylation of nucleoside monophosphates by nucleoside triphosphates. At this time, there was considerable interest in the results and methods I had obtained during my stay in England. A good deal of attention was being paid in particular to the demonstration that “synthetic” oligonucleotides could be synthesized by enzyme-catalyzed nucleotide exchange reactions (7). Before long, I learned about the discovery of polynucleotide phosphorylase in *Azotobacter vinelandii* by Marianne Grunberg-Manago and Ochoa at New York University. The same enzyme was independently discovered in *Escherichia coli* by Uri Littauer and Kornberg.

At the time, I was one of only a few individuals who had the knowledge and experience required to study this enzyme and its products. Ochoa proposed that we collaborate and I accepted. Early in the course of the collaboration, a very able and pleasant postdoctoral fellow, Maxine Singer, joined my laboratory. She contributed greatly to the studies and made the association enjoyable. We put to good use all that I had learned in England about polynucleotides. One of our important findings was that short oligonucleotides could serve as primers for polynucleotide phosphorylase (9). Some time later, Singer and I used polynucleotide phosphorylase to prepare polynucleotides and oligoribonucleotides that Nirenberg used in his work on the genetic code. Singer continued to work on polynucleotide phosphorylase when she became an independent investigator.

The elegant organic synthesis of oligonucleotides by Khorana was not available until a later period. Therefore, when working on the genetic code, it was an advantage to be able to use enzymatic methods.

Russell Hilmoe remained my able and intelligent technician for many productive years; he was particularly good at adapting to new situations. Marie Lipsett, who had a good grasp of physical chemistry, joined the laboratory group; she collaborated with Dan Bradley on the study of complex formation between oligonucleotides and homopolymers. The flow of visitors continued as many people began to investigate nucleic acid enzymology. Littauer and I. R. (Bob) Lehman visited from Kornberg’s department in St. Louis. Gobind Khorana’s occasional visits were a joy as they gave me a chance to observe the development of his work and share in his good company as well as collaborate. Several times I also visited in Khorana’s laboratory. Audrey Stevens was an especially brilliant postdoctoral fellow; all on her own she was one of the people who simultaneously discovered RNA polymerase. Altogether, it was an enjoyable and exciting time. After some years, however, I decided to turn to a different problem: the properties of bacterial membranes.

**New Fields**

Harold Neu, a medical postdoctoral fellow, joined me in the new investigations. The first problem he tackled was the location of ribonuclease in *E. coli*. At that time, a ribonuclease had been found associated with the 30 S ribosomes of the bacteria. Neu showed that the ribonuclease was actually in the periplasmic space between the cell membrane and the cell wall but
binds to the 30 S ribosomes when the cell is split open (10, 11). With special care, it was possible to obtain ribosomes free of ribonuclease. Thus, the ribonuclease is a periplasmic enzyme with no connection to ribosomes. In the course of this work, Nancy Nossal, a postdoctoral fellow, contributed to the development of Neu’s procedure for the osmotic shock of the cells (12). The protocol made it possible to recover enzymes in high yield from the periplasmic space of Gram-negative bacteria. The procedure has since been used in many laboratories. Neu, and later others, discovered a number of other periplasmic enzymes, all located in the space between the cell membrane and cell wall.

Anraku, a visitor from Japan, was very quiet but very effective and productive. He observed that Gram-negative bacteria able to transport D-galactose contain a specific periplasmic protein that can bind that sugar. A similar observation was made in the laboratory of Arthur Pardee. In the next few years, a large number of binding proteins were discovered in my laboratory and elsewhere. At NIH, several additional postdoctoral fellows contributed to this work. H. R. Dvorak, an M.D., had a special interest in metalloproteins. He and R. W. Brockman, a hard worker who visited the laboratory from Alabama, also worked on phosphatases released from \textit{E. coli} by osmotic shock.

**The Years at Cornell**

In 1967, Efraim Racker induced me to join the Department of Biochemistry at Cornell University. The move was the beginning of more than 30 pleasant and productive years in Ithaca. The first postdoctoral fellow to join the laboratory, George Dietz, was an able and pleasant young man who studied the uptake of hexose phosphates by \textit{E. coli}.

Joel Weiner, a graduate student from Canada, and Clem Furlong, a postdoctoral fellow, worked on amino acid transport in \textit{E. coli} including leucine-specific and glutamine-specific (13) periplasmic binding proteins. Furlong was an especially good experimentalist and was helpful with equipment problems. Weiner later became an outstanding member of the Canadian Biochemical Society. Ed Berger, a graduate student, carried out a landmark study showing that there are different mechanisms of energy coupling for the active transport of proline and glutamine in \textit{E. coli} (14); this work received much favorable attention. Another member of the early group at Cornell was postdoctoral fellow Barry Rosen. He studied basic amino acid transport in \textit{E. coli}, another process that involved a binding protein.

Other students, postdoctoral fellows, and visitors contributed to our growing understanding of the periplasmic space and transport. Susan Curtis looked at the mechanism of ribose uptake, which involved energy from ATP rather than an energized membrane. James Cowell noted a similar result for glycylglycine. Janet Wood, a very able Canadian, worked on L-leucine transport. J. B. Smith and a graduate student, Paul Sternweis, purified the two “minor” subunits of F_1-ATPase and examined their properties (15). I was able to help Smith during a period when jobs were difficult to get and was delighted when he began doing independent work. T. Kitagawa made an interesting finding when he showed that the osmotic shock procedure does not necessarily kill the cells; some cells remain viable. Stanley Dunn and Masamitsu Futai used their time in the laboratory purifying and reconstituting the \textit{E. coli} F_1-ATPase (16). Nizar Makan from India spent several postdoctoral years on exhaustive work that yielded evidence for metabolic processes that might be involved in permeabilization.

In 1975, I decided to gain more experience in animal cell research. A half-year sabbatical was granted and I spent it with Henry Rozengurt in London. In the ensuing years, I made six additional visits of several months each to the Rozengurt laboratory. On one of these visits, I observed that 3T6 cells, which are spontaneously transformed, leaked nucleotides when 50 \mu M ATP is added to the medium; the effect is highly specific for ATP. Many excellent investigators have since studied this phenomenon, and G. Weisman, I. Friedberg, and I reviewed this work in 1986 (17). Friedberg received his degree for the work in my laboratory in about 1980. The most recent years in my laboratory included Ding-ji Wang and Ning-na Huang. They showed that ATP, in concentrations of a few micromolar, was a mitogen and explored this important effect of extracellular ATP in a series of papers (18).

I want also to mention a few other people who were in my laboratory at various times and whose collaboration I value. They include R. G. Alfonzo from Venezuela, K. Jacobson, a skilled organic chemist, and the productive Fernando Gonzalez, a graduate student and postdoctoral fellow. Barun De was a persistent and hard worker. Ahmed Ahmed came to the United States on a number of occasions to learn modern biology; he is a well known Professor of Plant Science.
and Toxicology in his native Egypt. I was also fortunate to know Gary Weisman and to watch with pleasure as he developed into a leader in his field.

In the early 1980s I was able to spend 13 months (divided into short periods) back at NIH as a Fogarty Scholar in the laboratory of Claude Klee. It was good to be able to spend the entire day doing experiments at the bench. Klee is remarkable for being able to do experiments at the same time that she was running the Laboratory of Biochemistry in the National Cancer Institute.

## Conclusion

These reminiscences cover about 75 years. They are based on what I remember and no claims for accuracy are made. Selected references and reviews are included for the interested reader, and these sources also describe similar work done in other laboratories.

I have never forgotten how Professor W. O. Fenn arranged that I would be sole author on three papers describing work that I carried out in his laboratory. After all, he was the department head and I was only a medical student on a part-time physiology fellowship. On occasion, I tried to do the same for a student or postdoctoral fellow of mine. However, I stopped when a reviewing editor accused me of removing my name because I had no interest in the work.

Wonderful friendships are formed in research laboratories. Bernard Horecker was a friend for many years and a good source of advice; several of our joint papers are still referred to on occasion. Arthur Kornberg gave no end of guidance and inspiration, especially in the early years. My wife Adelaide and I will always have a special place in our hearts for Herb and Celia Tabor.

I have mentioned here nearly all of those who held positions in my laboratory over the years. The list is small. I prefer to work with a small group and always to do some experimental work myself.

I am especially pleased with the performance of women in my laboratory. They had difficulties in obtaining positions in my day.

Address correspondence to: lah9@cornell.edu.

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