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FROM BACTERIAL NUTRITION TO ENZYME STRUCTURE: A Personal Odyssey

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INTRODUCTION

As a former editor or member of the Editorial Board of the *Annual Review of Biochemistry* I helped to select authors of the prefatory chapters for more than 20 years. Now I am asked to write one myself. The first such chapter appeared in 1953 with the prefatorial statement: "This is the first of a series in which it is the hope of the Editorial Committee that our elders in biochemistry will give us through chapters of a historical and philosophical character the benefit of their long years of experience in biochemistry." With one exception, the chapters have appeared regularly ever since. Their authors comprise a group I am proud to join even while harboring some doubts about really belonging. Their autobiographical accounts have added much to our understanding of how and why they became biochemists, and how biochemistry itself developed. I shall continue in this tradition.

Background

My mother, née Hedwig Emma Ludwig, was born in Berlin, Germany in 1881 of poor parents who were converts to the Mormon Church. At the age of eight, following her mother's death, she was brought by missionaries to Utah, where she was adopted by the Stauffer family, who owned a farm near Montpelier, Idaho. My father, Heber C. Snell, grew up on a farm in Spanish Fork, Utah. The two met while both were serving as Mormon missionaries in the Chicago area, and were married in 1905 shortly after their return west.

My father's interests changed substantially over time. He received a B. A. degree in English from Brigham Young University in 1912 and much later an M. A. degree in psychology from the University of Utah (in 1923). His interest in religion and biblical history led him to pursue a PhD in New Testament history from the University of Chicago Divinity School. Graduate study was mostly accomplished during summer leaves from his work as a teacher or school administrator. The degree was finally granted in 1940. This long-term exposure to biblical scholarship "infected" him (as he used to joke) with a "scientific" attitude toward the Bible and its sources, and a healthy skepticism regarding the accuracy of revelation, which rubbed off on all of his children and contributed to a critical attitude toward both doctrinal religion and other spheres of endeavor. Because of his studies and occasional job changes the family moved frequently. The fourth of five children, I was born in Salt Lake City in 1914. Within a year we moved to Wyoming's Big Horn Basin (first Cowley, then Lovell) where I attended grades 1–3, then successively to Salt Lake City (grade 4), Ephraim, Utah (grades 5–7), and finally to Provo, Utah (grades 8–12). Our move to Provo was motivated by the necessity (as our parents saw it) of serially supporting five children through college—there were no scholarships—on an income entirely inadequate for

this purpose. By living at home we could all afford to attend Brigham Young University. My father lived in Provo during summers, but continued to teach at a junior college (Snow College) in Ephraim during the long session of the school year, often returning to Provo on weekends. In addition to family, my mother took care of up to three boarders (university students) to help with expenses during this period.

An excellent teacher at Provo High School, Mr. Hatch, had aroused my enthusiasm for chemistry, and I decided to make it my college major. As insurance against unemployment (the Great Depression still lingered on) I reluctantly included the courses necessary for a teacher's certificate in Utah secondary schools, an action that precluded my taking several attractive biology courses, and probably reduced my chances of obtaining various graduate fellowships for which I applied upon graduation from BYU. Fortunately, the certificate was not needed. At the last moment, obviously as an alternate, I received an offer of a Wisconsin Alumni Research Foundation scholarship for $400. I immediately quit my summer construction job and headed for Madison.

WISCONSIN 1: 1935–1939

In Madison my first interview was with the chairman of the committee on scholarships and fellowships, Professor W. H. Peterson, a biochemist interested especially in microbial metabolism. He encouraged me to investigate other groups in Madison as a possible "home" (my scholarship was unrestricted), but offered me a spot in his laboratory should I wish to take it. At BYU my interests had leaned toward organic chemistry; I had had no courses in biochemistry—none were then offered. However, because the term had already started and I didn't wish to lose more time, I decided to accept Peterson's offer. This decision, taken on the spur of the moment without adequate information, was one I have never regretted.

Thesis Research: Bacterial Growth Factors

Professor Peterson had intensively studied fermentations by bacterial cultures isolated from fermented plant products such as silage or sauerkraut. These organisms, mostly lactic acid bacteria, required complex media for growth. We agreed that as a thesis problem I would attempt to identify some of their nutritional requirements. It was a propitious time for such studies. Orla-Jensen & Snog-Kjaer (1) had just reported that a newly isolated vitamin for animals, riboflavin, also was required for growth of some lactic acid bacteria, and Tatum et al (2) had shown that some propionic acid bacteria required thiamine for growth. Thus the only pure B-vitamins then known also were required by bacteria. Might yet undetected bacterial growth factors also be important in

animal nutrition? The possibility, hardly acknowledged at the time, fitted well with the intense interest in animal nutrition then current at Wisconsin.

We students rarely reported directly to Professor Peterson, but we profited greatly from his weekly journal club–seminar. We were also helped by his advanced students and postdoctoral fellows. During my first graduate year, I was housed with Drs. E. L. Tatum, Harland G. Wood, M. J. Johnson, and others, in a laboratory in Agricultural Hall. The next year, I shared a basement laboratory in the Agricultural Chemistry (later rechristened Biochemistry) building with D. W. Woolley and Dr. F. M. Strong. These colleagues supplied intellectual stimulation of a high order. I was particularly impressed with Harland Wood's attempts to convince Peterson's group that carbon dioxide fixation by the heterotrophic propionic acid bacteria was real. Despite impeccable data, his efforts met initially with limited success. If scientific dogma was so difficult to correct, how much more difficult this must be in areas where there are no experimental data!

ACETATE It is rather humbling to reread one's first publication in the light of knowledge accumulated during the ensuing 50 years. Mine was an attempt to define certain requirements for growth of *Lactobacillus delbrueckii* (3). To follow growth, we simply titrated the acid produced after an appropriate time. Later, when photoelectric colorimeters became available (and affordable) direct turbidity measurements were also used. Since almost all of the substances later identified as essential nutrients were either unknown, unavailable, or both, we could either depend on luck to find a crude medium in which a single nutrient was growth limiting, or, alternatively, attempt to remove or destroy one or more of the essential unknown growth factors of an adequate medium without lowering others below the concentrations required for growth. In an attempt at the first of these approaches we found that *L. delbrueckii* grew poorly on a peptone-glucose-salts medium, but grew well when a potato extract was added. The effective substance in the potato extract was partly extracted by ether. Its identity was never established, but we found that the growth response to the ether extract was improved substantially by addition of acetate. This unexciting but key finding, noted only in passing at the time, led us to add acetate to all media subsequently used in nutritional studies with lactic acid bacteria. Attempts to explain its effectiveness (other buffers were ineffective) were renewed several years later in Texas by a thesis student, Beverly Guirard, who discovered a much more potent "acetate-replacing factor" in natural materials (4). Pursuit of these observations led to its isolation in pure form by Lester Reed, who named it "lipoic acid" (5). The same substance was discovered independently by others as an essential growth factor ("protogen") for *Tetrahymena geleii* in work leading to its synthesis (6) and as a cofactor

for oxidation of pyruvic acid by certain streptococci (7). The latter function explains why either acetate or lipoate suffices for growth of most lactic acid bacteria: added acetate supplies the acetate required as a biosynthetic intermediate, thus bypassing the need for its synthesis. Since these organisms lack the Kreb's cycle they do not need lipoate for α-ketoglutarate oxidation. Had we not added acetate to these early media, characterization of additional nutrients would have been delayed until a suitable supplement containing lipoate was available.

PANTOTHENATE, NICOTINATE, RIBOFLAVIN Addition of acetate to our basal medium made possible comparatively rapid progress. Frank Strong had just returned from a postdoctoral year in Karrer's laboratory, where riboflavin was synthesized in 1935, and had small amounts of the compound. In confirmation of Orla-Jensen's report, we found that its addition to the medium increased growth of our test organism (*Lactobacillus casei*), but blanks were high. Riboflavin is alkali-labile, but when we treated the peptone with alkali to remove it, no growth occurred even on addition of riboflavin unless a yeast or liver extract was also added. After two years of effort I had purified the growth-promoting substance about 1000-fold from liver extract; it was essential for growth of every one of 14 species of lactic acid bacteria tested (8). Its properties at this point resembled those described by R. J. Williams's group for a partially characterized growth factor for yeast which they called pantothenic acid (9). Cross-testing of concentrates showed apparent identity of the active substance in each; my best preparations were about 25% as active as concentrates that Williams adjudged to be almost pure. This identity was validated the following year when pure calcium pantothenate was synthesized in the Merck laboratories (10). The contact established with Professor Williams at that time led later to my first job.

An approach to complete elucidation of the requirements of these bacteria was now clear. As each nutrient was identified, it was added to the basal medium—in pure form, if possible, but otherwise as a concentrate. The medium could then be further refined by replacing its crude ingredients (e.g. alkali-treated peptone) by more highly purified components (e.g. acid-hydrolyzed casein). With the latter modification, for example, we identified tryptophan as one requirement, and nicotinic acid as another (8). We tried nicotinic acid because Knight (11), Mueller (12), and Elvehjem (13) had just identified it as an essential growth factor for *Staphylococcus aureus*, *Corynebacterium diphtheriae*, and dogs, respectively. Others showed almost immediately that pantothenic acid was the active substance present in a previously uncharacterized factor required in animal diets (14a,b). It was now obvious to all that bacterial growth factors were potential vitamins for animals; an academic pursuit now became of interest to industry as well.

MICROBIOLOGICAL ASSAY FOR RIBOFLAVIN As individual vitamins were identified, their determination in food became an important aspect of nutrition research. Because of the very small amounts present, chemical methods were notably inaccurate; biological assay with animals was satisfactory, but required purchase and upkeep of expensive animals over a period of weeks. Use of bacteria that required a given vitamin as assay organisms had been suggested earlier (15). Such methods had not been tested critically or widely used, but in principle seemed to offer a logical improvement in methodology. This was the genesis of our assay for riboflavin with *L. casei* as the test organism (16). A peptone medium was used from which riboflavin was removed by alkali treatment and pantothenate (and any other growth-limiting vitamins) was supplied by a yeast extract freed of riboflavin by first treating with lead acetate, then hydrogen sulfide (to remove the lead), and finally intense light. The assay was sensitive, gave quantitative recoveries of riboflavin added to test samples, and agreed with the results of animal assay of the same samples (16). It was highly successful and widely used, and served as the prototype for many similar methods later devised for each of the other B-vitamins and for many amino acids. As my major professor, Professor Peterson would normally have been included as an author of this paper. He refused because a micromethod for glucose he had once published "gave me nothing but trouble." Frank Strong and I first submitted the paper to the *Journal of Biological Chemistry,* which rejected it as being insufficiently biochemical and more suited for a bacteriological journal. We strongly disagreed; had the method relied upon a fluorimeter instead of bacteria as a detecting device no question of its suitability would have arisen. We published it instead in the Analytical Edition of *Industrial and Engineering Chemistry.* This experience served as a caution to me later, in my years on Editorial Boards of the *Journal of Biological Chemistry, Biochemistry,* and the *Journal of the American Chemical Society,* not to interpret the subject matter of biochemistry too narrowly. Although I received the PhD in 1938, the job situation was such that I remained in Madison for a postdoctoral year, during which I continued to study nutrition of *L. casei.*

The Norit Eluate Factor

The demonstrated requirements of *L. casei* for growth in a glucose-hydrolyzed casein (plus tryptophan)-salts medium now included acetate, riboflavin, pantothenate, and nicotinate. Growth on this medium did not continue on subculture unless yeast extract was added to supply still unidentified micronutrients. Adsorption of the extract on activated charcoal ("Norit") produced a filtrate and an eluate fraction, both of which were required for growth. On addition of the filtrate fraction the medium now became suitable for assay of the Norit eluate factor, which I succeeded in purifying about 1000-fold before

leaving Madison. The active principle was acidic (it was extracted by butanol from acidic but not from basic aqueous solution), but showed behavior toward precipitants that strongly resembled the purine bases and basic amino acids (17a,b).

TEXAS 1: 1939-1945

Biotin and Avidin

In early 1939 Professor R. J. Williams invited me to join his group as a Research Associate following his impending move from Oregon State University to the University of Texas. Our mutual interest in microbial growth factors made this a happy choice for me—I needed only to extend my interests to include yeast. I moved to Austin that September where I was to help purify a new growth factor for yeast, called "biotic acid" by Eakin and Williams. To exclude its identity with biotin, a potent yeast growth factor isolated three years earlier by Koegl & Toennis (18), Williams had requested and received a few micrograms of this vitamin from F. Koegl. Within a month we had shown that biotin (*a*) replaced biotic acid completely (19), (*b*) replaced a then unidentified growth factor for *Clostridium butylicum* (20), and (*c*) together with the newly isolated vitamin, pyridoxine, completely replaced the filtrate factor for *L. casei*. [Moeller had reported in 1938 that both pyridoxine and biotin were required by *Lactobacillus plantarum* (21a,b).] The yeast assay for biotin developed in connection with this work (19) permitted us to demonstrate the extremely tight binding of biotin by a protein from egg white, which we purified and named *avidin* (22a,b), and the release of biotin from the complex by heating. Together with Paul Gyorgy's group we then showed that avidin was the "toxic" protein in raw egg white previously known to produce egg white injury (biotin deficiency) in animals (23). At that time biotin was an extremely rare and precious substance. Although we suggested the possible use of avidin in its purification, we never envisioned that this vitamin would become a reagent for biotinylation of other biofactors, and that avidin would become useful for purification of such derivatized materials.

Folic Acid

Collapse of the biotic acid problem left us temporarily without an unidentified nutrient to study. Williams suggested that I further purify the Norit eluate factor for *L. casei,* but I had agreed with Professor Peterson to leave that problem in Madison. Instead, Herschel Mitchell and I turned our attention to less studied lactic acid bacteria. Several of these required a purine base plus uracil for growth. *Streptococcus lactis* R (now called *S. faecalis* R) did not grow with this supplement alone, but grew well when large amounts of

thymine or small amounts of yeast extract were added. We mounted a major effort to purify the active principle to homogeneity from a rich local source, spinach. With a steam kettle and filter press installed in the attic of the Chemistry Building we processed roughly four tons of spinach. A series of adsorption steps and heavy metal precipitations gave us a concentrate some 130,000 times more active than a standard liver extract in supporting growth of *S. faecalis* R (24). The active principle, which we named *folic acid,* also was highly active in supporting growth of *L. casei* under the conditions used for assay of the Norit eluate factor. We had rediscovered this same factor with another test organism! The matter became of little import when isolation of the crystalline growth factor and its identity with vitamin B_c [a chick growth factor discovered (25a,b) the same year as the Norit eluate factor] was reported from both Lederle Laboratories (26) and Parke Davis & Co. (27). A subsequent report from Lederle Laboratories describing its characterization and synthesis (28) bore 16 names on the masthead: an academic research laboratory clearly did not belong in this league! I took some comfort in the fact that the Lederle workers and others who subsequently isolated various forms of folic acid all had used *L. casei* or *S. faecalis,* the test organisms we introduced, for their studies.

Some Nonresearch Matters

Two events important to my career occurred in 1941. First, I married (on the Ides of March!) Mary Terrill, a senior in chemistry who worked part time in the Chemistry Library. Her background (Southerner, Episcopalian) and mine could hardly have been more different—a commonly touted recipe for marital discord. Fifty-one years, four children, and six grandchildren later we are still happily married.

Second, I was appointed an Assistant Professor of Chemistry (half time). My first teaching assignment was an undergraduate course in organic chemistry for petroleum engineers. I managed to stay one lecture ahead of the students and found petroleum chemistry surprisingly complex and interesting. Later, I initiated a graduate course in enzyme chemistry, and for a time was known facetiously by some of my associates as "the best enzyme chemist in Texas." Fortunately a genuine member of the species soon turned up in Houston.

Pyridoxal and Pyridoxamine

Folic acid was the last of the unidentified bacterial vitamins required by *L. casei* and *S. faecalis* R. Both organisms could now be grown in defined media and could be used for assay of any substance they required. We obtained startling results, however, when *S. faecalis* was applied to assay of pyridoxine (PN) in natural products. Values obtained were hundreds to thousands of times

higher than those obtained when the same samples were assayed with yeast or animals. The active substance (we called it "pseudopyridoxine") was elevated in tissues of animals fed extra PN, and its urinary excretion by a human adult (EES) was greatly enhanced by ingestion of PN (29). It finally became clear that PN per se was inactive in promoting growth of these bacteria; its apparent activity resulted from conversion of traces of the added PN to pseudopyridoxine during heat sterilization of the medium. Oxidation or amination procedures converted PN in part to an aldehyde or amine, respectively, both of which were highly active for *S. faecalis,* and which we therefore assumed to be interconverted in vivo (30). If this assumption was correct, there were only two possible structures for each compound. At this point, we enlisted the aid of Karl Folkers at Merck & Co. His group had synthesized PN earlier, and now synthesized our candidate compounds (31). Two of them showed the expected high activity (32); we called them *pyridoxal* (PL) and *pyridoxamine* (PM). The three forms of vitamin B6—PN, PL, and PM—were about equally active for yeast and animals; only PL was active for *L. casei,* while both PM and PL were active for *S. faecalis*. When appropriate standards were used, vitamin B6 assay with the latter organisms gave values fully in line with the total vitamin B6 content of tissues as determined by yeast or animal assay (33).

Some Functions of Vitamin B6

TRANSAMINATION Because autoclaving with the growth medium increased the activity of PN for lactic acid bacteria, we tried this same treatment on PL and PM. The effects were dramatic: activity of PL was reduced greatly for *L. casei* but was slightly increased for *S. faecalis*. Both changes were consistent with the transformation of PL to PM. This reaction was confirmed by heating glutamate with PL and isolating the products. The reaction corresponded to equation 1 and was fully reversible (34). It occurred also with other amino acids; coupling of two such reactions (e.g. 1 and 2) would give reaction 3, a fully reversible non-enzymatic transamination reaction in which PL and PM would act only as catalysts.

$$\text{Glutamate} + \text{PL} \leftrightarrow \text{PM} + \alpha\text{-Ketoglutarate} \qquad 1.$$

$$\text{Oxaloacetate} + \text{PM} \leftrightarrow \text{PL} + \text{Aspartate} \qquad 2.$$

$$\text{Glutamate} + \text{Oxaloacetate} \leftrightarrow \alpha\text{-Ketoglutarate} + \text{Aspartate} \qquad 3.$$

On the basis of these results, then unpublished, I suggested (32) that these compounds might play a similar role in enzymatic transamination—one of

my luckier prognostications. Full accounts of these and subsequent developments have appeared elsewhere (35).

SYNTHESIS OF D-ALANINE AND L-AMINO ACIDS A puzzling observation made during this period was that when large amounts of DL-alanine were added to the growth medium, *S. faecalis* grew without added vitamin B6. *L. casei* did not grow under these conditions, but if DL-alanine plus a vitamin B6–free enzymatic digest of casein were added, good growth ensued. To my amazement, D-alanine proved to be the growth-promoting agent under these conditions (36). Although D-glutamate was then known to be present in some bacterial capsules, there was no precedent for any D-amino acid being essential for growth of any organism. Our initial suggestion that D-alanine might act as a precursor for vitamin B6 synthesis proved to be quite wrong. Following my return to Wisconsin, my student, Joseph Holden, showed that cells grown with D-alanine and no vitamin B6 were devoid of the vitamin, whereas cells grown with vitamin B6 but no D-alanine contained D-alanine (37). We concluded that D-alanine was an essential cellular component, and that in these media, where all of the L-amino acids were supplied as hydrolyzed casein, synthesis of D-alanine was the major growth-limiting reaction for which vitamin B6 was required. This conclusion was nicely corroborated by Wood & Gunsalus, who independently showed about this time that bacterial alanine racemase was a vitamin B6–dependent enzyme (38). In 1954 during the tenure of a Guggenheim fellowship that I spent with Professor E. F. Gale in Cambridge, England, M. R. J. Salton showed me how to prepare cell walls of *L. casei*. These walls contained most of the cellular D-alanine (39); little or none was present in cellular protein. Thus was the importance of D-alanine for cell wall formation in bacteria first established.

These observations left the role of the enzymatic casein digest in the response of *L. casei* to D-alanine unexplained. Later, during his thesis work in Madison, Hayato Kihara fractionated the digest and showed that peptides of L-alanine were responsible for its activity (40). It turned out that in the absence of vitamin B6, L-alanine had to be supplied for growth, but its uptake was inhibited by D-alanine while uptake of L-alanine peptides was not affected. Many of the essential amino acids could be replaced by the corresponding ketoacids if vitamin B6 was present, but not if the vitamin was omitted (41). The growth test thus indicated that vitamin B6 was required for synthesis of each of the L-amino acids and opened the possibility, never sufficiently studied, of comparing the behavior of enzymes such as the transaminases in cell lysates with their behavior in growing cells under conditions where their activity limits growth (42).

WISCONSIN 2: 1945-1951

In the spring of 1945 Professor Elvehjem wrote to ask if I would be interested in returning to Madison as a faculty member. I felt highly complimented but also troubled. Texas had been good to me; I had been promoted to Associate Professor in 1943, and Professor Williams had provided an ideal work environment: congenial colleagues, good laboratory space, and essentially complete research independence with continued support through the Clayton Foundation. The chief drawbacks were the paucity of well-prepared graduate students and the long hot summers—neither air-conditioning nor fans were available during the war years. The weather finally decided the matter; I developed prickly heat and opted for Madison.

Teaching and Research

In Madison I taught the general biochemistry course for undergraduate majors and beginning graduate students. This course, my favorite, requires one to keep reasonably current in a large area without worrying about the intricacies that sometimes burden more specialized courses.

Wisconsin attracted highly qualified students, and I was fortunate to have several: Jesse Rabinowitz, David Metzler, Gene Brown, Harry Broquist, Robert MacLeod, and Hayato Kihara to name only a few. Their support fell to the professor in charge, so I was rapidly introduced to my least favorite activity, applying for research support. Support came from the Wisconsin Alumni Research Foundation, the G.I. bill (for returning veterans), and especially the National Institutes of Health, which funded most of my research without interruption from 1946 to my retirement in 1990. Widespread generous federal support permitted an unparalleled expansion in the biological sciences during this period. Unfortunately, some of my younger colleagues are learning only now a basic fact in biology: that in a closed system all growth curves must eventually plateau.

Research now took on a different aspect. To this point, I had been a laboratory participant—sometimes the only one—in almost all problems. Now, more and more, laboratory research became a student activity while I became more of a wandering office fixture, participating in research by remote control. Each student needed a separate thesis problem; the number of topics under study increased with the number of students. I discuss below only those results that appear most significant in retrospect.

PYRIDOXAMINE PHOSPHATE My first Wisconsin student, Jesse Rabinowitz, improved our assay for pyridoxal and pyridoxamine with *S. faecalis* and further developed the differential assay for all three forms of vitamin B6. In

the course of this work, he discovered the natural occurrence of pyridoxamine phosphate (PMP) as a major combined form of vitamin B6 that required hydrolysis prior to assay of the vitamin (43). Walter McNutt then showed that PMP was itself an essential growth factor for several lactobacilli, even in media that contained free pyridoxal or pyridoxamine (44), suggesting that these organisms were unable to phosphorylate free vitamin B6. We found later that one such organism was, in fact, extremely low in pyridoxal kinase (45).

PANTETHEINE-PANTETHINE A number of unexpected requirements appeared when we extended our investigations to bacteria from milk. Several of these organisms required oleic acid, deoxyribosides, or both for growth. One culture, *Lactobacillus bulgaricus,* grew in our "complete" medium only when whey was added. A visitor from Denmark, E. Hoff-Jorgensen, found that the organism required lactose (or other β-galactosides) as an energy source; neither glucose nor galactose was fermented readily. However, lactose alone was inactive in the absence of another unidentified factor, which we called the *L. bulgaricus* factor or LBF (46). LBF occurred in several chromatographically different forms, one of which we purified about 3000-fold to near homogeneity (47). The key finding that LBF could be replaced by very large amounts of pantothenate (48) led us to suspect that LBF might be a combined form of this vitamin. Coenzyme A, the only combined form of pantothenate then known, was inactive in our bacterial assay, but showed very high activity after digestion with intestinal phosphatase. The structure of this pantothenate-containing fragment of CoA was then unknown. Our best LBF concentrates were neutral (i.e. no free amino or carboxyl groups were present), contained sulfur, and on acid hydrolysis gave rise to a ninhydrin-positive substance that chromatographed with the sulfur compound in the hydrolysate. I speculated that this compound could be β-mercaptoethylamine (cysteamine) or its disulfide, cystamine, decarboxylation products of cysteine not then known to occur in nature, and prevailed upon E. L. Wittle at Parke, Davis and Co. to synthesize the amides of these compounds with pantothenic acid. These compounds were highly active; the diamide was chromatographically identical with one form of LBF. We named the disulfide *pantethine,* and the reduced thiol *pantetheine* (49a,b). The previously detected multiple forms of LBF appear to be mixed disulfides formed between pantetheine and various naturally occurring thiols; pantetheine itself was the hitherto unidentified fragment of CoA that contains the catalytically important sulfhydryl group discovered by Lynen during his studies of "active acetate" (50).

POLYAMINES An unusual requirement for growth of *Hemophilus parainfluenzae* on a peptone medium, NAD, had been identified by Lwoff &

Lwoff (51). As his thesis problem, E. J. Herbst set out to define other nutritional requirements of this organism. No growth was obtained in a "synthetic" medium complete with respect to known vitamins and amino acids, but good growth occurred on addition of yeast extract. The active substance proved to be a strongly basic, acid-stable compound that was volatile with steam. Off-the-shelf trials of naturally occurring amines with these properties showed that putrescine was highly active; putrescine was isolated and characterized from one good source, orange juice (52). The related compounds, spermine and spermidine, also were highly active. This was the first demonstration that these long-known and widely distributed polyamines play an essential role in living organisms. Other organisms have since been shown to require them as well, and several possible functional roles for them have been described (53), but the nature of their essential function in vivo is still unclear.

Return to Texas

The years in Madison were happy ones. After reconciling herself to the fact that the lakes actually froze over during the winter, Mary learned to skate with our two boys; however, after Texas the winters seemed interminable. Professionally things were going well. I had been promoted to Professor of Biochemistry in 1947 and research was progressing satisfactorily. However, I was dissatisfied with my research space, which was split between the Steenbock and Phillips laboratories and was not under my jurisdiction. In the meantime, a new and fully air-conditioned Experimental Science building was being completed in Austin, and I had been invited back as Professor of Chemistry with a part-time appointment in the Biochemical Institute. Following a minor (in retrospect) altercation with Professor Steenbock I decided to return to Austin, where I became the first occupant of excellent laboratory space in the Biochemistry wing of the new building. Hayato Kihara, who had only to write his thesis, volunteered to leave for Texas early; thanks to him the laboratory was almost operational upon our arrival. Several other students who had finished their course and residency requirements in Madison elected to finish their thesis research in Austin. To simplify granting of their degrees from Wisconsin, I remained on leave there until the end of the academic year in 1953.

TEXAS 2: 1951–1956

With the advent of air-conditioning, summer weather was now inconsequential; it may even have increased laboratory occupancy. In any case, research rapidly resumed where it had ended in Madison.

Peptides and Bacterial Growth

Most of our test organisms grew more heavily when media containing acid hydrolysates of protein or "complete" mixtures of amino acids were supplemented with enzymatic protein digests. Hayato Kihara, J. Mack Prescott, and Vincent Peters studied several instances of this behavior. All fell into three categories: (*a*) uptake of one amino acid was inhibited by presence of an antagonistic amino acid (40, 54); (*b*) for undetermined reasons, an organism lacked an efficient uptake mechanism for a given amino acid (55); or (*c*) the free amino acid underwent partial enzymatic destruction—only the portion escaping destruction was available for growth (56). In cases (*a*) and (*b*) presentation of the amino acid as appropriate peptides allowed its uptake by independent mechanisms not affected by the same inhibitors; in case (*c*) peptides were insusceptible to attack by the destructive enzymes. In all instances examined, the effective peptides were hydrolyzed by intracellular enzymes; in no case was any evidence found for their direct utilization.

Where uptake of several different essential amino acids is slowed by multiple amino acid imbalances in the growth medium the peptide requirement can appear surprisingly specific and its identification becomes difficult. "Strepogenin," about which a considerable literature had accumulated by 1950, is a case in point (57). Although single peptides that supplied the several limiting amino acids were present in protein digests, the multiple deficiencies were most easily remedied by a mixture of smaller peptides. I have always wondered whether peptide "requirements" of this nature represent the beginnings of the apparently more specific requirements for peptides exhibited by some animal cell cultures.

With clarification of the nature of growth stimulation of lactic acid bacteria by peptides, the nutritional problems presented by these organisms were largely solved. Other groups had shown that some of them require thiamine, vitamin B12, deoxyribosides, or mevalonic acid. The thesis problem assigned to me in 1935 had required 20 years for reasonably complete solution; the entire array of B-vitamins, which coincident work had shown animals to require also, was required by one or another of these bacteria. By supplying alternative assay organisms, the bacterial work had greatly speeded isolation and identification of many of these vitamins and their determination in foodstuffs, and had resulted in discovery of additional forms of several vitamins and other biofactors. The focus of my research now shifted to the metabolism of vitamin B6 and its functional roles in metabolism.

Pyridoxal-Catalyzed Reactions of Amino Acids

In extending our earlier studies of non-enzymatic transamination between pyridoxal and amino acids, David Metzler showed that transamination

(reaction 4) took place with most amino acids, was greatly accelerated by ions of copper, iron, or aluminum, and was fully reversible (58). With serine, α,β-elimination (reaction 5) occurred, while with threonine two competing reactions, 6 and 7, were observed (59). Another student, Joanne Olivard, showed that optically active amino acids were racemized (reaction 8) by a mechanism that did not require transamination (60). Govind Kalyankar demonstrated that α-aminoisobutyrate was decarboxylated (reactions 9 and 10), proving that, as in enzymatic decarboxylation (61), an α-H on the substrate was not prerequisite to catalysis of these non-enzymatic reactions.

$$RCHNH_2COO^- + PL \leftrightarrow RCOCOO^- + PM \quad 4.$$

$$CH_2OHCHNH_2COO^- \rightarrow CH_3COCOO^- + NH_3 \quad 5.$$

$$CH_3CHOHCHNH_2COO^- \rightarrow CH_3CH_2COCOO^- + NH_3 \quad 6.$$

$$CH_3CHOHCHNH_2COO^- \leftrightarrow CH_3CHO + H_2NCH_2COO^- \quad 7.$$

$$\text{L-Amino acid} \leftrightarrow \text{D-Amino acid} \quad 8.$$

$$(CH_3)_2CNH_2COOH \rightarrow CO_2 + (CH_3)_2CHNH_2 \quad 9.$$

$$(CH_3)_2CNH_2COOH + PL \rightarrow PM + CO_2 + CH_3COCH_3 \quad 10.$$

These studies thus revealed a remarkable correspondence between these non-enzymatic reactions and enzyme-catalyzed reactions dependent upon PLP as coenzyme. Pyridoxal itself could be considered a primitive, nonspecific protoenzyme; apoenzymes simply enhanced quantitatively and lent specificity to catalytic potentialities already present in the coenzyme. We could now test pyridoxal analogues in the non-enzymatic systems to determine the structural features necessary for catalysis. The heterocyclic nitrogen atom, the 3-hydroxy group, and the 4-formyl group of pyridoxal supplied the minimum essentials for catalysis. These findings permitted formulation of the now generally accepted textbook mechanism for catalysis by pyridoxal (62), according to which Schiff's base formation between pyridoxal and the amino acid labilizes directly one or more of the three groups surrounding the α-carbon. Thus reactions 4, 5, 6, and 8 result from release of the α-H as a proton, with variable disposition of the extra electron pair left in the complex; reaction 7 results from release of the R group, and reaction 9 from release of the carboxyl group. Reaction 10, like 9, requires release of the carboxyl group, but the extra electron pair localizes as in reaction 4 (61). A number of other reactions falling within these three classes also were studied.

Because of wartime disruptions of communications, we were unaware until later that Braunstein & Shemyakin (63) had published a mechanism for the action of PLP enzymes very similar to our own. The two proposals differed chiefly in our emphasis on direct, as opposed to indirect, labilization of groups about the α-carbon atom. I had supplied Professor Braunstein with pyridoxal immediately after the war, but we first met in Japan in 1958. Despite fairly frequent subsequent meetings at symposia on vitamin B6 enzymes, communication between us was never easy, I believe because of his deafness and what I'm told is my own abnormally quiet speaking voice.

The facile deamination of serine (reaction 5) in these systems suggested that the corresponding enzymatic reaction might be PLP dependent. Dave Metzler found that a serine deaminase from *Escherichia coli* was indeed a PLP enzyme, and was specific for D-serine (64). Neither non-enzymatic nor enzymatic labilization of the R group in reaction 7 requires an α-H atom: α-methylserine yielded alanine and formaldehyde when heated with pyridoxal and metal ions (65), while bacterial degradation of this amino acid is catalyzed by a PLP-dependent hydroxymethyl-transferase to yield D-alanine and methylene tetrahydrofolate (66).

CALIFORNIA: 1956–1976

A family joke had it that since Mary and I could not agree on the relative merits of North vs South we should move to the neutral West. This became a serious consideration when I was asked to become chairman of the Department of Biochemistry at the University of California at Berkeley. Although I'd heard rumors of unrest there, these did not reverberate loudly from 2000 miles away and the position seemed too attractive to decline. My two older sons (12 and 13 at the time) were against leaving Austin, but they were easily won over by a promise that we would learn to ski. (As a result, at age 42, I became an enthusiastic, if uninspired, skier for the next 33 years.) The family and my entire laboratory—four postdoctoral fellows and one graduate student—moved to Berkeley in the summer of 1956, where I met my retired predecessor as Chairman, Dr. H. O. L. Fischer, for the first time. In addition to being a distinguished biochemist, Fischer was one of the most genuine gentlemen I have ever met. However, I soon learned that the rumors of discord had substance and, not remarkably, revolved around faculty appointments, teaching loads, and space assignments. During several interesting but sometimes unpleasant years the problems were mostly resolved by (*a*) splitting the Department of Biochemistry completely from Professor Stanley's Virus Laboratory, with which it was thoroughly entwined, (*b*) absorbing into Biochemistry several outstanding members (Professors W. Z. Hassid, H. A. Barker, David Hackett) of the Plant Biochemistry Department

when that department moved to Davis, and (*c*) obtaining approval from the university administration (and some matching funds from the National Institutes of Health) for construction of a new biochemistry building, thus permitting expansion of both Biochemistry and the Virus Laboratory. I believe biochemistry on the Berkeley campus was strengthened by these moves. They provide in my mind a good example of how—given sustained effort, a certain amount of patience, a sympathetic administration, and money—all parties can benefit from conflict resolution.

It seemed almost pleasant to resume the normal routines of departmental administration, teaching, and research. However, whatever yen I had for administration was now quenched. A sabbatical leave after six years of service provided the opportunity to rotate the chairmanship, in this case to Professor Barker. We spent an enjoyable year in Feodor Lynen's laboratory in Munich, where I purified a decarboxylase involved in degradation of pyridoxine (67). One of my objectives—to learn spoken German—was largely foiled; Americans (Harland Wood, Richard Himes, Daniel Lane, Walter Bortz, among others) outnumbered Germans in the laboratory. Upon my return to Berkeley the next year, the new building was finished.

Research in California

Like Wisconsin, California attracted many outstanding graduate students and postdoctorates. Laboratory size was limited only by one's laboratory space, grant support, and personal inclinations. This abundance of students reflected not only the distinction of the faculty, but also the attractiveness of the physical environment.

As an outgrowth of previous activities, I began studies of the metabolism of pantothenate and vitamin B6, and the characterization of a number of enzymes of special interest. Only a few highlights are discussed here.

PANTOTHENATE DEGRADATION AND SYNTHESIS A soil pseudomonad that grew well on pantothenate as a sole source of carbon and nitrogen was isolated by Charles Goodhue. By use of resting cells and partially purified enzymes we established that pantothenate was degraded to α-ketoisovalerate via reactions shown by the broad arrows in Figure 1 (68). The degradative path shown for β-alanine had been established earlier by Hayaishi's group (69). Intermediates in the biosynthesis of pantothenate, shown by the narrow arrows of Figure 1, also had been established earlier but enzymes involved had not been studied. Joel Teller and Susan Powers showed that the first committed reaction was catalyzed by a tetrahydrofolate-dependent hydroxymethyltransferase (70). Synthesis and degradation of this vitamin thus follow completely different pathways.

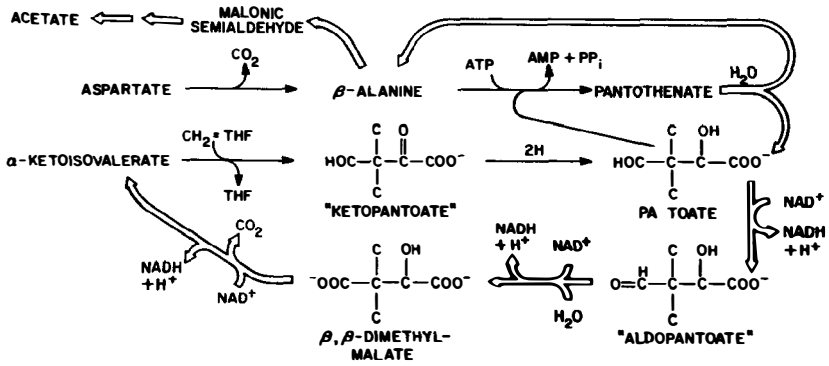

Figure 1 Degradation (broad arrows) and biosynthesis (narrow arrows) of pantothenate.

VITAMIN B6 DEGRADATION For some time we attempted, without any luck, to gain some insight into the biosynthesis of vitamin B6. When Dempsey (71) and Hill & Spenser (72) reported some success in this area, we abandoned these efforts, but continued our more rewarding studies of vitamin B6 degradation. These studies, begun in 1956, continued at irregular intervals until 1986. Two similar but entirely distinct pathways were found; intermediates involved and the enzymatic steps by which they are formed are shown in Figure 2. Pathway I was present in a pseudomonad isolated with pyridoxamine as carbon-nitrogen source, and is presumably followed for degradation of pyridoxal (an intermediate in the pathway) as well. Pathway II was found both in a pseudomonad and an *Arthrobacter* sp. isolated with pyridoxine as the carbon source (73). I had hoped to extend current understanding of the induction of long degradative pathways, now based mostly on studies of the β-ketoadipate pathway for degradation of homocyclic aromatic compounds (74), to heterocyclic compounds by studying induction of the separate enzymatic steps of Figure 2, but time ran out.

TRANSAMINASES Following the International Symposium on Enzymes, held in Tokyo and Kyoto in 1958, Dr. Hiroshi Wada came to my laboratory from Osaka University. This fortunate event initiated a series of visits by highly qualified Japanese fellows that extended over many years, and contributed greatly to my research program. At that time the validity for enzymatic transamination of the "shuttle" mechanism proposed for non-enzymatic transamination (reactions 1–3) was still being questioned. In support of this mechanism, Wada showed that glutamate-aspartate apotransaminase catalyzed reactions 1 and 2, thus demonstrating directly the two postulated half reactions of enzymatic transamination, but with PM and PL serving as loosely bound replacements for PMP and PLP (75). He then discovered and crystallized

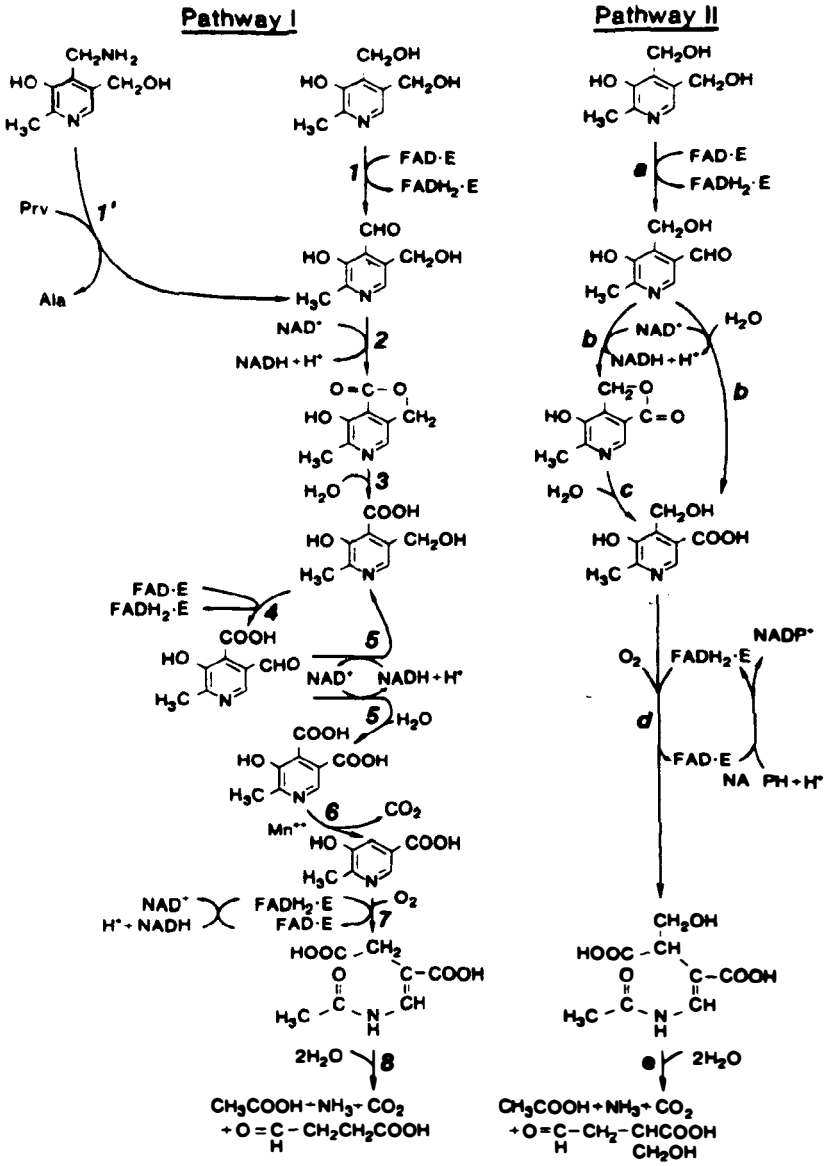

Figure 2 Enzymatic steps in degradation of vitamin B6 by *Pseudomonas* MA-1 (pathway I) and by *Arthrobacter* Cr-7 (pathway II). Reproduced with permission from *The Journal of Biological Chemistry* (73).

pyridoxamine-pyruvate transaminase (enzyme 1′ of Figure 2); its efficiency in catalyzing this reversible reaction convinced me that the phosphate group of PLP, although highly important for coenzyme-apoenzyme interaction, did not itself contribute to catalysis of transamination. In contrast, Helmreich's group has shown that the phosphate group is catalytically involved in phosphorylase action (76).

TRYPTOPHANASE According to our proposed mechanism for pyridoxal catalysis both β-elimination and addition reactions of serine should proceed through α-aminoacrylate as an intermediate. To examine this proposition, Henry Browder had begun purification of tryptophanase, which catalyzes reaction 11 (RH = indole), before we left Texas. However, his best preparations also catalyzed reaction 12, indicating possible contamination with tryptophan synthetase.

$$RCH_2CHNH_2COOH + H_2O \rightarrow RH + CH_3COCOOH + NH_3 \qquad 11.$$

$$\text{Indole} + \text{Serine} \rightarrow \text{Tryptophan} + H_2O \qquad 12.$$

To eliminate this possibility, we obtained from Charles Yanofsky a culture of *E. coli* B/1t7 carrying a deletion for the synthetase. Tryptophanase purified to crystallinity from this source by Austin Newton still catalyzed reaction 12; indeed, a mutant of *E. coli* B/1t7 in which tryptophanase was constitutive grew well without tryptophan if indole was supplied. Thus tryptophanase itself catalyzed reaction 12. It also catalyzed many other β-elimination reactions, e.g. where R in equation 11 was -OH, -SH, -OR′, -SR′, etc. When indole was present tryptophan was formed from each of these substrates as well (77). Yoshimasa Morino's kinetic analysis indicated that each of these reactions proceeded through a common intermediate, which could only be enzyme-bound α-aminoacrylate (78). Labilization of the α-H occurred in all instances independently of elimination of the R group. The postulated mechanism seemed thus to fit the experimental findings at all points. We had assumed that hydrolysis of the aminoacrylate intermediate to pyruvate and ammonia would be irreversible; however, Takehiko Watanabe found that reaction 11 was fully reversible when pyruvate and NH_3 were added in sufficient amount (79). Because of the variety of reactions catalyzed by this enzyme, and the wealth of information concerning its mechanism of action, several groups, including ours, have attempted to determine its three-dimensional structure. To date, suitable crystals have not been obtained. Fortunately my former student, Edith Wilson Miles, and her colleagues have crystallized and determined the structure of a related enzyme, tryptophan synthetase, whose

b-subunit catalyzes many of the same reactions as tryptophanase, and are successfully correlating its structure with its mechanism of action (80).

PYRUVOYL ENZYMES: HISTIDINE DECARBOXYLASE Of five inducible amino acid decarboxylases studied by Gale in crude bacterial extracts, three required an unidentified coenzyme later identified by I. C. Gunsalus as PLP, while two, glutamate decarboxylase (GluDCase) and histidine decarboxylase (HisDCase) appeared not to require this coenzyme. Shukuya & Schwert (81) later obtained pure GluDCase from *E. coli*; it was a PLP enzyme. Fully expecting HisDCase also to require PLP, despite a contrary assertion based on studies of crude preparations by Rodwell (82), we embarked on its purification from *Lactobacillus* 30a. To our surprise, the homogeneous enzyme, although inhibited by carbonyl reagents, neither contained nor required PLP (83). Dixon Riley identified the new prosthetic group as a pyruvoyl residue linked as an amide to the amino terminus of the larger of two dissimilar subunits (84). By borohydride reduction of a mixture of ^{14}C-histidine and enzyme, label was fixed to protein. Following acid hydrolysis, Paul Recsei identified two ^{14}C-compounds, reduced adducts of pyruvate with histamine and histidine, respectively (85), thus demonstrating that the enzyme acts via a Schiff's base mechanism analogous to that for PLP enzymes. HisDCases of two other gram-positive bacteria, *Lactobacillus buchneri* and *Clostridium perfringens,* also were pyruvoyl enzymes. In contrast, homogeneous HisDCases isolated for comparison from three different gram-negative organisms by Beverly Guirard were typical PLP enzymes. This curious occurrence of two types of HisDCase, one in each of two staining classes of bacteria, and each of about equal catalytic efficiency, is not understood. It must reflect some desired property of the individual enzyme rather than the nature of the organism, for the ornithine decarboxylase of the gram-positive *Lactobacillus* 30a is a PLP enzyme, while decarboxylases for adenosylmethionine and phosphatidylserine, both from the gram-negative *E. coli*, are pyruvoyl enzymes. I have reviewed these and subsequent developments recently (86, 87).

Life in Berkeley

The years in Berkeley were the most intellectually stimulating of my life. The University attracted distinguished visitors and speakers from all fields. Proper attention to one's own work required that one ignore many seminars and lectures of considerable interest. Other competitors for limited time included committee work (both local and national), editorial work, skiing weekends in the Sierras, and the considerable attractions—plays, music, restaurants—of San Francisco. During a trip to New York City to attend a committee meeting, my plane (TWA flight 42, Dec. 4, 1965) collided with an Eastern Airlines

plane somewhere over Connecticut. Somehow, minus 30 feet of the left wing, we landed safely at Kennedy airport. The incident led me to question why I was there at all, and also the necessity for many of our nationwide committees. As a result I began to limit my own committee involvements.

Meanwhile, campus agitation over the Vietnam war was increasing. My attitude toward the war was decidedly mixed. I recognized its futility, but with two sons in the armed forces, I also hoped for a successful outcome. In April of 1968 tragedy struck; our oldest son, Esmond E. Jr., was killed from ambush. This shattering event threw both Mary and me into a depressed state which lifted only gradually over a period of years. I took refuge in routine tasks—refining of lectures, writing up research results, editorial work for this publication (I was then its Editor), etc. A series of lectures at City University of New York that fall provided a much needed change of scenery. Normal life eventually resumed, but some of its former zest was gone.

In 1976 family considerations dictated my move back to the University of Texas, this time as chairman of the Department of Microbiology with a joint appointment as Professor of Chemistry. I recognized that many would question my sanity, as they had earlier when I returned to Texas from Wisconsin. Only a few who knew Austin realized that fate really wasn't so unkind.

TEXAS 3: 1976-1990

Since biochemistry is central to the biological sciences and I had used microorganisms as research tools for 40 years, I was not particularly discomfited by my sudden incarnation as a microbiologist, especially since I would teach only biochemistry courses in the Chemistry or Microbiology departments. However, I was not happy with my tenure as chairman. Vacancies in faculty ranks needed filling. Good scientists were in high demand, and the Texas system was such that by the time we could offer a position the best candidates were no longer available. Attempts to initiate a molecular biology program were vitiated by disagreement among the various departments and by administrative indifference. After four years, upon reaching administrative retirement age, I was pleased to return full time to research and teaching. The last three summers of my term as chairman I had spent as a Humboldt Senior Scientist with Ernst Helmreich in Wuerzburg or with Helmut Holzer in Freiburg. These stays were personally rewarding, but too short to permit much research.

Research Activities

Much of the work on enzymatic degradation of vitamin B6, discussed earlier (see Figure 2), was conducted by students (Ivy Jong, Yu-Chen Lee, M. S. Huynh, Michael Nelson) and postdoctoral fellows (Ganesh Kishore, T. Yagi)

in Texas. My principal interest, however, was in clarification of the relationship between the pyruvoyl-dependent and the PLP-dependent histidine decarboxylases, and the origin of the pyruvoyl residue in the former enzyme. Since these topics have been reviewed recently (86, 87), only a brief outline is given here.

PROHISTIDINE DECARBOXYLASE AND ITS ACTIVATION The fact that the pyruvoyl residue of HisDCase from *Lactobacillus* 30a arose from serine (84) implied existence of an inactive proenzyme. This proenzyme (proHisDCase) was first detected by Paul Recsei in a mutant of *Lactobacillus* 30a where its activation was slow (86). Unlike HisDCase, which contains pyruvate and two dissimilar subunits, proHisDCase contains only one type of subunit (π) and no pyruvate; activation proceeds spontaneously near neutral pH. Through the efforts of Paul Recsei, Haruo Misono, and Paul van Poelje we found that activation occurs by a unique intramolecular, nonhydrolytic, monovalent cation-dependent, serinolysis reaction (Figure 3), with simultaneous formation of the pyruvoyl group, NH_3, and the α and β chains of HisDCase. The reaction involves an interesting transfer of oxygen from the hydroxyl group of one serine residue of the π chain to the carboxy terminus of the newly formed β chain (86, 87).

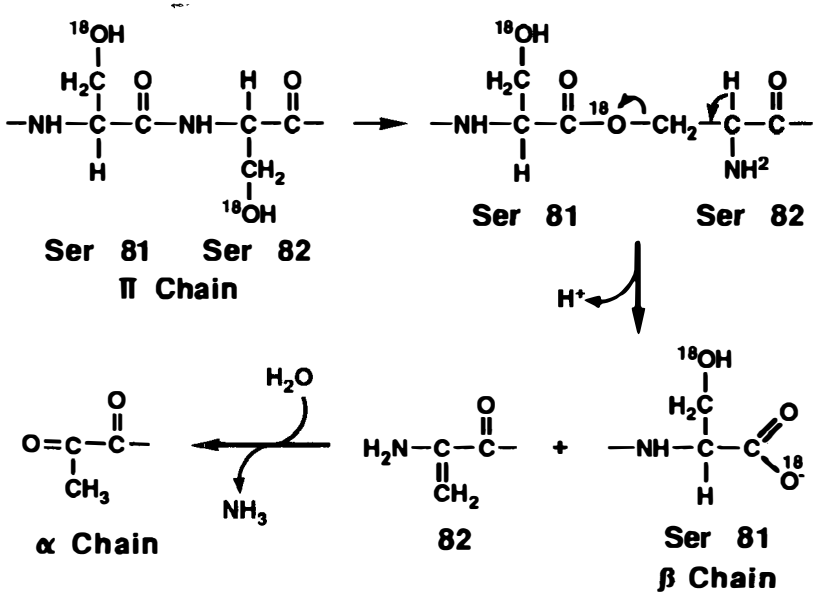

Figure 3 Reaction pathway in conversion of a single π chain of prohistidine decarboxylase, π_6, to the α and β chains of pyruvoyl-dependent histidine decarboxylase, $(\alpha\beta)_6$

SEQUENCE AND STRUCTURE OF HISTIDINE DECARBOXYLASES Paul Recsei and George Chang had crystallized the pyruvate-dependent HisDCase, and my colleague, Marvin Hackert, thought the crystals might be suitable for X-ray studies. While he and his coworkers began crystallographic work, we (Q. K. Huynh, Gloria Vaaler, Paul Recsei) began sequencing the enzyme by peptide sequencing techniques. I was rapidly approaching retirement age, and was pleased when another colleague, Jon Robertus, expressed interest in sequencing the gene for this enzyme preliminary to a study of related problems. The happy results of these efforts was a complete sequence for HisDCase and proHisDCase from both *Lactobacillus* 30a (88) and *C. perfringens* (89) and a crystallographic structure for the former enzyme that permitted identification of its active site and a more complete analysis of its mechanism of action (90). The postulated roles of various amino acid residues in substrate binding and catalysis of decarboxylation are consistent with results of site-specific amino acid replacements obtained by both the Robertus group (91) and my own (92).

I found these results sufficiently exciting to postpone retirement for a few more years. I remembered Professor Peterson from my student days saying "We don't know the structure of any protein and we never will." Fifty years later we were determining such structures ourselves, results none the less satisfying for being obtained by procedures that had become almost routine. Similar studies of the PLP-dependent HisDCases from *Morganella morganii, Klebsiella planticola,* and *Enterobacter aerogenes* showed that their amino acid sequences were from 80 to 85% identical, but showed no homology with the pyruvoyl-dependent HisDCases (93). While the pyruvoyl enzymes appeared to depend on a critical glutamate residue as a proton donor for decarboxylation, the PLP enzymes used a lysine residue for both this function and for displacing newly formed histamine from the enzyme (94). Unfortunately, we have not yet obtained crystals of the PLP enzymes suitable for X-ray work.

Winding Down

In accordance with my belief that one should retire before colleagues think it advisable, I retired completely in 1990. Bacterial nutrition had proved a rewarding research topic. Its study had led to discovery of several new biofactors of importance to both bacteria and animals, and had provided methods for their determination. It had also provided clues to the metabolic functions and modes of action of these new biofactors, which in turn had led to corroborative studies of PLP enzymes and to discovery of an additional class of carbonyl enzymes, the pyruvoyl enzymes. Equally satisfying had been the interactions such studies provided with students and colleagues the world over, and the appreciation of different viewpoints that these interactions bring.

Paul Boyer remarked someplace that he hadn't expected biochemistry to provide a means to see the world, but that is how it turned out. I concur. International Congresses, symposia, research leaves, sometimes associated with short vacations, have taken us to five continents; the other two we've added on our own. Retirement comes at a time when methodology permits productive pursuit of almost any imaginable problem. Although one regrets withdrawing from research at such a time, hitherto neglected aspects of life also are attractive. I anticipate no difficulty in filling the remaining years with rewarding activities, while continuing to keep an appreciative eye on developments in biochemistry.

ACKNOWLEDGMENTS

Over the 52 years of my scientific life about 100 individuals have contributed to projects in my laboratory. Only a few of them have been mentioned in the text, but they all have my thanks and gratitude. A few longer-term associates have been especially helpful in assuring continuity of projects and in maintaining a workable laboratory. Dr. Hayato Kihara filled this function during early years; Dr. Beverly Guirard has done so for most of the past 40 years. They both deserve my very special thanks.

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