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MEMORIES OF MICROBES AND METABOLISM

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ABSTRACT

As Jackie Gleason was wont to say: "How sweet it really is!" And—reflecting on the 1940s–1980s, when studies of microbial nutrition revealed exciting structure-function relationships of the B-complex vitamins with relevance to metabolism in humans—it really is. A chemistry degree from Beloit College and a doctorate in biochemistry from the University of Wisconsin set the stage for my life's work at Lederle Laboratories, the University of Illinois, and Vanderbilt University. At Lederle my research contributed to folic acid chemistry: coenzyme forms and function; antimetabolites and cancer chemotherapy. My subsequent university studies centered on lysine biosynthesis and metabolism, e.g. its precursor role in carnitine and in indolizidine alkaloids of physiological interest. There were also many opportunities to reach out and give something back to the system via teaching and diverse service activities, all of which has led to a happy, fulfilling career, one for which I am ever thankful.

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Introduction

Having, for some 10 years, been an Associate Editor of the *Annual Review of Nutrition* and a participant in the Editorial Committee's annual task of selecting

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authors for prefatory chapters, I note that the tables have been turned: I have been asked to contribute to the 1997 volume. I must seek to make this my best writing, for it will keep company with prefatory chapters written by my peers, namely Esmond Snell (27), my PhD mentor; Tom Jukes (21), my boss during those halcyon days at Lederle; and Bill Darby (12), who brought me to Vanderbilt, where I spent the longest period of my professional life. Were it not for these men who have been so influential in my career, I probably would not be writing this article at all.

Early Years, High School, and College Days

In 1924 in Chicago my family broke up, a situation unusual in that day. Happily, I found a lovely home with my two aunts and my grandmother in Rockford, Illinois. My grandmother, a Swedish immigrant and a staunch Lutheran, ruled with a matriarchal hand well into her 90s. Her eldest daughter, Esther Pearson, a grade school teacher, was the breadwinner and my "father figure." Her top salary—in the deep depression years—of $200 a month impressed me, and I noted we were one of the few families on the block with steady employment. My grandmother developed pernicious anemia but was sustained by injections of liver extract given by a doctor who made house calls (a vanished breed). Little did I dream that one day I would be lecturing medical students on the clinical and biochemical aspects of vitamin B_{l2}.

High school days were fun days for me, especially playing clarinet in the concert band. Happily, I had good enough grades to obtain admission to Beloit College with a $100 tuition scholarship each year. Money I had earned from magazine and paper routes and summer furniture-factory jobs combined to defray tuition costs, $300 per year. I lived at home during those college years (1936–1940) and commuted with other students to Beloit, Wisconsin, some 20 miles north, in retired Greyhound buses. This was hardly the glamorous college life depicted in the movies—Jack Oakie in a racoon coat, a coed on each arm, rooting for The Team. But such commuting provided quiet study time at home in the evening and an opportunity to "keep tabs" on my Rockford College girl friend, Marion Englof, whom I subsequently married.

Beloit College was a small, liberal arts school with high academic standards and a chemistry department noted for the fact that many of its students went on to do graduate work. Although I thought I wanted to be a mathematics teacher and took education courses (too many!) to meet secondary school requirements, I found chemistry much more interesting than the abstract, higher mathematics. This was due particularly to Paul Boutwell, Professor of Chemistry and University of Wisconsin graduate (biochemistry): He was an inspiring teacher and a warm, enthusiastic person as well. He made the textbooks of JB Conant, *Organic Chemistry,* and B Harrow, *Biochemistry for Medical and*

Dental Students, come alive. I got hooked on biochemistry when we performed a proximate analysis on osage oranges plucked from country hedges and isolated cystine from barbershop hair sweepings. This was a new kind of chemistry for me, an introduction to natural-product chemistry, which I found fascinating, a feeling I have retained all my life.

A Year at the Big "U"; World War II Years

After graduating from Beloit College I found employment in a local paint factory, where I was introduced to the intricacies of paint formulation. But Dr. Boutwell, who was operating behind the scenes, had other ideas for my future. One day I received an unsolicited letter from the Department of Biochemistry at the University of Wisconsin offering me a graduate fellowship, $720 per year, and a tuition waver. Elated, I immediately visited the Wisconsin campus. I talked with the biochemistry professors, had the temerity to turn down an offer to work in Dr. Elvehjem's laboratory (a large room full of barking, black-tongued dogs scared me!), and instead opted for the microbial chemistry group of Dr. WH Peterson and Dr. Marvin J Johnson. Little did I realize what a momentous decision that would prove to be. It set the stage for the major focus of my subsequent research career, i.e. the use of microbes as tools for nutritional/biochemical investigation. Subsequently I realized I had stumbled into a rarified academic atmosphere: I learned that Ed Tatum, Duane Woolley, and Esmond Snell had been former tenants of this laboratory. It is easy to remember Woolley because, although he had moved on to a position at the Rockefeller Institute, he had left behind a cold room (the only cold room) still reeking of spoiled fish, which hearkened back to his research on thiaminase and Chastek paralysis.

My fellowship was provided by the Red Star Yeast Co. of Milwaukee, and the research concerned efforts to produce a high-thiamine baker's yeast. The fortification of foodstuffs, e.g. the addition of niacin to baking flour, was just coming into vogue and served as a model for our work. Jim VanLanen, a postdoctoral fellow, had all the fun with the biosynthetic studies, while I, when not pursuing a heavy course load, ran (daytime, nighttime, and in-between time) all the thiamine assays on his yeast samples. When the data looked promising, we drove to Milwaukee and attempted direct extrapolation of our findings in the Red Star Yeast pilot plant. After a year, I had had enough of this. I had acquired sufficient credits for a MS degree and had a publication assured. So, in August 1941, with World War II on the horizon, I accepted a job as a research assistant, for $1500/year, at Lederle Laboratories, Pearl River, New York.

At Lederle I found myself in a newly forming research unit, the particular concern of which was the discovery of nutritional factors required by man and higher animals. One project in particular that interested me was an effort to

produce riboflavin by fermentation for use as an animal-feed supplement. I remembered Professor Peterson's lectures that the mold *Eremothecium ashbyii* was reputed to produce riboflavin in exceptionally high yield. We obtained the culture from the Baarn Culture Collection in Nazi-occupied Holland: It glowed on arrival! In the first experiment, the mold produced 100 μg of riboflavin per ml, a yield that with persistant effort rose some 10- to 20-fold! Furthermore, the culture grew well on a waste liver fraction, a by-product of B-complex vitamin refining. It was from such results that full commercialization—construction of a fermentation plant and recruitment of a knowledgable staff—and the expertise required for the coming antibiotic era were derived. As for me, I received a $300 raise and an extra week of Christmas vacation to travel home to Rockford and get engaged. I thought: "Gee, industry is great!" Additional Lederle experience in these war years included production of serum albumin from blood donated by the Red Cross and preparation of antitoxins for tetanus and for gas gangrene and toxoids for the armed services. These diverse experiences vividly demonstrated to me how biochemical and bacteriological "book learning" could be applied to the preparation of products useful to man. But I sensed the need for more basic training and, after the war, returned to Madison for additional graduate work.

Back to the University of Wisconsin, 1946–1949

I had learned of a young, up-and-coming new staff member, Esmond E Snell, in the biochemistry department at the University of Wisconsin whose research interest was bacterial nutrition and metabolism. Moreover, he too had been a student of Prof. Peterson's. This sounded like "all in the family" to me, and happily, I was readmitted to the department with Dr. Snell as my PhD mentor. This was, however, not an entirely pleasant time to be going to college. The campus was flooded with soldiers returning to school under the aegis of the GI Bill. Housing was difficult. I had married by this time, and for a year my wife, Marion, and our little boy, Steve, had to live with Marion's parents in Rockford, where Marion taught school. I finally found a lonely room for myself in East Madison, miles from the campus, and—painfully—learned to become a student again. Classrooms were overcrowded, necessitating the scheduling of evening classes, and I found myself squeezed into space provided in Prof. Steenbock's laboratory. But my research was exciting and involved studies of the role of biotin and folic acid in bacterial metabolism. By growing the fastidious lactobacilli on completely defined media, I saw how, by judicious juggling of relevant nutrients and by observing resultant effects on growth, it was possible to draw strong inferences about vitamin function. Our data suggested relationships between biotin and fatty acid synthesis, and folic acid and one-carbon metabolism, results that were subsequently borne out by many

others as biochemical tools and techniques became available to unravel the complexities of metabolic pathways. I had six publications to show for my thesis efforts, which in 1949 served as an entry to an excellent research position in the Nutrition and Physiology Research Section, Lederle Laboratories.

There were, during those Wisconsin years, many things to be learned outside the classroom as well. For example, sometimes in the late evening, Dr. Steenbock would leave his office and come over and reminisce with me about "the good 'ol days" (some of which—i.e. those of McCollum/Steenbock's gone by—weren't so good! I have often thought how fitting it was that Dr. Steenbock's last student, Hector DeLuca, should—in effect—conclude the Steenbock era with his major contributions to vitamin D active forms and functions). And down the hall, the ever-colorful KP Link held forth (pipe-smoking and attired in blue jeans, work shirt, and red bandana) as he proceeded with his fascinating studies of the hemorrhagic factor (dicumarol) in spoiled sweet clover. Here, too, the precise role of dicumarol has only become clear in recent times, following the elucidation of the vitamin K cycle and the demonstration of its disruption by dicumarol, studies in which John Suttie, a student of Paul Philips, figured importantly. And Snell's laboratory was humming, with other of his students making major strides in such areas as elucidation of vitamin B_6 forms and their functional role, putrescine and polyamine metabolism, mineral metabolism, *Lactobacillus bulgaricus* factor (LBF, pantotheine) and relation to coenzyme A, etc. WL Williams did the initial work on LBF, but perhaps more important, I discovered that Bill played rhythm guitar! We managed to withstand the pressures of graduate school by playing Dixieland jazz (clarinet/guitar) on weekends in Steenbock's lab when we were reasonably sure he was out of town.

Life in Industry, Lederle Laboratories, 1949–1958

The Nutrition and Physiology Research Section at Lederle, under the leadership of Drs. Jukes and Stokstad, was an independent, close-knit group. It included animal nutritionists, physiologists, microbiologists, biochemists, and organic chemists. Much of our activity, described by Jukes earlier in this series (21), was centered on the identification of unknown growth factors, animal protein factor and the antibiotic growth effect, folic acid antimetabolites, and cancer chemotherapy. I supervised a microbiological assay laboratory, monitoring the activity of relevant samples submitted by the staff on the above projects, and so had broad exposure to exciting areas of nutritional research. Some position for a fresh PhD!

One of my biggest thrills was working on an experiment that had been set up to learn the chemical nature of citrovorum factor (CF), a growth factor required by *Leuconostoc citrovorum*, which could be replaced by a high level of folic

acid. In a move based both on hints in the literature and on our own hunches, we dissolved folic acid in formic acid and added Zn dust to generate hydrogen gas. Much to our elation, the folic acid solution became thousands of times more potent for growth of *L. citrovorum*. It was clear that reduction was essential for CF activity. 10-Formyl folic acid was no more active than folic acid, but when reduced with Zn dust and HCl, it became as active as folate when the latter was reduced with Zn dust and HCOOH. Months later, John Brockman and other chemists—working without benefit of nuclear magnetic resonance, mass spectrometry, etc—concluded that CF was 5-formyltetrahydrofolic acid (3). During the course of these chemical investigations, labile derivatives of folate became available, e.g. tetrahydrofolate (THFA), 10-formyl-THFA, and 5,10-methylene-THFA. It was realized that these folate forms might have coenzyme roles in reactions of one-carbon metabolism, e.g. in the biosynthesis of purines and thymine. But, fortunately, CF—now termed leucovorin—became available in large quantities and, in contrast to the aforementioned labile THFA derivatives, was stable; it became widely used by many investigators as a ready source for generating folate coenzyme forms appropriate for relevant reactions of one-carbon metabolism.

Another thrilling aspect of this work was our finding that CF was effective in counteracting the effects of the folic acid reductase inhibitors: 2,4-diaminofolic acid (aminopterin) and 4-amino-10-methylpteroylglutamic acid (methotrexate) (7, and references therein). Here again, because of the stability of leucoverin, it became useful in "CF rescue" in the clinical management of certain types of cancer by methotrexate chemotherapy. And so one can see that the dividends accruing from looking at the nature of the folic acid requirement of the lowly lactobacillus, *L. citrovorum*, were great indeed, leading to major insights into the role of reduced forms of folic acid in one-carbon metabolism, and also serving in helping to understand the role of methotrexate in cancer chemotherapy (20).

And then, some research accomplishments came about by sheer accident. For example, Seymour Hutner, a dedicated protozoologist, convinced us that protozoal nutrition might relate significantly to nutrition of higher animals. As a result, we tried to identify in liver extract a factor required for growth of *Crithidia fasiculata*. One day my technician reported that the protozoan no longer required the liver factor. On questioning the technician it was discovered that a fresh vitamin mix had been employed that inadvertantly contained a 1000-fold more folic acid than was needed. This clue led immediately to the finding that *C. fasiculata* would give a growth response to high levels of folic acid, but also to certain unconjugated pteridines as well (5). With such leads, Patterson et al (24) subsequently isolated 2-amino-4-hydroxy-6 (1,2-dihydroxypropyl)pteridine, termed biopterin, from 4000 liters of human urine taken from donors who consumed milligram quantities of folic acid. Although

we found that it was not required for growth of higher animals, biopterin became of great interest as subsequent research, particularly from Seymour Kaufman's laboratory (22), showed that at the tetrahydro level it functions as the coenzyme in the complex multienzyme phenylalanine and tryptophan hydroxylating systems. The work takes on important clinical significance, e.g. in relation to phenylketonuria. The end products of these hydroxylating reactions are the tyrosine-derived neurotransmitters, dopamine and norepinephrine, and the tryptophan-derived neurotransmitter, serotonin, suggesting that tetrahydrobiopterin is essential for normal brain development and function.

The isolation, elucidation of structure, and synthesis of thioctic acid (lipoic acid, 1,2-dithiolane-3-valeric acid) was another major accomplishment of the Lederle group during this period. Initial work from the laboratories of IC Gunsalus (University of Illinois) and LJ Reed (University of Texas) on lipoic acid function ultimately led to an understanding of its catalytic role in the α-keto acid dehydrogenase multienzyme complexes that catalyze the oxidative decarboxylation of pyruvate, α-ketoglutarate, and the branched-chain α-keto acids (cf 25). This work underscores the fundamental importance of lipoic acid in the derivation of energy from carbohydrate and branched-chain amino acid catabolism.

The Lederle contribution to the above research endeavors constituted what might be termed "blue sky" research, namely, basic work in an industrial environment that led to compounds of major biological interest but that, from Lederle's standpoint, did not lead to any obvious commercialization. But not to worry—Lederle was blessed with the discovery of the first broad-spectrum antibiotic, aureomycin, which was of immense clinical and commercial value. Moreover, following the refining of the antibiotic from *Streptomyces aureofaciens* fermentation, the mycelial residue was found to be a rich source of animal protein factor (forms of vitamin B_{12}) and traces (parts per million basis) of aureomycin. On drying, this product, termed Aurofac, gave substantial growth responses in farm animals (especially in swine and poultry) fed plant protein diets, e.g. corn/soybeans. Tom Jukes and Bob Stokstad were the prime movers and shakers in this enterprise, about which much has been written (cf 21). It is now estimated that the amount of antibiotic in animal feed is on the order of 20 million pounds annually, twice as much as for humans (TH Jukes, personal communication).

I was involved in the antibiotic-animal feed program in only a tangential way, being called on, for example, to determine levels of vitamin B_{12} and antibiotic in feeds; to determine levels of aureomycin, if any, in animal tissues following antibiotic feeding, etc. I recall meeting with Food and Drug Administration (FDA) scientists on a Lederle farm on the Del Marva peninsula in the eastern United States, where we sacrificed numbers of poultry that had been fed varying levels of antibiotic. I conscientiously obtained tissue samples to bring back to

my laboratory for aureomycin assays. The FDA team did the same, but they also loaded all the residual poultry (which included turkeys) into their automobiles for home consumption (Thanksgiving Day was fast approaching). I haven't worried much about aureomycin and antibiotic feeding relative to residual tissue levels, emergence of resistance strains, etc, since (40 plus years)!

During this period, I had a wonderful personal and professional relationship with Bob Stokstad. His outlook on life, sense of humor, sound scientific judgment, ability to handle both basic and applied problems, sense of common decency, etc, were magnificent attributes for confronting the problems of the day. Bob and Tom made a tremendous team. In that atmosphere things got accomplished. In fact we did too well; by the late 1950s, it seemed there were no more vitamins to be found. A chicken, for example, could grow and reproduce on a completely defined synthetic diet. We had worked our way out of a job. But the world of intermediary metabolism loomed bright as a major field of investigation, and in the fall of 1958 I moved to the dairy science department at the University of Illinois to pursue this interest.

Life in a College of Agriculture, University of Illinois, 1958–1969

Dr. Glenn Salisbury, at the University of Illinois, had set up a unique department where basic science could flourish in the midst of work that had a more direct application to dairy science. Although I was a city boy and could not milk a cow, this didn't make a particle of difference to Glenn, and I was very comfortble in this agricultural setting. Furthermore, rapport was excellent with the biochemists, organic chemists, and microbiologists on campus, which included some world-class scientists. Pleasantries could be exchanged with HH Mitchell and WC Rose, "giants" encountered on campus.

At Lederle we had discovered that synthetic α-ketoadipic acid and α-aminoadipic acid were excellent precursors of L-lysine in baker's yeast fermentation [e.g. yields up to 20% lysine, dry weight basis (6)]. We pursued the biosynthetic and nutritional aspects of this discovery. It is interesting to note that in the 1960s there were still amino acid biosynthetic pathways to elucidate. (Observation: Biochemistry is a young science!) We had an excellent yeast biosynthetic system to exploit, and a series of yeast and *Neurospora* lysine auxotrophs to aid in the construction of the homocitrate-aminoadipate pathway of lysine biosynthesis (Figure 1) (18). Ketoadipate derives from homocitrate via reactions analogous to early steps in the tricarboxylic acid cycle (Figure 1). Ketoadipate is then aminated via transamination with glutamate to give aminoadipate, which on reduction to the semialdehyde condenses with glutamate to form saccharopine [6-N-(glutaryl-2)-lysine]. The latter then undergoes cleavage, yielding lysine and α-ketoglutarate (α-KG). A practical aspect of these

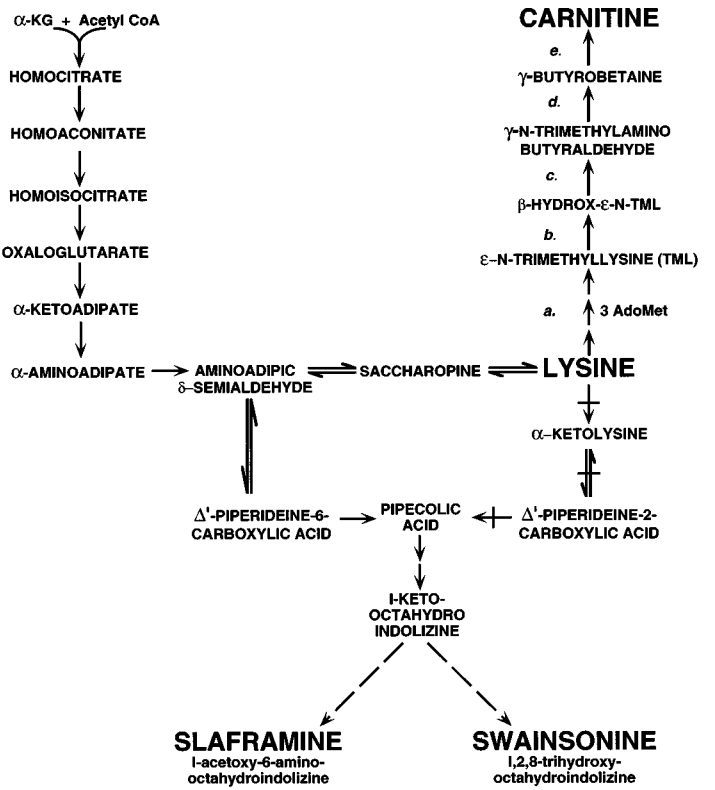


Figure 1 Pathways of lysine biosynthesis and metabolism in fungi: a summary of our adventures. Abreviations: KG, ketoglutarate; CoA, coenzyme A; AdoMet, adenosyl methionine; TML, trimethyllysine.

transformations is that synthetic ketoadipate is aminated asymmetrically, forming L-aminoadipate, and yielding L-lysine as the final product. Subsequent study showed that such "yeast lysine" is nutritionally available for growth of lysine-deficient rats (15).

The discovery of saccharopine as an intermediate of lysine biosynthesis in fungi (23) attracted wide attention from both a biochemical and a biological standpoint. As reviewed elsewhere (8, and references therein), biochemical studies of purified yeast and fungal systems showed that aminoadipic semialdehyde reductase catalyzes the condensation and reduction reaction

$$\text{aminoadipic semialdehyde} + \text{GLU} + \text{NADPH,H}^+ \rightarrow \text{saccharopine} + \text{NADP}^+ + \text{H}_2\text{0} \qquad \text{(Reaction 1)}$$

to give the stable intermediate saccharopine. The latter is then oxidized by saccharopine dehydrogenase,

$$\text{saccharopine} + \text{NAD}^+ + \text{H}_20 \rightarrow \text{lysine} + \alpha\text{-KG} + \text{NADH} + \text{H}^+ \qquad \text{(Reaction 2)}$$

yielding lysine and α-KG. The sum of these two reactions,

$$\text{aminoadipic semialdehyde} + \text{GLU} \rightarrow \text{lysine} + \alpha\text{-KG} \qquad \text{(Reaction 3)}$$

illustrates that the net effect of these two enzymes is to bring about a classical transamination; but it is unique in that pyridine nucleotides rather than vitamin B_6 coenzymes participate in the catalysis.

Soon after the discovery of the role of saccharopine in lysine biosynthesis in fungi, it was recognized that saccharopine was the first intermediate of lysine catabolism in higher animals and man (cf references in 8). Formerly it had been thought that such catabolism proceeded via a circuitous route involving pipecolic acid (Figure 1). Compelling evidence that the major route of lysine breakdown in the human is via saccharopine came from the discovery of genetic diseases in infants with impairments in saccharopine formation (reverse Reaction 2) or degradation (reverse Reaction 1), resulting in hyperlysinemia (11). Lysine catabolism in animals involves, then, a reversal of the fungal biosynthetic pathway to ketoadipate (Figure 1) and subsequent breakdown to CO_2 and H_2O. This research is but one illustration of an investigation of a series of metabolic transformations in microorganisms that subsequently impacted significantly on the elucidation of biochemical events in humans.

In the early 1960s, several midwestern experiment stations, including the University of Illinois', reported incidents of excessive salivation (slobbering) in dairy cattle consuming forages (e.g. red clover) contaminated with the fungus *Rhizoctonia leguminicola.* It appeared that a mycotoxin was involved, which my student SD Aust isolated from *R. leguminicola* mycelium (1). In excellent collaboration with Dr. K Rinehart, we showed that the mycotoxin, termed slaframine, was an indolizidine alkaloid, 1-acetoxy-6-aminooctahydroindolizine (Figure 2) (17). Slaframine induced salivation in many species, e.g. guinea pigs, cats, and dairy cattle on a parts per million basis. Aust found that slaframine required activation for biological activity, and also that the alkaloid was derived from lysine and pipecolic acid metabolism (1). This latter was welcome news given our interest in lysine metabolism, and it led to long-term biosynthetic studies of the indolizidine alkaloids of *R. leguminicola.* Our research on slaframine was of obvious interest to our dairy science colleagues, e.g. much work followed on effects of slaframine on ruminant digestive function (10). On a more general basis, attention was focused on the indolizidine alkaloids as a class of compounds with unique biological activity, as is further elaborated below (cf Figure 2).

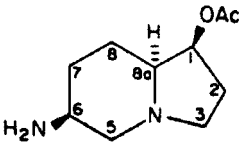
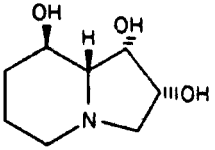
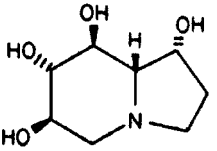
Alkaloid structure	Occurrence	Biological action
 I. Slaframine	*Rhizoctonia leguminicola* cells. A fungal contaminant of legume forages.	"Salivation factor." A parasympathomimetic secretagogue.
 II. Swainsonine	*Swainsona canescens* (Darling pea); spotted locoweed (*Astragalus lentiginosus*); *Rhizoctonia leguminicola*; *Metarhizium anisopliae*.	A mannose analogue; inhibits Golgi mannosidase II, disrupting N-asparagine-linked glycoprotein biosynthesis.
 III. Castanospermine	Seeds of the tree *Castanospermum australe*.	A glucose analogue; inhibits glucosidase I, disrupting N-asparagine-linked glycoprotein biosynthesis.

Figure 2 Slaframine, swainsonine, and castanospermine: structure, occurrence and biological action. Slaframine, (1S,6S,8aS)-1-acetoxy-6-aminooctahydroindolizine; swainsonine, (1S,2R,8R, 8aR)-1,2,8-trihydroxyoctahydroindolizine; castanospermine, (1R,6R,7S,8S,8aS)-1,6,7,8-tetrahydrooctahydroindolizine. Reprinted with permission, from Reference 4a.

Life in a School of Medicine, Vanderbilt University, 1969–1987

My decision to leave Illinois for the biochemistry department at Vanderbilt was difficult to make. The University of Illinois is a magnificent university, and the state of Illinois is my native state. But Glenn Salisbury, whom I greatly admired and who had opened the door to academia for me, was moving on to a higher administrative post. We had just lost our eldest son, Steve, in Vietnam, and I thought a change of scene was in order. And then along came Bill Darby who, with all his charm, could sell refrigerators to Eskimos! He convinced me that "Vanderbilt was the place."

At Vanderbilt, the biochemistry department reminded me a bit of the Wisconsin scene (circa 1940s), with many of the staff working on nutritional problems, e.g. John Coniglio, lipids; Frank Chytil and Dave Ong, retinoids and cellular retinol-binding proteins; Conrad Wagner, folate metabolism and folate

binding proteins; and myself, amino acid metabolism. Jan van Eys (carbohydrates) and Harold Sandstead (zinc nutrition) were also at Vanderbilt (early 1970s). Strong support for graduate and postdoctoral students was derived from a National Institutes of Health (NIH) training grant, and the nutrition milieu was subsequently enriched with a Clinical Nutrition Research Center grant (to Harry Greene, the Director), which provided for a nutritional assessment laboratory. The basic biochemistry course for graduate and medical students, in which nutrition and metabolism was stressed, was team taught in important part by the aforementioned staff. Moreover, in later years, large blocks of laboratory time were secured for nutrition lectures, clinical correlations, demonstrations, and hands-on animal experimentation.

My research at Vanderbilt took an early, exciting turn and, to my delight, allowed us to keep our focus on lysine metabolism. I became interested in carnitine biosynthesis on reading that ε-N-trimethyllysine was excreted in man. It seemed conceivable that γ-butyrobetaine, a known precursor of carnitine, could arise from the metabolism of trimethyllysine if the latter were to lose carbons number 1 and 2 (Figure 3). We initially studied carnitine biosynthesis in a *Neurospora crassa* lysine auxotroph. With appropriate isotopically labeled lysine, methionine, or trimethyllysine as test precursors, it was shown that carbon atoms 3, 4, 5, and 6, and the 6-amino group of lysine, formed the carbon-nitrogen backbone of carnitine (Figure 3), and methionine was the source of methyl groups for trimethyllysine and hence of carnitine (19). These findings were soon found to also be true in the rat (29). But in the rat, the methylation of

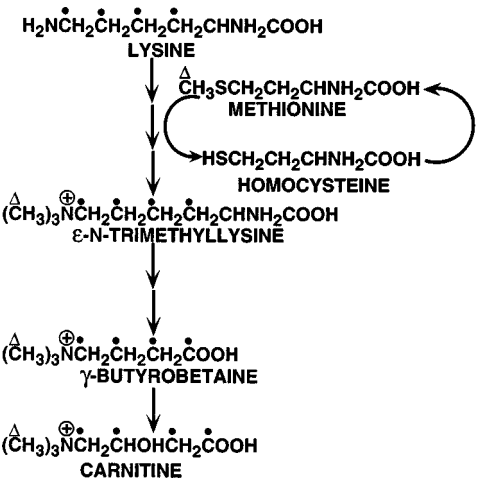


Figure 3 Early view of carnitine biosynthesis from lysine and trimethyllysine.

lysine to trimethyllysine occurs as a posttranslational modification of peptide-bound lysine, which is then subsequently released by lysosomal hydrolase to enter the carnitine pathway (Figure 1, Reactions *a, b, c, d, e*). Reactions *b* and *e* are complex oxygenase systems requiring O_2, α-KG, ascorbate, and ferrous ion as cofactors. Reactions *c* and *d* require pyridoxal 5′-phosphate (PLP) and NAD, respectively. Some specifics—identification of certain of these carnitine precursors, the nature of the enzyme transformations involved, etc—required several years to elucidate (cf 16, and references therein). In addition to our own input, the laboratories of S Lindstedt (University of Gothenburg), LM Henderson (University of Minnesota), and C Hoppel (Case Western Reserve University) contributed importantly in this regard. Dietary deficiencies of the amino acid precursors or the cofactors required in carnitine biosynthesis might be expected to call forth a degree of carnitine deficiency in higher animals. Indeed, such expectations have been borne out in ensuing work from several laboratories. Early we showed, for example, that rats fed a 20% wheat gluten diet limited in lysine grew poorly, were anemic and hypoproteinemic, and in general were found to have about a one-third lower level of carnitine per gram of skeletal muscle and heart muscle than the control group had (28). The term "conditionally essential nutrient" has now been introduced (13) as a way of considering seemingly nonessential nutrients such as carnitine and choline.

A major impetus to carnitine research in the period I describe was the recognition of its role in the intramitochondrial transport of fatty acids, a process essential for subsequent fatty acid oxidation and energy release (2). This development unleashed a Pandora's Box of biochemical and clinical investigations with respect to the implications of carnitine metabolism in health and disease (14). The work on the biosynthesis of carnitine that I have briefly described relates to nutritional and enzymatic events that have impacted, and will continue to impact, these investigations.

Some of the excitement in divining metabolic pathways is to witness the economy that nature employs in fashioning diverse molecules. For example, the carbon atoms of lysine (Figure 1) derive from acetate and α-KG, and the amino groups derive from the amino groups. What a difference it would make from a global nutrition point of view if higher animals could effect such an economical synthesis of the essential amino acid lysine! There is a certain irony in such thinking, for—as was discussed—higher animals are able to catabolize lysine via the reverse of the biosynthetic pathway (Figure 1), i.e. via saccharopine and ketoadipate. And pipecolate [which we now know (30) arises from lysine via saccharopine and aminoadipic semialdehyde and its anhydride (Figure 1)] is utilized for indolizidine ring biosynthesis simply by the addition of two additional carbons atoms (namely carbons 2 and 3 of the latter ring system) from malonate (9).

We continued studies of indolizidine biosynthesis in *R. leguminicola* and subsequently found a second alkaloid, swainsonine, 1,2,8-trihydroxyoctahydroindolizine (cf Figure 2 for structure) was also produced by this fungus (26). This fortuitous finding put us into the mainstream of swainsonine investigations. As was reviewed elsewhere (4), (*a*) an Australian group had isolated swainsonine from legumes of the genus *Swansona* and identified the alkaloid as the toxic agent when these plants were consumed by livestock; (*b*) swainsonine was found to be a potent inhibitor of α-mannosidase and the toxicosis observed in livestock explained in terms of the induction of a lysosomal storage disease similar to genetically determined mannosidosis in humans and cattle; and (*c*) in this country swainsonine was found to be present in *Astragalus* genera, plants long known to be associated with locoweed poisoning of cattle in the western United States. We were able to supply swainsonine in large quantities via *R. leguminicola* fermentation to the Poisonous Plants Laboratory, US Department of Agriculture, Logan, Utah, for large-animal experimentation in establishing the relationship between locoism and swainsonine toxicosis. Swainsonine is also proving to be a useful experimental tool for blocking glycoprotein assembly and is being studied as a possible metastatic agent.

Our work on indolizidine alkaloid biosynthesis often seemed quite remote from the real world, but it constantly presented intriguing questions for graduate students. For example, 1-ketooctahydroindolizine (Figure 1) is at a branch point in the indolizidine pathways producing either slaframine, a parasympathomimetic secretogogue, or swainsonine, a potent α-mannosidase inhibitor. The swainsonine yields were always several-fold higher than those of slaframine. What is the nature of the regulatory mechanisms governing the extent of such syntheses? Also, slaframine and swainsonine differ importantly in chirality at C-1 and C-8a (cf structures in Figure 2). How do such differences bear on structure/function relationships? It can be appreciated that the questions posed in our biosynthetic studies are fundamental questions that could also have an important bearing on the yields and activity of biologicals (e.g. antibiotics, steroids, etc) produced in industrial fermentation.

Another fascinating development in indolizidine alkaloid research was the realization that certain of these N-heterocycles can be viewed as hexose analogues in which the oxygen atom of the C-1,C-5 pyranose bridge is replaced by nitrogen. Castanospermine (Figure 2) is an excellent example wherein the stereochemical relationships of the hydroxyl groups in the 6-membered piperidine ring are precisely the same as in glucose, a 6-membered pyranose ring. Indeed, castanospermine functions as a glucose analogue (Figure 2) and inhibits glucosidase I, thereby disrupting a critical stage of glycoprotein synthesis. And it is thought that swainsonine (Figure 2) functions as a mannose analogue in inhibiting α-mannosidase II, because of the structural similarity of its protonated

form to the mannosyl cation (cf references in 4). These of course are not the only examples of antimetabolites being produced by microorganisms, but they serve as a reminder that the antimetabolite era, usually associated with the discovery of the sulfonamides, is not the exclusive domain of the organic chemist.

During my years of swainsonine research at Vanderbilt, I enjoyed a close chemical and biochemical collaboration with Drs. Tom and Connie Harris of the chemistry department and with Dr. Oscar Touster in the department of molecular biology, who, I am happy to say, had groups vigorously working in this field as well. With my Wisconsin heritage, and given KP Link and the spoiled sweet clover-dicumarol saga, perhaps it was foreordained that our investigations of the *R. leguminicola* spoiled red clover should lead to slaframine and swainsonine, with their toxic effects in livestock!

Additional Reflections on Academe

Embarking on an academic career, now nearly 40 years ago, I soon learned that professors do more than just teach and do research. Their third major activity is service, which in my case took the particular form of committee work (locally and nationally), consultations (especially study sections), and editorial boards. At first I thought such activity would make serious inroads on my research time and when the phone rang that I should play "hard to get." But as I became involved, I saw that I was associated with very top-flight people, and that in general our endeavors were tremendous learning experiences and opportunities to help improve the quality and direction of nutritional science. I would like to mention a few of these experiences.

One of my most rewarding activities was participating in 1965 with an International Committee on Nutrition for National Defense (ICNND) team in assessing the nutritional status of the Nigerian population. For me, a bench-level nutritional biochemist, this experience was an introduction to human nutrition, i.e. seeing kwashiorkor in a live infant, not merely as a textbook illustration; appreciating the work of people like Howerde Sauberlich, who contributed so much to the analytical methodology involved; and getting some grasp of what people like Arnie Schaeffer were doing in the intricate planning of such a complex operation. Another fascinating African adventure was to visit with Bill Darby his Naval Medical Research Unit III operation in Cairo, which afforded me a close look at nutritional conditions in the field, coupled with laboratory follow-up, and tours of the colorful Casbah in the cool of evening. My long association with the American Institute of Nutrition and its committees and Council—with such stalwarts as Lucille Hurley, Hamish Munro, Dorris Calloway, Mal Nesheim, and Dick Allison, to name a few—provided an effective framework for strengthening nutrition at a national level. And I am particularly proud of having been a member of the PEW Foundation National

Nutrition program committee (chaired by Mal Nesheim) at a time when nutrition program support was extremely difficult to obtain. We were able to create five Centers of Excellence of Nutrition across the nation, seeking to bring together existing institutional strengths to sustain teaching and research programs to serve as models of excellence in nutrition. I was also privileged to have served for 10 years, the formative years, as an Associate Editor of the *Annual Review of Nutrition*, with Dr. Darby and then Dr. RE Olson as Editors. And my apprenticeship in such matters was as Bob Olson's Associate Editor of *Nutrition Reviews* for seven years. What a way to keep abreast of the best in current nutrition literature!

But it hasn't been all work. Attendance at nutrition meetings in such colorful settings as San Diego, Quebec, New Orleans, Rio de Janeiro, Kyoto, Bangkok, and Upsala, to name a few, provide some of my fondest memories. For example, the Rio de Janeiro meeting included a side trip to Machu Picho, the falls of the Iguassui River, and Lake Titicaca high in the Bolivian Andes. And the Gordon Research Conferences on Vitamins and Metabolism, which I attended frequently (1950–1970) in charming New England settings, were superb examples of successfully combining business and pleasure; they contributed greatly to our research directions and to the effectiveness of my teaching. I well recall, with a smile, a Gordon Conference evening session in New London, New Hampshire, on a Fourth of July eve. Vincent duVigneaud was holding forth, proclaiming in no uncertain terms that methyl groups arose "from without," i.e. from dietary methionine and subsequent in vivo transmethylation. But a feisty Joseph Stekol insisted that methyl groups could be formed "from within," i.e. via de novo synthesis. As duVigneaud spoke, cherry bombs went off in the nearby village, with blasts that reverberated in the conference hall as though the wrath of God would descend on those who would embrace de novo methyl group synthesis. "VduV," given his flare for the dramatic, loved it! And so did we!

About Retirement; Concluding Remarks

On retirement I found it difficult to break away from the excitement of bench-level research. In 1990, I was afforded the opportunity to work in Dr. S Aust's laboratory, Utah State University, Logan, Utah, for three months. Knowing a good thing when I see it, I stayed for three years! I continued to work on the problems of fungal metabolism that Steve and I had started years ago at the University of Illinois. But perhaps more important, I had the opportunity daily to interact with students, participate in seminars, give occasional lectures, etc. Subsequently I collaborated with Ann Sorenson, from the Department of Nutrition and Food Science, in preparing a series of video tapes on nutrition and metabolism, seeking to enliven such presentations with personal anecdotes

from "the good ol' days." I hope these "lectures" will be used both locally and more widely to disseminate the "gospel of nutrition." In addition, at my doorstep was 37-mile-long Logan Canyon, with all its natural beauty to enjoy. I decided that "retirement" wasn't so bad after all!

A current TV commercial promoting a nutritional supplement for the elderly intones, "It's a great time to be silver." Although I have little hair left to be silver, if the ad implies that "the silver-haired generation" is fortunate, I agree. To appear on the scene with a PhD degree from the University of Wisconsin shortly after World War II was exceedingly opportune. The country had gone through the war years under tight rationing, which created an insatiable demand for consumer goods. We were in for a long period of economic growth. Like many, "I liked Ike." Nixonomics and Reaganomics, with their danger signals, were far distant. The Sputnik launch put the United States behind in the space race, and a concerned Congress directed generous funds to the National Science Foundation and the NIH to bolster US science. And the metabolic maps on our office walls were incomplete, just waiting for people like me to finish them! I couldn't have imagined when Tom Jukes called me into his office in the mid-1950s and asked if I would be interested in looking at ways of producing l-lysine economically via biological means that many years and some 20 or so PhD and MS theses later, Figure 1—in which my students were major contributors—would appear!

And now as I look back on nearly half a century of activity in industrial and academic settings, I feel a lot like one of my students who in his PhD thesis acknowledgment wrote: "One does not reach this stage of a professional career without the help of God and his fellowman." To this—as I think of those I have named herein, especially my wife, Marion, who has helped me so immeasurably over the years, and all those students, postdocs, and lab personnel without whom my life's mission among the microbes would have been impossible—I can only say, "Amen!"

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