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Biochemical and Biophysical Research Communications 312 (2003) 81–83
BBRC
www.elsevier.com/locate/ybbrc

The summer of '51

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Received 25 September 2003

Brookhaven National Laboratory, formerly Camp Upton, is situated on Long Island roughly at equal distance between New York City and Montauk Point. Upton, a US Army induction center in the great wars of 1917 and 1941, was transferred to the US Atomic Energy Commission (Department of Energy), which in turn, contracted Associated Universities, a consortium of northeastern universities, to establish a laboratory to investigate peaceful applications of atomic energy. To this end, four departments, biology, physics, chemistry, and medicine, were authorized. I won a doctoral degree in the Department of Botany, University of Illinois, in June 1947 and gained employment in the Brookhaven Biology Department 3 months later.

At the first meeting with the departmental chairman, I was informed that one responsibility of biology personnel was to supply ^{14}C-labeled compounds to labs of the associated universities. A number of mammalian physiologists and biochemists had requested radioactive simple sugars. I had not expected assignment to a dedicated project, but that experience, without realization then, set the direction of my career.

In as much as barium carbonate was the sole radioactive compound available and I was the token botanist, photosynthesis in leaves was a practical approach to the production of ^{14}C-labeled sucrose, glucose, and fructose. Plants were grown in a commercial greenhouse about 10 miles from the lab site. My method was included in *Biochemical Preparations* [1]. Gratification ensued when the procedure was checked in the Urbana lab of a former biochemistry instructor, Herbert E. Carter.

A supplier of gratis ^{14}C-tagged compounds was recompensed. Samples were exchanged for glucoses chemically synthesized with label in C-1 (aldehyde), C-2, or C-6. Sugars isolated after lengthy periods of photosynthesis were uniformly labeled. On the other hand, feeding of $^{14}CO_2$ in the dark to leaves resulted in [3,4-^{14}C]glucose.

The biosynthetic project behind, it became clear that research options had to be evaluated. My preference was metabolic events in higher plants, but an on-site greenhouse would not be erected for at least 3 to 5 years. I undertook a literature survey (New York City public library) in order to conclude a doable approach making use of my labeled sugars. Few publications [2–4] notwithstanding, it became evident that isotopic distribution within a compound resulting during metabolism of glucose is valuable evidence for defining the pathway by which the compound is formed. I decided to study the mechanism of lactic acid formation by *Lactobacillus casei*, a homofermentative lactic bacterium using glucose labeled with ^{14}C in various positions. Shortly thereafter, presumably due to publication of our results with *L. casei* [5], my association with Gunny and his students began in the fall of 1950 with the arrival of Ralph DeMoss, my first visitor. Ralph, later chair of the Department of Microbiology, University of Illinois, with Gunny and another graduate student, Raymond C. Bard, concluded from fermentation balances (CO_2, ethanol, and lactate) and enzymatic assays that *Leuconostoc mesenteroides* dissimilated glucose via a pathway differing from the Embden–Meyerhof glycolysis scheme [6].

Ralph's and my personal traits, work habits, and individual technical expertise coordinated well. Under Gunny's mentorship, he had honed microbiological and biochemical methodologies, including preparation of bacterial cell-free extracts and enzymatic procedures. He provided guidance in setting up a laboratory equipped for biochemical investigations with bacteria, algae, fungi, and higher plants. My principal contribution was chemical degradative and radioisotopic procedures.

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doi:10.1016/j.bbrc.2003.09.202

Since Gunny was scheduled to carry out the isotopic distribution pattern of *Leuconostoc* the following summer, Ralph and I undertook an analysis of the fermentative pathway of glucose catabolism by *Pseudomonas lindneri.* Despite a molar stoichiometry of 2 CO_2 and 2 ethanol per glucose, isotopic data indicated a pathway unlike that in yeast. CO_2 in yeast is evolved from C-3 and 4 [4]. In *P. lindneri*, CO_2 arises from C-1 and C-4, while C-2, 3, 5, and 6 of glucose enter ethanol, and carbon atoms 2 and 5 become the carbinol carbons of ethanol [7]. Fructose is fermented by the same pathway [8]. Analysis of the data pointed to a fermentative pathway closely related or possibly identical to the pathway of glucose oxidation by *P. saccharophila* elucidated by Entner and Duodoroff [9].

Gunny, already recognized for his work on bacterial metabolism, arrived the first week of July 1951. Forthwith, he located on-site housing in the "old" officers' quarters, where I had been billeted 4 years earlier. While he dined in the lab cafeteria that evening, we scripted a project with a 60-day limit, including submission of manuscript. Within the week, a daily timetable of 2 PM to 2 AM evolved including weekends, a schedule that tested severely my wife and our marriage of 8 months. The exception was one Saturday afternoon when the bacterial suspension inoculated the previous day did not spew CO_2, an indicator of an inactive culture. A manuscript was submitted by mid-September 1951 [10].

In our first experiment with [1-^{14}C]glucose, we found that resting cell suspensions of *Leuconostoc* ferment glucose by a sequence similar to *P. lindneri* since the aldehyde carbon is quantitatively removed as CO_2. Unlike the pattern in *P. lindneri* we found that the methyl and carbinol carbons of ethanol are derived from carbons 2 and 3 in that order, and the carboxyl, α- and β-carbons of lactate from carbons 4, 5, and 6. We could also show that [2-^{14}C]acetate increases the rate of fermentation and is reduced to ethanol. From the specific radioactivities and quantity of isotope found in the ethanol, it was concluded that the added acetate is not in equilibrium with the acetate formed by the cells or acetate is not an obligatory intermediate in ethanol formulation. Our results indicated that the mechanism whereby glucose is converted to lactate, CO_2, and ethanol involves a new pathway, and that although the classical Embden–Meyerhof scheme may function in part, a considerable digression from this scheme occurs. The sequence has been designated in the literature as the pentose phosphate–transketolase pathway. It includes a portion of the direct oxidation reactions investigated by Warburg, Dickens, Lipmann, Horecker, and S.S. Cohen coupled to additional metabolism of the resulting pentose phosphate based upon the enzymatic studies of Horecker and his colleagues. Of equal importance, for the determination of isotopic carbon patterns in carbohydrates, the *Leuconostoc* method yields individual carbon atoms rather than pairs, a decided advantage over the *L. casei* fermentation [2,5].

The publication of my findings with Gunny resulted in an invitation to collaborate with Bernard Horecker, a co-editor with Gunny on the first editorial board of BBRC, on his in-depth study of the mechanism of conversion of pentose phosphate to hexose monophosphate in extracts of rat liver. In the collaborative effort carried out at the National Institutes of Health, the course of the reaction was followed in a rat liver extract with [1-^{14}C] and [2,3-^{14}C]ribose phosphate. The isotopic distribution pattern in the resulting glucoses determined with *Leuconostoc* contributed evidence to a transketolase–transaldolase series of reactions with sedoheptulose-7-P as intermediate [11].

The summer of 1953 brought Horecker to Brookhaven to repeat the experiments with pea leaf and pea root cell-free preparations [12]. From our isotopic data, we concluded that the conversion of pentose phosphate into hexose monophosphate by root extracts is similar to that described for rat liver extracts. However, the leaf preparations appear to contain an additional mechanism possibly due to the rapid formulation of uniformly labeled carbohydrates in photosynthesis.

Harry Beevers, who later became known for his pioneering work on the glyoxylate cycle, came that summer to investigate respiratory metabolism in plants making use of labeled sugars. So inspired were Harry and Bernie by *Leuconostoc*, they were moved to compose a ditty to the tune of "Clementine."

Leuconostoc in the side arm, glucose in the center well;
Tip it in with phosphate buffer, carbon one comes off like hell.
Through the use of *Leuconostoc*, we have carbons 1 through 6,
Pure and unadulterated; they don't mingle; they don't mix.
Refrain: To the counter, to the counter, to the counter like a shot!
Turn the switch on, see the lights flash! Is it cold or is it hot!
(Additional verses are available on request)

The reaction was carried out in a Warburg respiration apparatus. The lights in an ancient scaler counting in tens did flash.

My publications with Gunny and Bernie brought contact with Severo Ochoa, who with Feodor Lynen in fall of 1953 used the facilities in my lab to prepare radiolabeled sulfate of high specific radioactivity for inclusion into acetyl coenzyme A. Lynen tendered an invitation to present a seminar on bacterial fermentative pathways before his Department of Biochemistry, University of Munich. This was accomplished after my participation in the 1954 International Botanical Congress in Paris. Following the lecture, I was introduced by Lynen to Otto Kandler, a member of the University Botanical Institute. Together with his wife, Gertrude, during an extended stay at Brookhaven in

1955, a program was carried out using the *Leuconostoc* fermentation to determine isotopic distribution patterns in sugars isolated from algae and higher plants that had photoassimilated $^{14}CO_2$ for brief periods. The patterns were asymmetrical [C-4 > C-3 > C-1 = C-2 > C-5 = C-6] in contrast to a symmetrical one predicted by the Calvin–Benson–Bassham photosynthetic carbon reduction cycle [13]. Termed the Gibbs effect and quoted as an argument against the cycle, asymmetrical labeling may result from a lack of isotopic equilibrium between the three-carbon intermediates that combine to yield hexose phosphates or from a quirk during the recycling of carbon through the cycle [see 14] and is apparently not an indicator of modifications.

My final publication with Gunny was a collaborative effort with J.T. Sokatch, a graduate student in his lab. We recognized from earlier reports [5,10] that the homo- and heterofermentative lactic bacteria catabolize glucose by two different schemes. In this joint study, we surveyed other bacteria with similar end products. All five heterofermentative strains eliminated C-1 as CO_2 while the two homofermentative organisms utilized classical glycolysis [15].

In retrospect, the summer of 1951 was a turning point in my fledgling career. My graduate years were spent in a department of plant sciences wherein course selection was limited to one offering in biochemistry, a doctoral dissertation committee of two botanists (physiologist and anatomist), analytical chemist and agronomist, and last thesis research off-campus resulting in little exposure to fellow graduate students in the chemical and biochemical sciences. Nonetheless, when I was employed by Brookhaven, I was determined to pursue intermediary metabolism in plants but I lacked the knowledge fundamental to metabolic events and the practical proficiency for investigation. Gunny and the late Ralph DeMoss were visitors, but a reverse flow of information was set into effect—they became my mentors. I end this article with appreciation for their measured patience and unusual skills.

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