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Nutritional Scientist or Biochemist?

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Abstract

When invited by the editors to provide a prefatory article for the *Annual Review of Nutrition*, I attempted to decide what might be unique about my experiences as a nutritional biochemist. Although a large proportion of contemporary nutritional scientists were trained as biochemists, the impact of the historical research efforts related to nutrition within the Biochemistry Department at the University of Wisconsin 50 to 60 years ago was, I think, unique, and I have tried to summarize that historical focus. My scientific training was rather standard, but I have tried to review the two major, but greatly different, areas of research that I have been involved in over my career: inorganic fluorides as an industrial pollutant and the metabolic role of vitamin K. I have also had the opportunity to become involved with the activities of the societies representing the nutritional sciences (American Society for Nutrition), biochemistry (American Society for Biochemistry and Molecular Biology), Federation of American Societies for Experimental Biology, the Food and Nutrition Board, the Board on Agriculture and Natural Resources, and the U.S. Department of Agriculture National Agricultural Research, Extension, Education, and Economics. These interactions can be productive or frustrating but are always time-consuming.

Contents

NUTRITIONAL RESEARCH IN THE UNIVERSITY OF WISCONSIN BIOCHEMISTRY DEPARTMENT

The Biochemistry Department at the University of Wisconsin was very involved in the beginnings of the science of nutrition. A chemist, Stephen M. Babcock, was brought from the Agricultural Experiment Station in Geneva, New York, in 1888 to chair the Department of Agricultural Chemistry on the Madison campus. Although he is best known for his development of the Babcock Test for the butterfat content of milk, he was an early advocate of the theory that the nutritional value of a diet could not be determined by chemical analysis alone, and he was the major impetus behind what came to be called the Wisconsin Single-Grain Experiment. The published report of this effort, University Bulletin No. 17, entitled "Physiological Effect on Growth and Reproduction of Rations Balanced from Restricted Sources," was largely under the supervision of Edwin Bret Hart, who had been hired from Geneva by Babcock to assume the chairmanship of the department in 1906. Babcock had conducted a preliminary two-cow experiment, one consuming a ration obtained entirely from the oat plant and the other consuming a ration with the same protein and energy composition from the corn plant. The oat-fed cow died, and the other cow survived and was transferred to a standard ration.

Under Hart's supervision, a four-year study utilizing young heifers that were fed chemically similar rations consisting of oats, corn, or wheat, or a mixture of the three sources, was initiated. The study was carried out through two gestation cycles, and only the corn-fed cattle maintained a good appearance and were capable of producing healthy calves.

Two other younger members of the department, Elmer McCollum and a graduate student, Harry Steenbock, were involved in this historic study, which clearly established that there were constituents of foods that were essential to the health of animals and that had not yet been identified. Hart's interest in nutritional issues was broad and included the use of inorganic phosphate sources for domestic animals, the importance of iodine in goiter prevention, the role of copper in promoting hemoglobin synthesis from iron, fluoride toxicity associated with the utilization of rock phosphate in animal rations, and the use of urea as a nitrogen source for ruminants. His associations with Steenbock and McCollum in their studies of vitamins A, C, and D had a large impact on the Biochemistry Department, particularly in the area of nutrition. Steenbock, Paul Phillips, and Conrad Elvehjem had all received their Ph.D. degree under Hart's supervision, and these three trained six future faculty of the department: Carl Baumann and Hector DeLuca with Steenbock, Alf Harper with Elvehjem, and Hank Lardy, Bill Hoekstra, and myself with Phillips. Although Lardy's work involved some aspects of nutrition, his expertise would have been labeled as metabolism by most of his colleagues. The other five were very heavily involved in studies with a major focus on nutrition.

E. V. McCollum, who was trained as a chemist, was hired by Hart in 1907 and was one of the authors of the Wisconsin Single-Grain Experiment. His subsequent studies followed-up observations from the single-grain experiment, and he developed the first rat colony used in nutritional experiments. While he was investigating the impact of varying the source of fats in the diet of rats, he discovered the factor called "fat-soluble A" (vitamin A) as a growth-promoting factor in the nonsaponifiable fraction of the active sources of lipids. He later demonstrated the existence of a group of substances called "water-soluble B," which presented neurological symptoms in the rats. McCollum left Wisconsin in 1917 to chair the Department of Chemical Hygiene in the newly established School of Public Health at Johns Hopkins University.

I never met these three prominent nutritional scientists of the early twentieth century. Harry Steenbock, however, was still active when I joined the department in 1961, and his office was very close to Paul Phillips's office during the period of time that I was a graduate student in his laboratory. Steenbock joined the faculty of the department in 1916. Retirement at age 70 was mandatory at that time, and when he reached 70 in 1956, the Board of Regents officially declared him an active member of the faculty with no limit. His presence in the department continued for some time.

Steenbock was a prolific scientist with wide interests in vitamin A and its precursors and the properties of nutritional anemia. He was, however, most known for his career-long studies of vitamin D and, in particular, his demonstration that the "growth-promoting and calcifying properties" of a ration could be enhanced by its exposure to ultraviolet light. He recognized the commercial value of this discovery as a treatment for rickets and sought a patent for the process, in which he indicated that the patent would be handled through the University of Wisconsin. He had previously unsuccessfully attempted to convince the University to patent the addition of vitamin A to margarine to support the Wisconsin dairy industry, but the University had little interest. The wording of the irradiation patent did put pressure on the administration and led to the formation of the Wisconsin Alumni Research Foundation. This entity handles patent applications from campus scientists and has been used as a model on many campuses. A fraction of income from a patent is transferred to the inventors, but the majority of income is returned by the Wisconsin Alumni Research Foundation to the University through the Dean of the Graduate School and is largely used to support the efforts of research scientists.

Conrad Elvehjem joined the department in 1930 and directed a laboratory that was focused on the action of a number of trace minerals and components of the vitamin B complex. These efforts were assisted by the laboratories of Frank Strong and Karl Paul Link and led to Elvehjem's identification of nicotinic acid as a liver component that would cure canine black tongue. The identification occurred during a period when the disease pellagra was widespread, and the translation to clinical practice was rapid and very successful. Elvehjem was very actively involved in national nutrition policy as well as in administrative activities on the Madison campus. He succeeded Hart as chair of the Biochemistry Department in 1944 and also served as dean of the Graduate School for much of this period. He became President of the University of Wisconsin in 1958 but retained an office in the Biochemistry Department during that period of his administrative responsibility. His later work was directed mainly toward the assessment of amino acid imbalance and later metabolism.

The two other more senior nutrition-related faculty in the Biochemistry Department when I joined it were Carl Baumann and Paul Phillips. Baumann's work covered a wide range of interests: vitamin A, amino acid metabolism, carcinogen action, and tumor metabolism, as well as a substantial effort in selenium function and metabolism. During the later portion of his career, much of his effort was directed toward sterol metabolism. Phillips had been a student of Hart in the late 1920s, when the toxic aspects of high-fluoride rock phosphate as an

animal feed were being studied, and Phillips returned to this problem following the end of the World War II in 1945. The rapid construction of aluminum smelters during the war resulted in emission of fluorides from this process and the accumulation of fluorides in forage at levels that were toxic to grazing animals. Two other students and I worked on this project, but Phillips's research interests were rather broad. His earlier work included the development of an egg yolk and phosphate buffer that preserved the motility and fertility of bull sperm and led to the rapid widespread use of artificial insemination by the dairy cattle industry. He was also involved in extensive studies of mineral and protein requirements, the influence of diet on the development of dental caries utilizing the cotton rat as a model, and the function of a number of trace minerals.

NUTRITIONAL RESEARCH IN THE EARLY 1960s

Entering a prominent Biochemistry Department as a new assistant professor was not a difficult transition for me. I knew the faculty and they knew me. What I did not realize at that time was the extent to which the faculty members were interrelated. In the summer of 1961 there were 18 faculty members, including myself, and all had received a Ph.D. degree from the University of Wisconsin-Madison, including 8 from the Biochemistry Department. Fifty years ago it was much more common to hire new faculty from their degree institution, but departments at other institutions were unlikely to have been as "home grown" as our department was in 1961. Almost all scientists now and some at that time would probably indicate that this was a bad hiring practice and that a department should bring in faculty with differing views and strengths. This is certainly a valid assessment, but in most cases the newly hired Biochemistry Department faculty were pursuing areas of science that differed substantially from those of their mentors. It did have an important positive factor: The faculty knew who they were hiring, and they had had a long time to assess their potential. The current faculty search system is long and complicated, depends a great deal on the opinions of others, and ends up with a hire of someone who may not be well known by other department members. The practice of hiring mainly from the Madison campus was likely also used to prevent the loss of individuals to other institutions. At the time I joined the faculty there was a great deal of strength in a number of biochemical research areas, and of the 18 departmental faculty members in the fall 1961, 8 were or have since been elected to the National Academy of Sciences.

By 1961, the majority of the historical figures of the department's nutritional efforts had retired. Elvehjem retained an interest in the department but was busy being president of the University, and Phillips was nearing retirement. Any decrease in a major commitment to nutrition-related research was reversed in the late 1950s with the hiring of Alf Harper, Bill Hoekstra, and Hector DeLuca.

Alf continued the focus on amino acids started by Elvehjem and developed a strong program directed toward the impact of amino acid balance on food intake and metabolism with an emphasis on branched-chain amino acids. He also took up Elvehjem's interest in nutrition policy through his activities within the Food and Nutrition Board and other national and international organizations. Bill Hoekstra developed a rather broad research program with an early interest in zinc requirements and metabolism and gastric ulcers in swine, and he mentored a number of joint Ph.D. students with Animal Sciences Department faculty to investigate the postmortem changes in porcine muscle. He also developed an interest in antioxidants, starting with vitamin E and continuing to the identification of selenium as a component of glutathione peroxidase.

The most exciting nutrition-related research efforts coming out of the department in the 1960s were the advances in our understanding of the role of vitamin D in promoting bone health in Hector's lab. His program studied the role of parathyroid hormone in

calcium homeostasis and moved us toward an understanding of the metabolism of vitamin D. By the late 1960s, his laboratory had identified 25-hydroxycholecalciferol (25-OH-D_3) as a hepatic metabolite of vitamin D, and by 1971 they had identified a kidney metabolite, 1,25-$(OH)_2D_3$ as the active form of vitamin D. These findings led to an active program of drug discovery and subsequently to a major study of the other physiological responses to alterations in vitamin D status. These findings were a major factor in the successful efforts to obtain a program project grant entitled "Fat-soluble Vitamins" from the National Institutes of Health (NIH)/Diabetes and Digestive and Kidney Diseases that Hector and I and various other colleagues shared for 30 years.

PERSONAL TRAINING AND RESEARCH ACTIVITIES

Undergraduate and Graduate Training

I received my undergraduate and graduate training at the University of Wisconsin. I was a typical rural Wisconsin farm boy and had attended a one-room country school that was only a 10–15 minute walk from our home. There were 12–18 students each year, and after eight years I rode the bus to Galesville and entered a high school class of 50 students. I do not remember a lot about what I learned, but I do remember being told by the teacher in charge of the choir that I would have to leave as "I could not match my tones," and being told by the principal's secretary when I graduated that "you are a very smart kid, but unless you learn to spell you will not amount to much." I still cannot sing or spell.

I entered the College of Agriculture at the University at Madison without any real idea of what I wanted to do. My high school ag teacher was very active in participating in animal and meat judging contests, and as I had met some of the University of Wisconsin Animal Science faculty, I soon joined the College's meat judging team. I also had some affiliation with the history of nutrition at Madison when I entered as a freshman. When Babcock, who might be called the father of nutrition at the University of Wisconsin, died, he left his house, which was only one block away from the University campus, to the University. It was to be used as a house for male students interested in agriculture, and it was run as a co-op by the students who lived there, with only minor involvement with the College of Agriculture administration. We hired a cook, split up the house maintenance, and shared what was probably the cheapest place to live on campus. It was a great place to live, although it could be somewhat cold in the winter, as only six could sleep on the second floor, and the rest were relegated to an unheated attic.

When I entered the University, the chair of the Dairy Science Department was my assigned advisor, and I was put into a course program typical of new students. I think it was during my freshman year that he suggested that I pursue a more basic science-based program and transferred me to the Biochemistry Department, with Paul Phillips as my advisor. I completed an undergraduate degree in Biochemistry, not a common degree at that time, and also worked as student labor in Phillips's laboratory feeding cotton rats, which were used in a dental caries study that Charlie Taketa was supervising. I do not remember seriously looking at other graduate programs at that time, and I remained in Phillips's lab and took over the fluoride toxicity project that Russ Miller had started. Hoekstra had some space in Phillips's laboratory, DeLuca's lab was next to us, and Baumann was one floor down. Because of this, most of the students I knew well were to some extent doing work related to nutrition. At that time the only general nutrition course in the College of Agriculture was a graduate course, essentially a seminar taught by the biochemistry faculty who had a nutritional interest. There would, however, have been animal nutrition courses in Animal Science, Dairy Science, and Poultry Science, and nutrition courses in the School of Home Economics.

Paul Phillips was an easy mentor to work with. You talked about what you were going to

do and what the results were. He assumed that you were going to deal with problems along the way. His students were given more freedom to push their projects ahead than most, and his approach seemed to work, as a large number of his students have had very successful careers.

Postdoctoral Training

I graduated from the University of Wisconsin Department of Biochemistry in the spring of 1960 and was offered an appointment in the department. Prior to joining the department, I spent a year as a postdoc with Dr. T. S. Work at Mill Hill, the London campus of the English National Institute for Medical Research. This was a period when the field of protein biosynthesis was just emerging, and I joined one of Dr. Work's postdocs, who was studying mitochondrial proteins, in particular the pathway by which they were synthesized and reached their location within this organelle.

Mill Hill was a good laboratory to work in but was somewhat different from what I was used to. Scientists tended to come in somewhat later in the morning than I normally did, had a short coffee break in the morning, a rather lengthy lunch, and an afternoon tea before leaving not that much later. Evening work in the laboratory was not common and was not encouraged. At that time a substantial amount of class separation was still in place. Technicians had only recently been given the right to wear white lab coats rather than green. In some laboratories, but not in Work's, the technicians who cleaned up a lab bench and brought out the bottles for a weekly or semiweekly TGIF session were not asked to stay. The coffee room/lounge near the lunch cafeteria was also off-limits to the technicians and office staff. This did not seem reasonable to me, but in retrospect it was probably not that bad. There was a substantial amount of interaction between scientists from different laboratories within the Institute following lunch that was probably enhanced by the policy. Overall, the laboratory at Mill Hill was a good place to spend a year. Although there was not a very high-energy atmosphere in the laboratories, my coworkers were doing good science, and they were easy to interact with. A nutrition laboratory was located in a building that was separate from the main building, and although I did know some of the scientists who worked there, I do not recall ever getting very interested in what they were doing.

Fluoride Toxicity Studies

I returned from London in the summer of 1961, joined the Biochemistry Department in September, and started teaching an undergraduate biochemistry course with three lectures and two laboratory sessions each week. This was somewhat different from the current treatment of new faculty, who are usually given a year before they assume any instructional responsibilities. Paul Phillips was nearing retirement, and my first research effort was to take over his fluoride toxicity project. This was an industrial toxicology problem based mainly on the aluminum smelting and phosphate rock–processing industries. The emissions from these plants contained high levels of hydrogen fluoride or soluble fluoride particulates that were taken up by or accumulated on vegetation that was consumed by grazing animals. A series of long-term studies of cattle was utilized to define the various responses of cattle to increased concentrations of ingested fluorides and to establish safe levels of consumption. These studies and some studies of the cellular response to fluoride increases continued to represent a portion of my research program for about 30 years.

This project was more of an industrial environmental toxicity problem than a simple scientific study of the impact of excessive fluoride ingestion by animals. The major industry involved was the smelting of pure aluminum from aluminum oxide. Very little aluminum was produced in the late 1930s, but production increased rapidly during the war, and as new plants were constructed, little attention was given to the control of fluoride emissions. After the war there was more concern about the possible impact that this may have had on the cattle in the area. There is no doubt that

animals in close proximity to the smelters were damaged, and companies—with or without a lawsuit—did compensate the owners. The industries involved felt that there was a need for research to assess the impact of fluoride ingestion on cattle and contacted Paul Phillips because of his previous experience with the effects of high fluoride phosphate supplements on cattle. The time spent with grant proposals and reports was much different than now. About a dozen companies were involved in supporting the project, and they would meet once a year in Madison to find out what we were doing. The companies would send their environmental control leader and sometimes a veterinarian and a lawyer or two. Over the years we evolved a format for the meeting that seemed to work very well. I would usually meet a few of them for dinner the evening before the meeting. We would spend most of the next day reviewing what my research group had done during the past year, they would all come out to my house for drinks after the meeting, and we would go out for dinner together. The next morning I would tell the companies' representatives what I planned to do the next year, and they would leave by noon. A detailed work plan or budget was not needed by them or the Dean. I did get to know some of them very well, and for a period of time I was rather heavily involved with the environmental aspects of this rather limited problem. I served as an expert witness in a number of lawsuits, consulted for a number of the sponsors by assessing the impact of fluoride emissions on the cattle around their plant sites, and participated in assessing the desirability of new plant sites. Aluminum production had become a multinational industry, and much of the involvement was outside of the United States. I had the opportunity to work with the agriculture/environmental agencies of a number of states in attempts to define safe levels of intake, and I proposed a regulation (8), which was based on the fluoride content of vegetation, that was adopted by a number of them. The most sensitive indicators of excessive fluoride ingestion are the effects on developing dentition, which are easily observed as a decrease in the quality and depth of incisor enamel. These alterations can be scored, and for a number of the sponsors of the fluoride project, I inspected herds of cattle in areas close to these plants, and largely on the basis of these dental scores, I made a decision as to whether they should compensate the herd owners for presumed "damage" to their animals. There are essentially no data that would indicate that the productivity of animals with moderate dental fluorosis was impaired, and although the herd owners did not ask for a productivity assessment, they were compensated for impaired productivity. Cattle inspections were usually conducted in the presence of a local veterinarian, and disagreements with the herd owners or their advocates were few.

Although this research project was not very nutritional or biochemical, I found it interesting, and I gained an understanding of large industry that is often lacking in an academic setting. I also learned that it is essentially impossible to satisfy an avid environmentalist, and an outcome that was at one time a goal to solve problems was not sufficient once it was met and soon became a target for a new goal. I also learned that if you raised questions about new regulations for a clean earth, you were not treated kindly by the environmental community.

Vitamin K–Related Research

The majority of my research career has involved efforts to understand the metabolic role of vitamin K. Many people have told me that they assume that this direction was based on my interaction with K. P. Link, as dicoumarol, the anticoagulant he isolated and characterized from moldy sweet clover hay, was known to be an antagonist of vitamin K. This was not the case, but Link also developed the extensively used anticoagulant warfarin, and Mark Hermodson, one of Link's last students, did much of his thesis work with me, assessing the metabolism of warfarin and the vitamin K requirements of warfarin-resistant rats. I had developed an interest in protein synthesis at Mill Hill and was looking for a good problem to study. By the early 1960s the basic pathway of protein synthesis in animals

was understood, and scientists were attempting to understand how the syntheses of specific proteins were regulated. I spent most of a couple of weeks in the library and found a number of areas that were very active. Glucocorticoids were being shown to influence the synthesis of a number of proteins, and alterations in the nutrient content of diets were being shown to impact the activity of specific enzymes. I became rather interested in the ability of alterations in iron intake to regulate ferritin production, but in retrospect I think that the leader in this field, Hamish Munro, would have squashed me. The activity of the procoagulant prothrombin was known to be dependent on sufficient vitamin K, but the metabolic role of the vitamin in regulating it was not known. As the action of vitamin K could be antagonized by warfarin, it seemed to me to be an approachable problem, and I decided to give it a try.

At this point, in mid 1964, I wrote an NIH grant entitled "Regulation of Prothrombin Synthesis by Vitamin K" and asked for $17,417 a year. I received this amount and was probably the luckiest person to have ever received an NIH grant. I knew nothing about coagulation, did not know any hematologists working in this field, and had never done an experiment in this area. There is no doubt that this grant would never have seen a reviewer if they had triaged grants in those days. This was the only RO-1 I ever received, as it was replaced by a very productive program project grant led by Hector DeLuca and focused on fat-soluble vitamins, which along with Hector's vitamin D work and my vitamin K work included vitamin E and other antioxidants research directed by Bill Hoekstra. This grant and a second Program Project Grant of mine that involved the coagulation-based hematologists in the Medical School were my major sources of support for the next 35 years.

Little research that substantially advanced an understanding of the metabolic and nutritional roles of vitamin K was published from the late 1930s, when it had been identified and characterized by groups led by Dam, Almquist, and Doisy, until the early 1950s. At that time, three additional plasma procoagulants, Factors VII, IX, and X, were identified, and along with prothrombin (Factor II), they came to be known as the vitamin K–dependent clotting factors. At the time that I began to work in this field, both Bob Olson at St. Louis University and Connor Johnson at the University of Illinois were looking at the role of vitamin K in the production of these proteins; very importantly, Caen Hemker's laboratory at the University of Leiden in The Netherlands had identified a circulating, inactive form of prothrombin in the plasma of patients treated with oral anticoagulants. Much of the focus in our laboratory during the mid 1960s was directed toward the question of whether vitamin K was regulating the rate of synthesis of the vitamin K–dependent proteins or if it was in some manner converting precursors of these proteins to the biologically active forms. This question was clearly answered when we were able to demonstrate that vitamin K–deficient rats given vitamin K and cycloheximide to block protein synthesis were capable of elevating circulating prothrombin to about 50% of the concentration of normal rats in one hour and that this pool of prothrombin contained essentially none of the radioactive amino acids administered to the rats at the same time. This observation (7) clearly defined a precursor to active prothrombin conversion and was very dependent on the efforts of Dhami Shah, a research associate who had come to me from Johnson's laboratory and who was with my lab for 25 years.

The finding that there was a biologically inactive form of prothrombin in the plasma of patients treated with warfarin indicated that a vitamin K–dependent modification of a liver prothrombin precursor was needed to form active prothrombin and that at least some of the inactive forms reached the circulation. Johan Stenflo at the University of Lund in Sweden found that large quantities of this protein could be obtained from the plasma of warfarin-treated cattle. He characterized the protein in an attempt to determine what the modification might be. Gary Nelsestuen, a postdoc of mine, and Stenflo were both able

to demonstrate that the difference in the two proteins was the inability of the "abnormal" form to bind Ca^{2+} ions. With this information available, researchers in the field were directing their efforts toward the identification of some type of calcium-binding prosthetic group that would need vitamin K for its attachment to prothrombin. The general hypothesis was correct, but the modification of prothrombin was much simpler than what most of us working on this problem were probably looking for.

All research scientists spend a lot of time at meetings, and occasionally we do become part of a knowledge-changing event. In the spring of 1974 I attended a Boorhaave meeting in Leiden in The Netherlands. It was a rather small meeting, and it was the first time I had met Johan Stenflo. Following a reception and dinner and a lengthy comparison of Oude (old) and Jonge (young) Genever, Johan informed a few of us that he had demonstrated the presence of a new amino acid, γ-carboxyglutamic acid (Gla) in prothrombin, but not in the biologically inactive form of the protein present in warfarin-treated cattle. Steffan Magnusson, a Danish protein chemist who was sequencing prothrombin and who was collaborating with a mass spectrometry group in England, was also at the meeting. The morning after Johan's presentation, Steffan stood up and said, "I have called my colleagues in Cambridge, and we are sure that this cannot be correct." It was, however, correct. Using a somewhat different approach in characterizing Gla, Nelsestuen, who was then a faculty member at the University of Minnesota and was not at this meeting, published the characterization of Gla a short time after Johan.

The demonstration of Gla residues in biologically active prothrombin but not in an inactive form present in warfarin-treated animals did establish a role for vitamin K in this process, but it did not clearly define the role. Our lab had demonstrated that a precursor to prothrombin did accumulate in the microsomal fraction of liver in vitamin K–deficient or warfarin-treated rats and that incubation of this crude fraction with vitamin K would result in the generation of biologically active prothrombin. It did not take a great deal of thought to return from The Netherlands and add radioactive CO_2 to our incubations. It was found that $^{14}CO_2$ was incorporated into proteins in the presence of vitamin K, that the majority of the radioactivity was present in prothrombin, and that it was in the amino terminal domain of prothrombin (2). Acid hydrolysis of the radioactive prothrombin led to a loss of 50% of the radioactivity, with the remaining activity present as radioactive glutamic acid. This was consistent with the known loss of one of the γ-carboxyl groups of Gla during acid hydrolysis.

The activity observed in the crude microsomal preparation was called the vitamin K–dependent carboxylase in our early publications, and although the term γ-glutamyl carboxylase might have been more appropriate, the original term has stuck. Fairly early studies demonstrated that the reduced form of the vitamin was the active substrate, that the reaction required O_2 and HCO_3^-, and that the enzyme retained activity if solubilized in a number of detergents. This enabled a determination of the concentration of the various substrates. Much of the early work was conducted by Jim Sadowski, a graduate student who after a postdoc started a vitamin K research program at Tufts USDA Nutrition Center, and Chuck Esmon, a postdoc who has continued to study the inflammatory response with a focus on the vitamin K–dependent factor, protein C, at the Oklahoma Research Foundation. Our early work was greatly aided by a collaboration with Dan Rich, a peptide researcher in the University of Wisconsin School of Pharmacy who developed low-molecular-weight peptides with GluGlu sequences that functioned as substrates for the carboxylase (9). The peptide FLEEL became a standard substrate used by anyone studying the carboxylase.

A major metabolite of vitamin K is its 2,3-epoxide, which had been earlier characterized by John Matschiner at St. Louis University and shown to circulate and be present in tissues at low levels; these levels increased substantively following warfarin administration. Early

studies of the carboxylase determined that this metabolite was generated by the carboxylase and that the energy to drive the carboxylation was derived from the oxidation of vitamin KH_2 by O_2 to form this metabolite (6). Much of the work in my laboratory in the late 1970s was directed toward an assessment of the substrate concentrations involved and stoichiometry of the products formed. These studies led to an understanding of an enzymatic pathway that is centered on the ability of the enzyme to utilize O_2 and vitamin KH_2 to abstract a hydrogen and generate a carbanion on the Glu substrate, which was attacked by CO_2 or picked up by a proton (4, 5, 10). Whenever I consider the extremely crude enzyme preparation that was used in these studies, I find it amazing that similar studies carried out 15 years later utilizing recombinant carboxylase yielded essentially similar data.

When Gla was discovered as an amino acid in at least a few proteins, I had assumed without much thought that it was probably decarboxylated and then metabolized like glutamic acid, so I decided that I would identify the enzyme involved. I obtained some Gla that was ^{14}C labeled in γ-carboxyl groups and got some help from Alf Harper, whose laboratory was next to mine and who had the equipment needed to trap any CO_2 that might be exhaled following decarboxylation. We injected the [^{14}C]Gla into rats and started to measure exhaled $^{14}CO_2$. After two hours we had only a trace of ^{14}CO in the trapped CO_2, but 60% of the injected dose was in the urine. I probably should have expected this, considering the metabolism of some other posttranslationally derived amino acids, but these data led to another vitamin K–related enzyme.

Gla excretion in the adult human is in the range of 50 μmol/day, and vitamin K intake is in the range of 0.2–0.3 μmol/day, and the bioavailability is probably no better than 20%. As it takes a mole of vitamin K to generate a mole of Gla, each mole of vitamin K epoxide formed by the carboxylase must be extensively recycled to the reduced form of vitamin K. The enzyme responsible for this was called the vitamin K epoxide reductase and is now more commonly known as vitamin K oxide reductase (VKOR). The action of this enzyme was a research area in which our laboratory was involved for a number of years, as were a few other labs that were interested in vitamin K–related problems, including that of Reidar Wallin at Wake Forest, who had been a postdoc in my lab. Although it had been known for some time that warfarin antagonized the production of active prothrombin, it was not until VKOR was discovered that it was known that its action was not at the level of the carboxylase, but rather it inhibited VKOR and limited the concentration of vitamin KH_2 available to the carboxylase. This aspect of VKOR research was also an active project in our lab. Although most of the research efforts in my laboratory have been more biochemical than nutritional, collaborations with University of Wisconsin clinicians Frank Greer and Neil Binkley have led to studies of the need for vitamin K in infants and the possible role of the vitamin in the control of osteoporosis (1, 3).

NONRESEARCH ACTIVITIES

When Does a Biochemist Become a Nutritionist?

The faculty that I was most closely associated with when I joined the department was the group that at that time would have been called nutritional biochemists. Bill Hoekstra, Hector DeLuca, Alf Harper, and Carl Baumann were working on the biochemical end of nutrient metabolism. I am not sure when I joined the nutrition society [American Institute of Nutrition (AIN) at that time, then American Society of Nutritional Sciences (ASNS), now American Society for Nutrition (ASN)], but I do remember that it was after I had joined the American Society for Biochemistry (ASB) [now the American Society for Biochemistry and Molecular Biology (ASBMB)]. Scientific societies were much more critical when choosing their members at that time, and the ASB was reluctant to admit those who had previously

joined the AIN. There was a national move in the late 1960s for universities to revise the role of their schools of home economics, and on the Madison campus in 1969, this led to the movement of a portion of the Foods and Nutrition group of the School of Home Economics to a new Department of Nutritional Sciences within the College of Agriculture. Hoekstra and Harper from Biochemistry, Earl Shrago from Medicine, and N. J. Benevenga from Animal Science were given joint appointments within the new department, with Alf Harper assuming the chairmanship. I was not aware of the discussions leading to this major change and was not involved in the Department of Nutritional Sciences on the Madison campus for the next 20 years.

By 1988, Alf Harper had stepped down from the chairmanship of the Nutritional Sciences Department, and the position was held by other members of the department for short periods. Much more so than at most institutions, a large number of departmental chairs are selected from within the department, and the dean was again attempting to fill the position but was having a difficult time finding a consensus candidate, with the faculty essentially evenly divided between two of its members. This is a particularly difficult problem at Wisconsin, where faculty have a major input on selection of chairs and are required to vote on their support of the current chair each year. I was attending a Thrombosis/Haemostasis Gordon Conference when the dean called me and indicated that the faculty was tired of the current split and that they would be satisfied if I would take the chairmanship. After some discussion with the Dean and the faculty, I agreed to take the position. My appointment stayed in the Biochemistry Department, and they very generously cut my teaching responsibilities to nearly nothing. As it is only a few minutes' walk between the two buildings, it all worked out well. The department had been working on the development of an Interdepartmental Graduate Program in Nutritional Sciences (IGPNS), which was approved soon after I became involved. These programs, which are active on a number of campuses, seem to be the best way to bring together graduate students who may have an appointment in another department but have interests in nutrition and a related thesis project. The IGPNS program enables them to get the broad training they desire and enhances the possibility of obtaining NIH support for students and postdocs.

Nonresearch-Associated Activities

At this point in my career, I thought that as long as I was going to head up a nutrition department, I should become more involved with the nutrition society, the AIN. I took a rather direct approach. I called a friend, who called a member of the nominating committee and got my name on the ballot. Then, following the annual officer election, I was voted a member of the AIN Council. A couple of years later I assumed the presidency and became rather heavily involved with AIN activities. During this period, AIN changed its name to the American Society of Nutritional Sciences (ASNS), which seemed to be a good idea, and initiated the Research Interest Sections (RIS), a large number of groups that formed around common research interests. Dale Romsos developed and pushed this effort, and I was not very supportive of it. Dale was right, and it was a good idea. As in all societies of this type, the current leaders are involved and visible while the majority of the members do not have much impact on the direction their society is going and justifiably can feel left out. The RIS groups have brought together individuals with similar interests and have provided a much larger fraction of the membership with an opportunity to be involved. My association with the ASNS leadership did give me an opportunity to interact with a much broader group of "nutritionists" with diverse research interests and also led to appointments to various other boards, committees, etc., all interesting and some more productive than others.

One aspect of the Society's activities that I did become intensely involved in was its publication, *The Journal of Nutrition*. Willard Visek at the University of Illinois in

Urbana-Champaign had been the editor-in-chief for some time and had started to make the shift from the historical model of a single editor making decisions on a wide range of research activities to the use of a number of associate editors, each covering specific research areas. Al Merrill and Bob Cousins were the first associate editors, and I agreed to be what I think was the third associate editor. When Willard retired from his position, I assumed the editor-in-chief position. I had written a letter to the search committee telling them that I thought that with some changes, the number of submissions could be increased by 20% during my five-year term as editor-in-chief. I appointed a few more associate editors and found that we were to able significantly decrease the time from manuscript submission to publication after we got the authors to believe that the deadlines were real. With the combined efforts of Dick Allison, the Society's executive officer, Karen King, the Society's publications manager, and Kathy Harden, the assistant editor who had worked with Willard, we were able to put *The Journal of Nutrition* online and to handle manuscripts electronically. Everyone involved in the publication efforts worked hard and seemed to enjoy it, and at the end of the fourth year, we had doubled the number of manuscript submissions.

My involvement with the ASNS also led to my service as the Society's representative on the Federation of American Societies for Experimental Biology (FASEB) board and eventually a term as president of FASEB. FASEB was founded by the American Physiological Society, the American Society of Biological Chemists, the American Society for Pharmacology and Experimental Therapeutics, and the Society for Experimental Pathology in 1913. The American Institute of Nutrition joined this group in 1940, and the American Association of Immunologists in 1942. These six initial societies held their annual meetings concurrently and worked together on common problems. The membership of these groups expanded rapidly after World War II, and although much of FASEB's efforts were directed toward the annual FASEB meeting held in Atlantic City each spring, the Federation expanded its activities by changing the focus of *Federation Proceedings* and initiating an Office of Public Affairs. By the mid 1950s, this office was moved to the current Bethesda location.

As the activities of the member societies increased, additional office space was needed, and the Bethesda campus was substantially enlarged to accommodate the needs of the member societies. FASEB eventually nearly disintegrated owing to the addition of a couple of new societies, the societal concerns regarding the fiscal handling of the yearly meeting, and the lack of a clear understanding of the responsibilities and obligations of the societies and FASEB. A small meeting of society representatives in 1989 resulted in major changes in the relationship between the societies and FASEB, and public affairs activities were expanded and placed under the control of the scientists appointed to the FASEB board by the member societies. The restructuring of FASEB also led to the dissolution of the FASEB meeting as the home for the annual meeting of the member societies. Five of the original societies did, however, form a new identity, Experimental Biology, which serves as a home for those FASEB societies or non-FASEB societies to hold joint meetings. This has been a successful move, and I was privileged to be a member of the board that developed this entity.

I found that service in the leadership of FASEB was interesting and rewarding, but very time consuming. The public affairs efforts of FASEB are supported by a competent staff, but the elected officers serve as FASEB's face and voice. FASEB has a typical societal route of president-elect, president, and past-president, and when I was involved (1995–98), I was lucky to have Ralph Bradshaw from the Biochemistry Society as the president before me. We worked together for two years during a period when FASEB was expanding its membership by admitting a number of smaller societies, protecting and expanding its role in federal funding advocacy, and enlarging its variability to the numerous groups involved in the biomedical

research community. During the years I was involved, the NIH budget doubled over a five-year period, some of the societies expressed a desire to enhance the public affairs of their society rather than those of the Federation, and activities of the FASEB leadership on Capitol Hill were expanded. Numerous organizations are advocates for increased funding for biological research and are involved in science policy, and FASEB has the finances and people to be involved at a level where they are in contact with the decision makers. Although the politicians make the final decisions, the combined efforts of the FASEB's scientific community members are vital in obtaining the best possible outcome for research scientists, and I feel that being involved with this group was well worth my time.

CHANGES IN THE SCIENTIFIC COMMUNITY OVER A 50-YEAR PERIOD

Major changes have occurred in the way that academic science is managed from the time that I became involved. Fifty years ago, most of the faculty in the Biochemistry Department could understand what their colleagues were doing, and the majority could have switched their efforts to research in an area someone else was involved in and make real progress. Today this would be very difficult. This also impacts the hiring process. At one time the faculty sat down together and decided who might best fill the positions made available by retirements and then hired those individuals. I am personally aware that I must be one of the luckiest scientists in the country. My Ph.D. research was in an area that most people would classify as animal science (or today it might be called environmental science) and that very few biochemists were interested in, and I ended up with a faculty appointment in one of the most prestigious biochemistry departments in the country. Today the hiring process involves a lengthy and expensive procedure of multiple letters of support and visits overseen by a bureaucratic administration. We are also faced with the split that I am sure all departments have: Do we want the "best" biochemist available or someone who will fill a research area we feel we need? The "best" biochemist, of course, turns out to be someone who is working within an area that each "best scientist" advocate is interested in.

It also seems inevitable that academic departments will lose their stature in the university system. In a large department, there are many faculty members whose interests are not centered in the department but rather within a smaller unit, which may be a cross-campus institute, degree program, joint appointment, or simply an informal group of faculty with a similar research focus. It has also become difficult for departments to hire in a manner that would maintain productivity in an area they want to continue in or to move in a direction they have decided to focus on. The deans and/or higher administration are increasingly holding positions to be used as they desire. At most institutions, higher administrations are pushing "cluster hires" (or a similar term), where a cross-campus search committee will bring in potential faculty members with a designated research focus and then negotiate an appointment with various departments. There is an incentive to accept cluster hires, as they are probably the only way to increase the number of faculty in a department. They can be very successful faculty members, but this process does make it difficult for departments to maintain a strong research focus and to be recognized for it. It is not a bad time to be retired from the system.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Contents

Indexes

Errata

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