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The functions of vitamin B_6: a beginning

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It was possible for a poor farm boy with no money to obtain a first-class education in the depths of the depression because New York State Supported Colleges of Cornell University were tuition-free to state residents. (Today in the richest country in the world, this is not possible.) If he were fortunate enough to have Professor I.C. Gunsalus, "Gunny," as his faculty advisor, he would take more courses in mathematics, chemistry, physics, etc., than most agricultural students. Several of my classmates who had Gunny as an advisor went on to distinguished careers in medicine, academic teaching and research, and industry.

The National Youth Administration paid 25 cents per hour for a maximum of 15 dollars per month. For the first 2 years I cleaned pens in the beef barn and groomed steers. My upper class years were spent correcting papers and setting up laboratories for the beginning bacteriology course. Waiting table at the fraternities and stoking a furnace provided food and some money.

After graduating in 1938, I attended the University of California, Davis, recently transferred from Berkeley, and then spent the next $2\frac{1}{2}$ years in a TB hospital in Oneonta, New York. Dr. Sherman and Gunny were kind enough to invite me back to Cornell as a graduate teaching assistant on my release, for which I received a my small stipend. In addition, a few graduate students were invited to live in the basement of Stocking Hall, the home of the Bacteriology Department, in exchange for monitoring the ammonia compressors used for refrigeration and the 25-year-old piping throughout the building.

The next $3\frac{1}{2}$ years were the most exciting and educational a young man could hope for. Gunny was always ready with advice, not always patient, but always searching for answers. His quick mind amazed me because his answers frequently came before I had finished the question. Interactions with other faculty members, particularly Professors Knaysi and Umbreit, and with other graduate students and assisting in laboratory teaching added to the learning process. Before a practical glass electrode was readily available, graduate students spent the week before classes opened assisting Gunny and Professor Stark in preparing buffers for students to determine pH with indicators in comparator blocks.

To be a graduate student at the right place and at the right time with the right advisor is fortunate indeed. When Gunny and UBC students Alex Wood and then Jack Campbell were exploiting growth for function, not maximal growth [1,2], Ernest F. Gale was showing a tyrosine decarboxylase in *Streptococcus faecalis* [3]. It was natural in the Bacteriology Department of James M. Sherman, the guru of streptococci [4], for Gunny and a graduate student to try to learn more about the requirements for this function.

The first problem was to find the conditions for maximum production of the decarboxylase and then those for growth with diminished activity. In a rich medium [5] complete with a tyrosine content, activity was increased near a limiting pH ~5. Using *S. faecalis*, strain 10C1, we developed a medium, synthetic except for acid-hydrolyzed, charcoal-treated gelatin. Requirement was found in a minus one protocol for added nicotinic acid and pyridoxine [5]. Cells grew well in the medium lacking pyridoxine but were devoid of tyrosine decarboxylase activity. Pyridoxine failed to restore activity unless autoclaved with cystine or otherwise treated [6].

In the early1940s, many young aspiring microbiologists having expertise with a variety of organisms interacted and exchanged data freely with their peers in microbiology and biochemistry. With the privilege of knowing Esmond Snell, then in far-off Texas and with

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travel restricted, we mailed roughs of prepared figures to him; the return mail brought two vials, two scratches and four, with the notation that the data would appear the next month in JBC Notes; please restrict circulation until then [7]. These were pyridoxal and pyridoxamine, the structures of "pseudopyridoxine" [8]. Pyridoxal reactivated apo-decarboxylase in cell suspensions prepared from the minimal medium. The data appeared in JBC Notes[1] [9] the month following the Snell report.

The dissociation constant for the pyridoxal-coenzyme–tyrosine decarboxylase could be approximated from the activity with increasing pyridoxal concentrations in cell suspensions [9]. A similar value was obtained from activity with increasing pyridoxal concentrations of cells grown in the synthetic medium [10]. Drying the cells in vacuo over Drierite produced a fluffy powder containing apo-tyrosine decarboxylase [10]. The activity was stable for long periods if the suspensions were kept over Drierite and provided a convenient source for coenzyme assay, further studies of enzyme function, reaction mechanism, etc. These "lyophilized" cells did not respond to pyridoxal unless ATP was also present [10]. Pyridoxal treated at room temperature with pyro-phosphoric acid produced an active cofactor and some of its properties were described [11].

Later we determined that cells of *S. faecalis* strain R grown in a pyridoxal-deficient medium were stimulated maximally in tyrosine decarboxylase activity only in the presence of both pyridoxal and ATP [10], rather than pyridoxal alone, as with *S. faecalis* strain 10C1 [6]. The strain R cells from a synthetic medium were free of the pyridoxal phosphate coenzyme [10,11], and when a cell-free extract was prepared, the decarboxylase was fully activated by the synthetic coenzyme [12]. The R strain with holo-enzyme from cells grown in a complete medium, prepared as an extract, could be resolved from the cofactor and reactivated on its addition [12].

All the members of the vitamin B_6 complex were shown to be convertible to co-decarboxylase [13]. With cell suspensions prepared from B_6-free media, pyridoxamine was found to replace pyridoxal in the presence of pyruvate—suggesting the formation of alanine by transamination, revealing a cofactor role for pyridoxamine phosphate [13]. Synthetic pyridoxal phosphate purified as the barium salt was made generally available, and spectral and other properties were reported [14].

Many studies of the roles of vitamin B_6 in the catalysis of amino acid reactions followed in the Cornell Laboratory and elsewhere. Soon more decarboxylases, lyases, desaminases, and multiple amino acid transaminases were reported. The cofactor was shown to be the 5-phosphoester, and soon the reaction mechanisms emerged. In this issue, Avery Wood, a principal contributor [15], documents continuing advances, among many additional reports from elsewhere.

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[1] JBC, *Journal of Biological Chemistry*. Notes received at JBC by 28th of the month appeared in the next issue. Notes were discontinued in May 1949, Volume **170**, a primary stimulus to start the BBRC. Accelerated publication of significant initiatives in JBC was restarted September 15, 1959, and continued with name changes in 1966 and 2000.