Howerde E. Sauberlich, 1919–2001

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If you happened to have been born and raised in Wisconsin in the early part of the last century and pursued academic and research activities at the University of Wisconsin, Madison, it seems that you would have had a good opportunity to participate in pioneering studies of macro- and micronutrients. Howerde E. Sauberlich joins a list of outstanding "Wisconsin" nutrition scientists including Drs. Elvehjem, McCollum, Schweigert and Steenbock.

Howerde was born on the family farm in Greenville, Wisconsin on January 23, 1919. Possibly because his mother was a school teacher, he completed his high school studies at the age of 16. His parents felt that he was too young for college and kept this extremely bright student on the farm. The family struggled to keep enough hired help because they were constantly being drafted into the Army; consequently, Howerde had to work very hard. He was impatient, even burning haystacks at night in the middle of the road to frighten motorists and possibly getting the attention of his parents, so that they finally let him enroll in Lawrence University when he was 21 y old. In 1944 he graduated summa cum laude and Phi Beta Kappa with a degree in physical chemistry. He was not going to be a farmer.

Howerde entered the University of Wisconsin and began his graduate studies, not in chemistry, but in the rapidly expanding fields of biochemistry and nutrition. Dr. Conrad Elvehjem was professor and chairman of the Department of Biochemistry in the College of Agriculture, so that Howerde was not completely removed from the farm. Dr. Elvehjem and his colleagues had recently isolated nicotinic acid from liver and identified it as the substance that cured “black tongue” disease in dogs, an animal model of the human disease, pellagra. Nicotinic acid was used by clinicians, including Dr. Tom Spies of the Hillman Hospital of Birmingham, Alabama, to produce dramatic reductions in the signs and symptoms of pellagra.

So, Howerde plunged into a department that was taking nutrition research from the “bench” to animals and ultimately to humans at a rapid pace. Flushed with success in finding the cure for pellagra, the faculty demanded that their graduate students be dedicated to hard work. Students did not take time off on Saturdays or even holidays. In the evening, students often walked backwards past the glass in the doors and windows of Dr. Elvehjem’s office, so if challenged, they could appear to be returning to, rather than leaving, the laboratory. They were driven. Indeed, the Wisconsin faculty suspected that many more nutrients essential for human health awaited isolation and characterization.

Dr. E. E. Snell and co-workers had developed microbiological assays for amino acids. One of Howerde’s first tasks was to improve the microbiological assay for tryptophane (this amino acid would lose its “e” about 10 y later). Tryptophane was of interest because of the growing evidence that it was a precursor of nicotinic acid. The microbiological assay has now virtually disappeared (except for folates), but it was once a powerful tool to detect nutrients in small amounts or in low concentrations. In this case, Lactobacillus arabininos is grown in a medium with all other nutrients except one amino acid. The extent of growth of the bacteria was then a function of the amount of this amino acid added to its medium. Thus, a quantitative assay could be performed. The results were pub-
lished in Science (1). Later in 1946, Howerde would use a microbiological assay for vitamin B-6 to study the effect of dietary protein levels on tissue levels of this vitamin in mice (2). These microbiological assays were of tremendous value to all scientists. Indeed, even chemists who published in the prestigious Journal of the American Chemical Society used microbiological assays to compare the properties of synthetic folates with those isolated from biosamples. The microbiological assays for folates that Howerde helped develop and that were later refined by many (including Drs. Jack Cooperman, Victor Herbert and Donald Horne) are still regarded as the "gold" standard. In 1946 he received an M.S. degree in Biochemistry.

One of the ways to determine amino acid requirements and utilization was to measure urinary excretion of amino acids in animals fed protein of varying quality. The theory was that essential amino acids would be excreted (i.e., wasted) when overall protein quality is poor. Working with Dr. C. A. Baumann, Howerde refined the use of Streptococcus faecalis, Lactobacillus arabinosis and Leuconostoc mesenteroides as assay organisms for amino acids (3,4). He received his Ph.D. in Biochemistry in 1948.

Most of the amino acids could be assayed microbiologically. One notable exception was alanine. Before leaving Wisconsin, Howerde developed an assay for alanine using Leuconostoc citrovorum. The assay medium actually contained folic acid; however, this organism grew slowly even in the presence of this nutrient. If various liver extracts (obtained from Eli Lilly) were added, a remarkable increase in growth occurred. The work was published and titled "A factor required for growth of Leuconostoc citrovorum" (5). This paper has been cited >300 times. This new substance would later be identified as 5-formyltetrahydrofolate but was then known as "citrovorum factor" or leucovorin. It is hard to disagree with the old adage that discoveries favor the prepared mind and the hard worker.

By 1949, Dr. Sauberlich had moved to the Laboratory of Animal Nutrition of the Alabama Agricultural Experimental Station of the Alabama Polytechnic Institute of Auburn, Alabama (later to be known as Auburn University). While at Auburn, he demonstrated that when folic acid was fed to rats and humans, the urine content of "citrovorum factor" increased dramatically. In 1952 he isolated "citrovorum factor" from kilograms of liver and spinach and (what I estimate), ~100 L of human urine (6). Hard work on the farm may have paid off here. He succeeded in crystallizing the compound and demonstrating that it was identical to the synthetic compound in all aspects with one exception; like Keresztesy and Silverman before him, Howerde thought that the synthetic compound was a mixture of optical isomers.

The discovery of leucovorin greatly affected medical practice. This was the first stable and fully reduced (i.e., tetrahydro-) folate that could be stored almost indefinitely as a dry calcium salt. This stability opened up potential uses for this folate. Leucovorin is now used in cancer chemotherapy in the "high-dose methotrexate-leucovorin rescue" protocol and with 5-flourouracil. On the one hand, in leucovorin-rescue therapy, 5-formyltetrahydrofolate repletes the pool of tetrahydrofolates used for the biosynthesis of purine and thymidylate. Thus, normal tissues may be rescued from methotrexate cytotoxicity.

On the other hand, 5,10-methylenetetrahydrofolate (derived from leucovorin) forms a thymidylate synthase-5-fluorodeoxyuridylate-5,10-methylenetetrahydrofolate complex and irreversibly inhibits this enzyme. Therefore, leucovorin enhances 5-flourouracil efficacy. Since 1966, over 1000 papers have been published describing the therapeutic uses of leucovorin. It is a rare event that a specific micronutrient has such an extensive effect on medical practice.

Dr. Sauberlich left Auburn University in 1957, briefly accepting positions at the University of Kentucky, University of Indonesia and Iowa State University before accepting a position as Chief of the Chemistry Division of the U.S. Army Medical Research and Nutrition Laboratory at the Fitzsimmons Hospital in Denver in 1960. Before leaving Alabama, he campaigned aggressively for fluoridation of water. Howerde was a forceful advocate of translating research into public health measures. At the University of Indonesia in Bogor, he was a member of a team sent by the State Department to train faculty and students in modern agricultural methods and research. After completing this assignment, the Sauberlich family, which included his wife Irene and two children, Melissa and Howerde II, traveled through 30 countries on their way home. Howerde was a well-traveled scientist.

At the Fitzsimmons Hospital, Dr. Sauberlich and co-workers began to refine and popularize the use of stimulation indices for the assessment of human B-vitamin status. In a series of papers, many published in the newly created American Journal of Clinical Nutrition, he and his colleagues used erythrocyte transaminases to show that the enzyme activity of vitamin B-6–deficient subjects was stimulated by the in vitro addition of pyridoxal phosphate (7). On the basis of these human experiments and a review of the data of others, the Recommended Dietary Allowance for vitamin B-6 in adults was estimated to be 1.5–1.75 mg/d. This value has not changed substantially for nearly five decades. Using his own clinical data and data from many others, Dr. Sauberlich also promoted the use of the erythrocyte transketolase and glutathione reductase stimulation indices for the assessment of thiamin and riboflavin nutriture, respectively.

In Denver, Dr. Sauberlich's work was not limited to the B-vitamins. He studied the effect of ionizing radiation on proteolytic enzymes. In 1964 he received the Meritorious Civilian Service Award from the Department of the Army for his studies of the use of ionizing radiation in food preservation. He was especially proud of this award and displayed it in a prominent location in every Sauberlich home. In another series of papers, he described human vitamin C deficiency and the metabolism of radiolabeled vitamin C in scurvy.

In the early 1970s, the Sauberlichs moved to the west coast where Howerde held positions as Chief of Nutrition Technology, Letterman Army Institute of Research; adjunct Professor at the University of California, Berkeley; and director of the Western Human Nutrition Research Center in Presidio. In 1974 he published a heroic experiment in which eight subjects spent 1–2 y eating a semipurified diet low in vitamin A (8). After observing and documenting the clinical signs and symptoms of the deficiency, the subjects were repleted with β-carotene or retinol. This study sought to establish relationships between plasma and whole body vitamin A levels and dark vision adaptation, and is one of only a few well-controlled, long-term vitamin A studies in humans. As a result of experiments like these and his vast knowledge of the literature, the widely cited “Laboratory Tests for the Assessment of Nutritional Status,” by H. E. Sauberlich, R. P. Dowdy and J. H. Skala, was published in 1974 (9). It contained over 500 references and has been used as a reference by many researchers.

In 1982 he returned to Alabama, this time at the request of Dr. C. E. Butterworth who had recently transformed a nutrition "program" at the University of Alabama at Birmingham into a Department of Nutrition Sciences jointly supported by the Schools of Health Related Professions, Medicine and Dentistry. We all knew that an imposing figure in nutrition
research had come to us. By the time he settled in Alabama for the second time, he had received many awards including the Mead-Johnson Award for vitamin B-complex research, the McLeaster Award for research in human nutrition, the Borden Award and the Canadian Clinical Chemistry Society Award. We were impressed and even intimidated by his successes. Our graduate students would stand at attention in front of his open office door, without knocking or speaking, simply waiting for him to look up and acknowledge them. Yet Howerde welcomed the chance to interact with us and carried his achievements so casually that it belied the fact that he had authored hundreds of research papers, review articles and book chapters.

The last phase of Howerde’s career was eclectic, yet very productive. A team of investigators, including himself and Dr. C. E. Butterworth, described the reversal of cervical dysplasia by folic acid supplementation. This research in part laid the foundation for folate deficiency as a risk factor for cancer. This is now a very popular hypothesis. In collaboration with the University of Miami, he studied the nutriture of HIV-infected patients. Micronutrient deficiencies were suspected of being risk factors in the progression of HIV infection to AIDS and its clinical course. He was instrumental in establishing a Ph.D. program in our Department and was the first program director. In the face of heavy responsibilities, he still took time out to write chapters and reviews for Present Knowledge in Nutrition, Folic Acid Metabolism in Health and Disease and the Annual Review of Nutrition, to mention a few. In 1986 he was elected as a Fellow by the American Institute of Nutrition. In 1999 after years of dedicated work, he published the second edition of “Laboratory Tests for the Assessment of Nutritional Status” (10), which contains nearly 500 pages, including over 3000 references.

Howerde devotedly tended his garden at his home in the suburbs of Birmingham, perhaps in a sense returning to his agrarian roots. He fought a continuous battle with a flower-eating ground hog that he eventually named Oscar. On the one hand, he resolved to trap the critter and on the other, he admired its evasive nature and tenacity. In April 2001, he received the Conrad A. Elvehjem Award for Public Service in Nutrition. This delightful, self-effacing and very hard-working man was in his office two weeks before his death on May 18, 2001 working on several manuscripts. All around his office, three-foot high stacks of reprints had been strategically placed and, within a few minutes, he could locate an article or a reference. I often requested an article or reference or the name of an investigator in a particular area. However, I believe that he secretly enjoyed being interrupted so that he could tell you about his scientific (and other) adventures and experiences—we will miss that.

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LITERATURE CITED