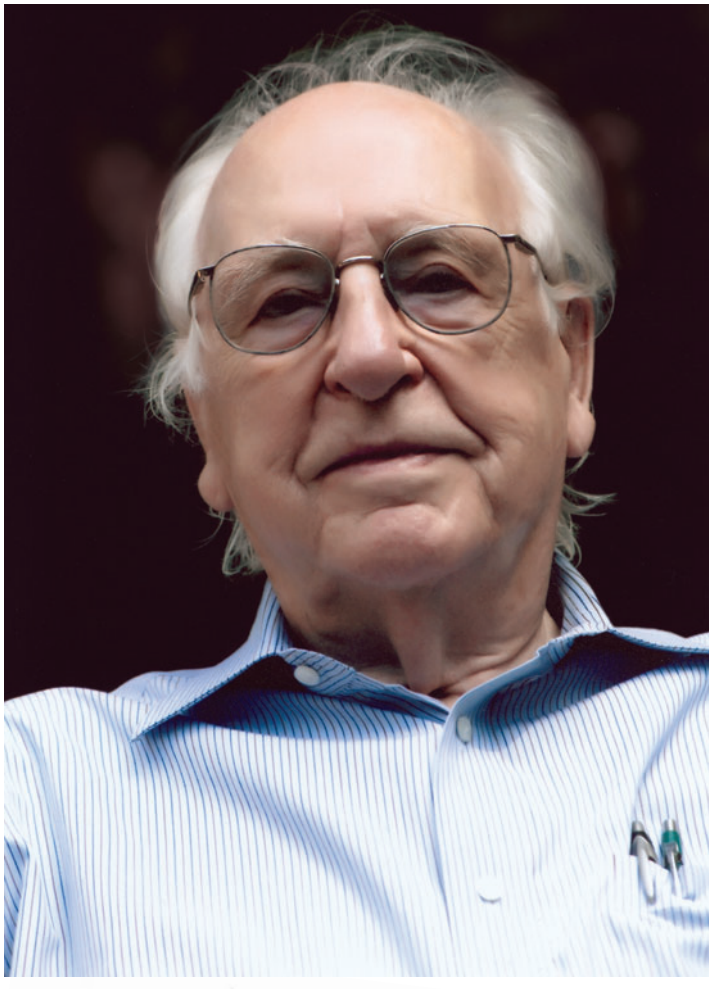


Jan G. Djungdahl

A Life with Acetogens, Thermophiles, and Cellulolytic Anaerobes

Lars G. Ljungdahl

Department of Biochemistry and Molecular Biology, The University of Georgia, Athens, Georgia 30602; email: larsljd@bmb.uga.edu

Annu. Rev. Microbiol. 2009. 63:1–25

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi: 10.1146/annurev.micro.091208.073617

0066-4227/09/1013-0001$20.00

Key Words

CO_2 fixation, acetyl-CoA pathway, corrinoids, tetrahydrofolates, clostridia, anaerobic fungi, cellulosomes

Abstract

Frankly, I was surprised to receive an invitation to write a prefatory chapter for the *Annual Review of Microbiology*. I have read several such chapters written by outstanding researchers, many of whom I know and admire. I did not think I belonged to such a preeminent group. In my view, my contributions to the physiology and biochemistry of anaerobic thermophilic bacteria and, more lately, to anaerobic fungi are modest compared to the contribution made by other authors of prefatory chapters. I am honored to write about my life and my work, and I hope that those who read this chapter will sense how exciting and rewarding they have been.

Contents

PROLOGUE

One does not go through life in a vacuum; you meet people who influence you. I have been fortunate to have met many people who have led me to new opportunities. From my early career in Sweden, first as a technician at the Karolinska Institute, the medical school in Stockholm, and then as a research chemist at the Stockholm Brewery Company, to my graduate studies and work as an assistant professor at Case Western Reserve University, and finally as professor of biochemistry and Georgia Power Distinguished Professor in Biotechnology at The University of Georgia, it is the mentors, colleagues, friends, family, and students who have been vital to whatever success I have had.

Most of my work has centered on the pathway for the total synthesis of acetate from carbon dioxide as it occurs in *Clostridium thermoaceticum* (renamed *Moorella thermoacetica*), a project first suggested to me by Harland G. Wood when I started graduate studies in 1958. He simply said, "There is more to carbon dioxide fixation than the Calvin cycle and you may prove it with *C. thermoaceticum*." Wood was right. Our studies led to the elucidation of the acetyl-CoA pathway, which has now been found in more than 100 bacteria and may be responsible for 20% of the yearly fixation of carbon dioxide on Earth, making it both ecologically and environmentally important. A second project, the degradation of lignocellulose, is for me the most long-standing. It started during my tenure at the Stockholm Brewery Company, and I worked cooperatively with Lennart Enebo of the Royal Institute of Technology in Stockholm. In 1949 he isolated a thermophilic cellulolytic anaerobic bacterium, which he called *Clostridium thermocellulaseum*. We compared cellulases from barley and *C. thermocellulaseum*, both of which actively hydrolyzed barley husks. The long-term idea was to obtain a cellulase that hydrolyzes complex carbohydrates of barley, forming sugars that could be fermented. Similar ideas are now popular, for example, the conversion of cellulose and hemicelluloses into sugars that can be fermented to ethanol, an alternative to gasoline.

FAMILY BACKGROUND

Although my parents were poor as children and did not graduate from grade school, they were enterprising and industrious. They had one rule

for me that served me well throughout life: I could do anything I wanted except things I would be ashamed to tell them about.

Both of my parents came from rather large families and, from an early age, had to help care for and support their siblings. This influenced my mother and her sisters to have just one child to whom they could afford to give their best. As a result, my "siblings" were actually my cousins; one of which, Rune Stjernholm, had a major influence on my life. He encouraged me to study chemistry, to further my education at Case Western Reserve University, and to join Harland Wood's laboratory. Rune had a successful career in the United States. Among other accomplishments, he served for 17 years as Chairman and Head of the Department of Biochemistry at Tulane University in New Orleans. He passed away March 6, 2008.

EDUCATION IN SWEDEN 1939–1945

My parents expected me to follow in their footsteps and become a skilled worker. Higher education was not considered. But, when I reached the sixth grade, my teacher told me to apply to a four-year technical secondary school with emphasis in mathematics, physics, chemistry, and mechanical engineering. This became the springboard to my career in science.

My education in Sweden was bracketed by World War II. The day I was supposed to begin my four years at Stockholm Stads Tekniska Mellanskola (The City of Stockholm's Technical Secondary School, or TM)—September 1, 1939—was also the day that Germany attacked Poland. I graduated from TM in 1943. TM was an excellent school for me and I loved it. Mathematics, physics, chemistry, and mechanical engineering were emphasized, as were the Swedish and English languages. I did well in the sciences but had trouble with English, a problem that is still with me. I speak English with a Swedish accent, and my Swedish now has a definite American/English slant.

After graduating from TM, I started working as a technician at the Karolinska Institute (KI). I also enrolled in evening classes at Stockholm's Technical Institute (STI) to continue my studies toward an engineering degree in chemical technology. Oddly enough, the day I was taking an oral exam for the degree—May 5, 1945—was the day Germany capitulated. During my exam, five of my teachers questioned me on broad aspects of chemistry. During the examination loud noises were coming from the street outside, with people happily and excitedly marching, waving flags, and screaming that the war was over. One of the teachers suggested, "Lars seems to do O.K., let's finish and join the fun outside."

THE DREAM JOB FOR A BUDDING BIOCHEMIST: KAROLINSKA INSTITUTE 1943–1947

Upon my graduation from TM in 1943 I needed a job and luck was on my side. During my early teens I was a member of the Boy Scouts. My Scout Leader, Torsten Thaning, was a physician engaged in research at KI. He knew about my interest in chemistry and arranged for me to meet with Eric Jorpes, a professor of chemistry at KI. I must have had a strong recommendation from T. Thaning, because Jorpes rather quickly offered me a job as a laboratory assistant with a salary of SEK 200 per month (about $40). I started July 1, 1943 and stayed until September 30, 1946, when I entered compulsory service in the Swedish Army for training as military police and demolition expert.

Laboratory Assistant

My first job in science—at age 16—was in the department of medical chemistry at KI, one of the most prestigious medical schools in the world. It was a dream come true. KI was established in 1810, and its departments of medical chemistry and anatomy were located in a beautiful building in the center of Stockholm on the island of Kungsholmen. Later, the Stockholm City Townhall was built next to KI. The Townhall is well known among Nobel Prize winners,

since the dinners celebrating their awards are given every year in its Blue Hall. (Alfred Nobel in 1895 designated KI as the institution to select the winners of the Prize in Physiology and Medicine.)

When I started working at KI, some of the students working toward MD degrees, a degree which in Sweden includes research and a doctoral thesis, were Peter Reichard, Sune Bergström, Pehr Edman, Stig Åqvist, Olof Jalling, Victor Mutt, Harry Boström, Sven Gardell, and Carl-Göran Heden, all of whom have made notable contributions to biochemistry. In fact, Sune Bergström received the Nobel Prize in 1982.

Work in Jorpes' laboratory involved several peptide hormones including insulin, gastrin, renin, angiotensin (also named hypertensin), and secretin. In addition, Jorpes worked with heparin. My first work entailed helping to prepare heparin from liver and other tissues. As I gained experience, Jorpes assigned me to work with Edman on angiotensin.

Working with Pehr Edman: Proteins and Amino Acids

Edman is perhaps the most well-known protein chemist I worked with at KI. He developed the procedure for sequencing peptides and proteins using phenylisothiocyanate, known as the Edman degradation (22). Edman was a straightforward man who rarely showed much enthusiasm, but one day at the end of 1944, he showed up brimming with excitement about a just-published paper by Consden, Gordon, and Martin (11) that described a method for analyzing amino acids using paper chromatography. We used a modified version of their method to analyze angiotensin. The results indicated that angiotensin has 10 different amino acids, including some extra histidine, and that the paper chromatographic system works well with peptides (21). I am convinced that Edman's future interest in protein sequencing was formed during our work characterizing angiotensin. It was Edman who introduced me not only to biochemistry but also to the joy of science. An interesting biography of Edman is available (59).

STOCKHOLM BREWERY COMPANY AND COMING TO THE UNITED STATES 1947–1958

From Yeast and Barley to an Anaerobic, Thermophilic, Cellulolytic Bacterium

When I finished my compulsory military service in 1947, I wanted to work as a chemical engineer. I got work as a research chemist at the Central Laboratory of the Stockholm Brewery Company, where I stayed until 1958.

The Central Laboratory, headed by Evald Sandegren, had two separate units, one dedicated to biochemistry and the other to microbiology. Lennart Enebo was in charge of the microbiology unit and he introduced me to microbiology. Sandegren was very interested in research. He told me, "Know you can learn a lot about life studying yeast and barley." I worked on several interesting projects. One was the study of free amino acids in brewing materials and their fate during the brewing process. Results included finding γ-amino-butyric acid (GABA) and several peptides in barley, wort, and beer, which were taken up by yeast (41). The presence of GABA in beer interested me. This amino acid has not been found in protein, but it is present in the brain and in the central nervous system, where it acts as a negative neurotransmitter. I still wonder if the relaxing effect of a glass of beer is due in part to the presence of GABA.

Another project pursued with Enebo became the foundation of one part of my future research: the enzymatic hydrolysis of cellulose/hemicellulose for the production of fermentable sugars. It started with the idea that cell walls are difficult to penetrate and that enzymes hydrolyzing starch and protein may have difficulty reaching their substrates inside the barley. Thus, an understanding of the action of cellulases and hemicellulases during the malting and mashing processes would be of

interest. Furthermore, the hydrolysis of the cellulose of barley would increase the amount of fermentable glucose and thus increase the utilization of the barley. The original idea of looking at cellulases of barley was proposed by Enebo, who had already initiated work on cellulose fermentation using thermophilic bacteria (25). He used a symbiotic culture consisting of three different bacteria, one of which was cellulolytic. This mixed culture vigorously fermented cellulose, but only when all three bacteria were together. The cellulolytic bacterium was not capable of rapid degradation of cellulose without the presence of the other two bacteria, which in turn were dependent on the supply of fermentable carbohydrate produced by the cellulolytic species. The three different bacteria were eventually obtained in pure cultures. Enebo characterized them and suggested the names *Clostridium thermocellulaseum* for the cellulolytic species and *Clostridium thermobutyricum* and *Bacillus thermolacticus* for the other bacteria (26). Enebo shared his excitement about his symbiotic mixed culture with me. It was my first exposure to anaerobic and thermophilic bacteria. Anaerobic and thermophilic microorganisms capable of CO_2 fixation and/or that have cellulolytic properties have since been and still are my main passions.

Our initial experiments with barley showed that at the beginning of the malting process the cellulase activity was very low, but that after 6 days it started to increase, such that after 15 days the cellulase activity in the green malt was 50 times greater than the cellulase activity in barley. The activity of cellulase purified from green malt was 40 times higher than that of the green malt. A problem during the purification, which involved ammonium sulfate precipitation followed by dialysis using cellophane dialysis sacks, was that the sacks were dissolved by the cellulase. We should have known better than to use cellophane sacks. The purified enzyme was activated by glucose, xylose, and a low concentration of cellobiose, but it was inactivated by cellobiose at concentrations over 1% and by lactose. The barley enzyme differed remarkably from the enzyme of *C. thermocellulaseum* in that its activity was much lower in its response to sugars, as were its pH and temperature optima. We concluded that cellulases from different sources, such as plants, bacteria, and fungi, have different properties (27).

Meeting Britt-Marie Swahn

Just before I began work at the Brewery, another life-changing event occurred. In 1947, during my military service, I was granted an unexpected leave on April 30, which is an important day in Sweden; it is the day on which the arrival of spring is celebrated. I called my friends but they all had dates, and I was left alone. My mother noticed my predicament. She gave me 10 kronor (crones) and told me to go to Skansen, a popular outdoor museum park in Stockholm. I noticed three nice looking girls, one of whom I recognized, as she often traveled on the same bus I did. She introduced me to her friends. One of them was Britt-Marie (Britten) Swahn. We started talking with each other and a world opened up for me. We married in August 1949 and were together until 1995, when I lost her to a brain tumor. We have two children. Ann-Sofie was born in 1953 and Per in 1957, both of whom have done very well in their careers in medicine and science. Britten had two sisters; we three husbands became the Swahn boys, and we had and continue to have a great relationship.

Coming to the United States

While I was working at the Stockholm Brewery Company, my cousin Rune was working at the University of Stockholm, Wenner-Gren Institute as a member of a research team led by Gösta Ehrensvärd. This team pioneered the use of isotopes and demonstrated the importance of acetate as a precursor for the biosynthesis of several amino acids. Their work drew the attention of Harland G. Wood, a pioneer and promoter of using carbon isotopes as tracers in biochemistry. During a visit in 1950 to the Wenner-Gren Institute, Harland Wood met Rune, realized his

skill and devotion to biochemical metabolism, and invited Rune to join him at Case Western Reserve University (CWRU) to work on the metabolism of propionic acid bacteria and to study for a PhD. Rune arrived at CWRU in 1952 and found himself at the world center for metabolic studies using isotope techniques. His metabolic experiments led to the discovery of several new enzymes, including the two key enzymes methylmalonyl-oxaloacetic transcarboxylase and methylmalonyl-CoA isomerase, involved in propionic acid formation. In 1956 Rune visited Sweden and I hinted that I would not mind doing as he had done, studying in the United States and earning a PhD. Rune must have taken my hint seriously and conveyed it to Harland Wood. In the fall of 1957 I received a short letter from Wood inviting me to work as a technician with him at CWRU and to study first for a Master's degree and eventually a PhD.

Britten and I thought of dozens of reasons to continue our life in Sweden, but with the encouragement of Evald Sandegren we decided to accept this not-to-be-missed opportunity. He suggested we stay for a couple of years and if it did not work out as expected, I would be welcomed back to the brewery. With Britten's strong support we accepted Wood's offer. On February 18, 1958, a cold but beautiful winter day, we left Stockholm by plane for New York and Cleveland, where we arrived two days later. We were exhausted, happy, and excited. Rune and his wife, Iris, helped us settle in and introduced Britten and me to the Swedish Cultural Society in Cleveland. We were stunned by the generosity of its members. With their help, we quickly settled into our new life. The day after arriving, February 21, I met Harland G. Wood for the first time.

CASE WESTERN RESERVE UNIVERSITY 1958–1967

I knew Wood had discovered CO_2 fixation by heterotrophic organisms and was a pioneer in using isotopes as markers for metabolic studies. I was warned that he was very demanding and dedicated to his work. He explained he needed me as his technician to help with isolation and degradation of radioactively labeled sugar phosphates. I mentioned my work with thermophilic bacteria and cellulases but also stressed that one of my priorities in coming to the United States was the pursuit of graduate studies. I liked Dr. Wood (he wanted me to call him that) from the beginning. Little did I know that we had a lifetime ahead of us to work on acetogens, and that we were to become good friends. *Clostridium thermoaceticum* was not mentioned at our first meeting.

After a few days it was time for me to work and to learn about the use of radioactive isotopes and how to degrade biological compounds to determine the incorporation and distribution of ^{14}C-labeled precursors in these compounds. At the time, Wood was working on two projects. There were still problems with the pathway involving the formation of propionic acid in *Propionibacterium shermanii*, the bacterium with which Wood had established heterotrophic CO_2 fixation. The second project was studying the metabolism of ^{14}C glucose in mammalian tissues, especially lactose synthesis in the mammary gland. He had already found that a substantial randomization of the ^{14}C glucose occurred, and he wanted to establish the mechanism of that as well as find a method to calculate the involvement of the pentose cycle (84). My job was to purify sugar phosphates and, after dephosphorylation, determine the distribution of ^{14}C among the carbons.

One study I was involved with was determining whether transaldolase catalyzes an exchange reaction between glyceraldehyde-3-phosphate and fructose-6-phosphate. If this exchange occurred, it would explain the unequal labeling of sugars observed in tracer studies. For substrates, we used 3-P-glycerate-1–^{14}C and glycerol-2–^{14}C. 3-P-glycerate was converted with glycerate kinase, and glyceraldehyde-3-P-dehydrogenase, to glyceraldehyde-3-P-1–^{14}C. Glycerol-2–^{14}C was converted with glycerokinase, glycerol-P-dehydrogenase, and triose-P-isomerase to glyceraldehyde-3P-2–^{14}C, and incubated with fructose-6-P and transaldolase.

The fructose-6-P now labeled was degraded, and 99% of the ^{14}C was located in C-4 of the fructose, with 3-P-glycerate-1–^{14}C as labeled substrate. Similarly, with glycerol-2–^{14}C the fructose was labeled almost exclusively in the C-5 position. The results clearly confirmed the exchange reaction between glyceraldehyde-3-P and fructose-6-P. The reaction can be used for the production of hexoses labeled in the C-4, C-5, and C-6 positions (43). The results appeared in my first publication from work done in the United States and I was very proud of it. Our work was criticized by a reviewer at *The Journal of Biochemical Chemistry* as describing unnecessary work since the outcome was obvious. I am a little old-fashioned and firmly believe one should conduct experiments even though the outcome can be predicted with some certainty. Results of experiments are correct, but that is not always true for predictions.

My newly learned expertise in the degradation of sugars was used to study metabolism in leukocytes. It resulted in a publication with Rune Stjernholm and Ernie Noble (56), which is the only publication I have coauthored with my cousin Rune.

In June 1958, I applied to graduate school at CWRU. My engineering degree from STI and my previous work experience qualified me for graduate school, and I signed up for the PhD program in biochemistry, with minors in chemistry and microbiology. My course load and my work as a technician meant that it would take eight years to finish the PhD requirements; I would be 40 before I graduated. After discussing the situation with Wood, I became a fulltime student and the faculty allowed me second-year status, which provided enough financial support.

Wood suggested that as my graduate project I should find the pathway of the synthesis of acetate from CO_2 in *Clostridium thermoaceticum*. I accepted that suggestion, since it involved CO_2 fixation and thermophily—two subjects of great interest that I continue to pursue today. Acetate synthesis became a project Wood and I collaborated on until his death in 1991.

Total Biosynthesis of Acetate from Carbon Dioxide by *Moorella thermoacetica* (*C. thermoaceticum*)

In 1942, Fontaine and coworkers described an anaerobic and thermophilic bacterium, *Clostridium thermoaceticum*, that ferments glucose, fructose, and xylose with acetate as the only product (28). In 1994 Collins et al. (10) renamed the bacterium *Moorella thermoacetica* (the new name will be used from this point forward). Two mechanisms were proposed for the formation of 3 mol of acetate from 1 mol of glucose. The first was a traditional C_3–C_3 cleavage of the glucose, forming pyruvate subsequently cleaved to yield 2 mol of acetate and 2 mol of CO_2, which were utilized to form the third mol of acetate. The second mechanism was a cleavage of glucose, forming three C_2 units converted to acetate. The first mechanism was favored and it was formulated as follows:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 8H$$

$$2CO_2 + 8H \rightarrow CH_3COOH + 2H_2O$$

$$\text{Sum}: C_6H_{12}O_6 \rightarrow 3CH_3COOH.$$

Fermentation of glucose in the presence of $^{14}CO_2$, as performed by Barker & Kamen (5), established that acetate was formed labeled. It was one of the earliest experiments in biology to use a radioactive isotope. Degradation of the acetate showed that ^{14}C was incorporated into the methyl and into the carboxyl groups. The result indicated that the first mechanism is the correct one. However, it was pointed out by Wood that the ^{14}C experiment by Barker and Kamen did not distinguish a mixture of acetate singly labeled in the methyl or the carboxyl group from acetate doubly labeled in the methyl and the carboxyl groups and formed totally from CO_2. Thus, it was essential to establish that both carbons in the same acetic acid molecule were derived from CO_2.

In a classical experiment, Wood fermented glucose in the presence of $^{13}CO_2$ at high

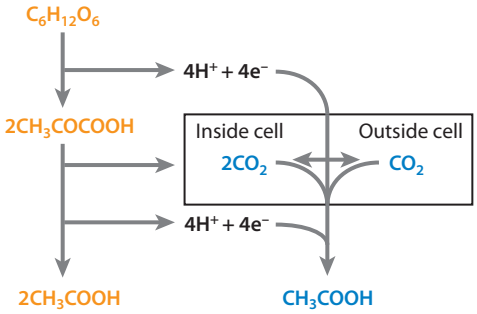


Figure 1

Homoacetate fermentation by *Moorella thermoacetica*. The diagram depicts the formation of two molecules of acetate from glucose and one molecule synthesized from CO_2.

concentration. The acetate formed was converted to acetylene, the mass of which was determined by mass spectrometry. Results clearly showed a total synthesis of acetate from CO_2. In addition they showed that there is an exchange reaction between the carboxyl group of acetate and carbon dioxide (82). Additional work in Wood's laboratory using labeled glucoses strongly indicated that glucose is fermented via the Embden-Meyerhof-Parnas glycolytic pathway with the formation of two C_3-compounds, most likely pyruvate, which is split into acetate and CO_2 (83). Kenneth Lentz, a graduate student who worked with Wood before I joined his lab, found that resting cell suspensions of *M. thermoacetica*, in addition to rapidly fixing CO_2 into both carbons of acetate, also catalyze a rapid exchange between $^{14}CO_2$ and formate. He also observed that labeled formate was a better precursor of the methyl group of acetate than of the carboxyl group (37). **Figure 1** summarizes the fermentation of glucose by *M. thermoacetica* as it was understood when I started my graduate studies.

When I began my graduate studies, the only known pathway for the synthesis of carbon-to-carbon bonds was the reductive pentose phosphate cycle, or the Calvin cycle, which occurs in autotrophic and photosynthetic organisms. In this cycle phosphorylated sugars play important roles (24). Formation of acetate from sugar phosphates is catalyzed by phosphoketolases. Thus, it seemed warranted to isolate sugar phosphates from the cells of *M. thermoacetica* after exposure to $^{14}CO_2$ and to determine the distribution of radioactivity in them. Another possible mechanism relates to the isotope exchange between $^{14}CO_2$ and formate as catalyzed by resting cells of *M. thermoacetica*, and to the finding by Kenneth Lentz (37) that formate may be a better precursor of the methyl group of acetate. Formate may be incorporated via tetrahydrofolates (THFs) into amino acids such as glycine and serine and possibly pyruvate and dicarboxylic acids, which may serve as intermediates in acetate synthesis. The involvement of these biochemicals in acetate synthesis was considered in a review by Wood and Stjernholm (86).

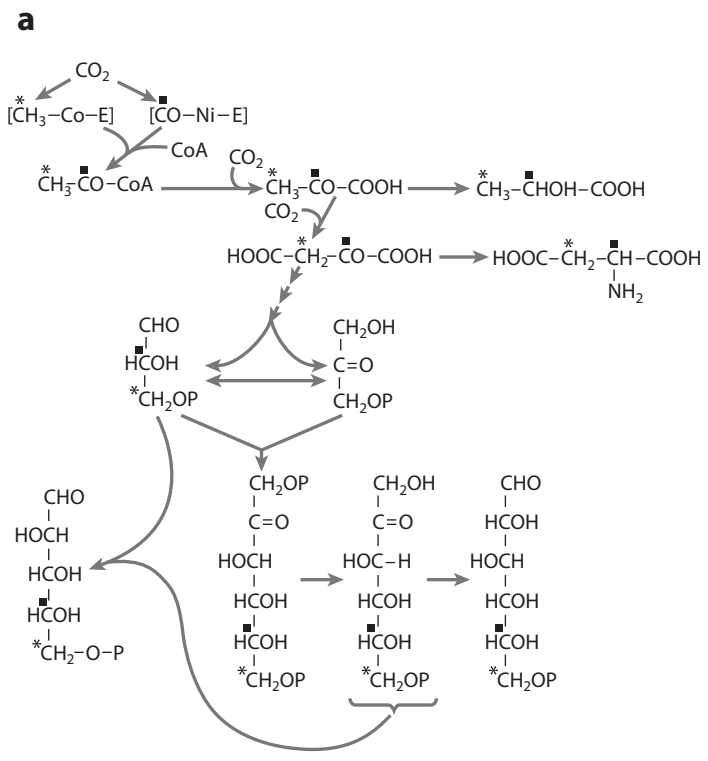
My first experiments were based on these speculations. Large amounts, 90 to 186 g, of *M. thermoacetica* cells, fermenting glucose or xylose anaerobically under N_2 at 55°C, were exposed to a 5-s pulse of highly labeled $^{14}CO_2$. Sugar phosphates, free amino acids, and carboxylic acids were isolated, and their specific radioactivities as well as the distribution of ^{14}C in them were determined (89). Only fructose-1,6-diphosphate, formate, and lactate had higher specific activities than acetate. The specific activities and distribution of ^{14}C in the sugar phosphates were such that they could not be precursors of acetate. Likewise, a pathway involving lactate, aspartate, serine, glycine, malate, and/or succinate could be excluded. The high activity of lactate related only to the carboxyl group, which was a reflection of a fast exchange reaction between the carboxyl group of pyruvate and $^{14}CO_2$. The high radioactivity of the formate was in agreement with the results previously obtained by Lentz and showed that formate could be a precursor of the methyl group of acetate. This prompted me to study the exchange reactions between pyruvate-CO_2 and formate-CO_2 using cell extracts from *M. thermoacetica* (42). The first attempt to find the reactions was negative. I quickly found that the reactions occurred only under strict anaerobic conditions and at temperatures coinciding with the growth temperature of

M. thermoacetica, which is between 40 and 65°C. Could it be that the growth temperature of a bacterium is related to the temperature stability of enzymes and other proteins from it? This observation excited me and strengthened my interest in how thermophiles require and can survive at high temperatures.

Several years after the publication of the pulse experiments, I reexamined the degradation data of the sugar phosphates, acetate, lactate, and aspartate and found they demonstrate that acetyl-CoA is a precursor of these compounds (**Figure 2**), and subsequently can be considered the primary building block for cell material in *M. thermoacetica* (45).

At the American Society of Microbiology meeting in Cleveland 1963, I had the opportunity to meet with Earl Stadtman from the National Institutes of Health (NIH). He was interested in *M. thermoacetica* and acetate synthesis from CO_2. I told him about the requirement of high temperature to catalyze the exchange reactions and that formate was a likely precursor of the methyl group. Stadtman in turn mentioned that methyl-vitamin B_{12} could play a role as methyl donor in acetate synthesis, similar to ^{14}C-methyl-B_{12} as substrate in the synthesis of methionine (31). Stadtman and coworkers soon showed it to be the case (63).

At the time, methyl-B_{12} had not been found in any biological material. Wood and I thought it would be possible to find it in *M. thermoacetica* if it were an intermediate in the synthesis of the methyl group of acetate from CO_2 and formate. We decided to repeat my first experiments using large amounts of *M. thermoacetica* cells fermenting glucose or xylose anaerobically under N_2 by exposing them to a 15-s pulse of highly labeled $^{14}CO_2$, and then isolating B_{12} or other corrinoids by acetone and phenol extractions by methods worked out in Konrad Bernhauer's laboratory at the Technische Hochschule in Stuttgart, Germany. Eckart Irion, who recently had obtained his PhD doing work on corrinoids in Bernhauer's laboratory, had just joined Wood's group and became very interested in our attempt to find methyl-B_{12} in



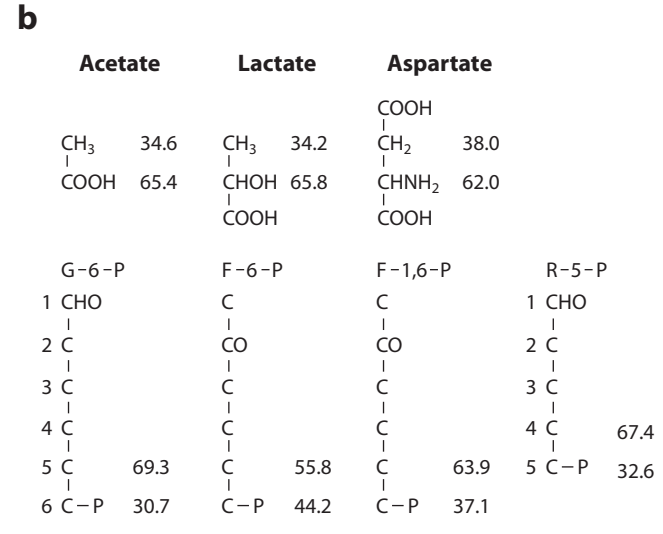


Figure 2

Panel *a* shows the pathway of incorporation of acetyl-CoA formed from CO_2 by *Moorella thermoacetica* into lactate, aspartate, and sugar phosphates. Panel *b* shows the distribution of ^{14}C in acetate formed from $^{14}CO_2$ by fixation via the acetyl-CoA pathway and the incorporation of this acetate into lactate, aspartate, and sugar phosphates. The pathway in panel *a* is based on the ratios of ^{14}C between the methyl and carboxyl groups in these compounds. Adapted from Reference 45.

M. thermoacetica and establish if it was an intermediate in the acetate synthesis from CO_2.

The work to isolate corrinoids from *M. thermoacetica* was successful. It was done in very dim light to prevent cleavage by light of any cobalt-carbon linkages such as those of Co-5′-deoxyadenosyl corrinoids, as found in coenzyme-B_{12}, and Co-methyl-B_{12}. We were working in the dark literally and figuratively—we couldn't see what we were doing and we didn't know what we would find. What we found was amazing. *M. thermoacetica* contained more than 20 different corrinoids in a combined amount of 70 μmol per 100 g of wet cells (33). Most of the corrinoids were of the coenzyme form with the 5′-deoxyadenosyl attached to the cobalt, and they appeared to be intermediates in the synthesis of complete B_{12} factors from cobyric acid. For us the most exciting part was isolating Co-(methyl)-5-methoxybenzimidazolylcobamide and Co-methylcobyric acid and finding that the Co-methyl groups had higher ^{14}C-specific activities than did the acetate formed in the same incubation. Both of these Co-methylcorrinoids served as precursors of the methyl group of acetate when tested in a cell extract of *M. thermoacetica* (39). Thus, the two Co-methylcorrinoids behaved as was expected by an intermediate between CO_2 and the methyl group of acetate. Cobyric acid and 5-methoxybenzimidazolylcobamide, or Bernhauer's Factor III, are the prominent corrinoids in *M. thermoacetica*. Factor III had been synthesized by Friedrich and Bernhauer (29) but had not been found before in nature.

With the knowledge that Co-methylcobamides satisfied the requirements as intermediates in acetate synthesis, we proceeded by demonstrating that cell extracts of *M. thermoacetica* in the presence of pyruvate convert the methyl group of 5–$^{14}CH_3$-tetrahydrofolate to the methyl group of acetate. We then speculated that the conversion of Co-methylcorrinoids to acetate may occur via a Co-carboxymethylcorrinoid, which we synthesized and found to be converted to acetate by cell extracts. On the basis of our findings, we presented a concept of the total synthesis of acetate from CO_2 (see **Figure 3**) (40). Accordingly, the methyl group of acetate is formed from CO_2 by reduction to formate, which is further reduced via several derivatives of THF to methyltetrahydrofolate, the methyl group of which is transferred to a corrinoid to form a methylcorrinoid. The speculation about the involvement of a Co-carboxymethylcorrinoid was wrong.

Diekert and Thauer (16) reported that *M. thermoacetica* contains carbon monoxide dehydrogenase, an Ni-Fe enzyme. Harold Drake (19) and Steve Ragsdale (67) (who was a former graduate student of mine) worked in Wood's laboratory and found that it was bifunctional: It had carbon dioxide dehydrogenase (CODH) and acetyl-CoA synthase (ACS) activity. The CODH/ACS catalyzes the condensation of the methyl group of the Co-methylcorrinoid, carbon monoxide formed from CO_2, and CoA yielding acetyl-CoA, in which the acetyl group is synthesized from CO_2. Subsequently, Ragsdale has done extensive and excellent work on CODH/ACS and has recently published a comprehensive review of this enzyme (64).

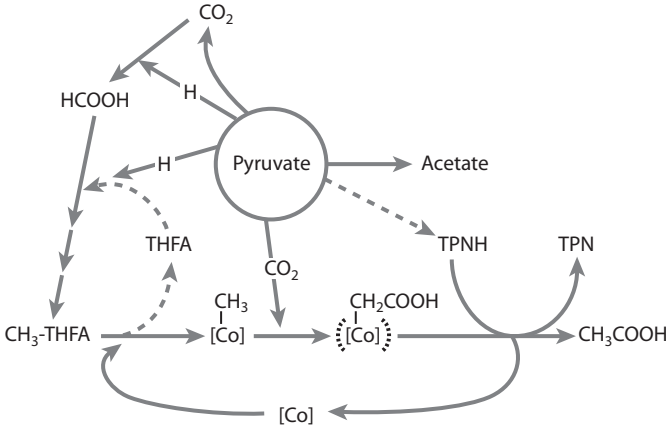


Figure 3

The first concept of the total synthesis of acetate involving formate, tetrahydrofolate derivatives, methylcorrinoid, and postulated carboxymethylcorrinoid as intermediates. [Co] is the cobalt of a corrinoid to which the methyl and carboxymethyl groups bind. THFA, tetrahydrofolic acid (40).

I received my PhD in January 1964 and became an assistant professor. But there was a fly in the ointment. As a graduate of CWRU, I had no prospect of a tenured position. Thus, in 1967, I began my search for a new position. I considered returning to Sweden but I received offers in the United States, one of which was from the newly formed department of biochemistry at the University of Georgia, Athens (UGA), which intrigued me. I accepted it. Before leaving CWRU, Wood and I divided up our research interests. Wood was to study the final reactions forming acetyl-CoA involving CODH/ACS, and I was to work on formate dehydrogenase and the formation of methyltetrahydrofolate and methylcorrinoids. In general we adhered to this agreement, but we left room for cooperation.

THE UNIVERSITY OF GEORGIA 1967–2005

A Promise to a Biochemist/ Microbiologist Fulfilled

In May 1967, while attending the ASM meeting in New York, I was walking on Broadway when I heard a voice calling, "Lars, you are looking for a job, aren't you?" It was Leon Campbell, a former professor of microbiology at CWRU. "If you are, just walk into the bar over there and introduce yourself to the bearded fellow at the counter and tell him I sent you. His name is Harry Peck." Peck, a graduate of CWRU who had studied with Howard Gest, was the chairman of the department of biochemistry at UGA. He was impressed by my work with Wood and by my connections with Lester Krampitz and Howard Gest. He invited me to visit UGA the following Tuesday to present a seminar. In the evening after my talk I was offered a job as assistant professor.

UGA had a large federal grant to develop excellence in biological sciences, and the state of Georgia was committed to expanding the university. From 1965 to 1967 more than 1100 new faculty members were hired. The department of biochemistry was moving into the newly completed Graduate Study Building and a fermentation plant was under construction. My impression was that UGA, especially the biological sciences programs, could develop into something impressive. I would be involved in building an excellent biochemistry department. I was also invited to join the department of microbiology, headed by William Jackson Payne. Peck and Payne were working on problems of great interest to me. Subsequent years have proven that Athens and the University of Georgia were a good fit for my family and me.

When I arrived at UGA the biochemistry department was still forming. Peck understood that it was not possible to attract, as he put it, the kings of biochemistry to our young department, but we could attract crown princes. We made ourselves known by inviting respected scientists for seminars and by giving recruitment parties at national meetings of biochemists and microbiologists. In addition to building a young and energetic faculty, several support services were set up. These included facilities for amino acid analyses and sequencing, mass spectroscopy, electron microscopy, NMR, EPR, X-ray crystallography, and antibody production; rather recently bioinformatics has been added. The early history of our department was chronicled by Milton Cormier in 2007 (12).

The new faculty was enthusiastic and eager to succeed. It displayed openness and willingness to cooperate not only within the department, but also throughout the biological sciences and the university as a whole. This openness and cooperation is still evident. For example, I have coauthored publications with 17 colleagues from the departments of biochemistry, microbiology, botany, and chemistry, and the Richard B. Russell Agricultural Research Center.

In 1978 the department had outgrown its original space and was housed in five locations. A new building dedicated to the departments of biochemistry and genetics was needed. Norman Giles and Sidney Kushner from genetics and Milton Cormier, Leon Dure, and I met with Fred Davidson, the president of UGA, who reacted favorably to our proposal.

We got the new building, and in 1991 the departments of biochemistry and genetics moved into it. As the department of biochemistry grew, the university was also expanding its focus in the biosciences. Peter Albersheim and Alan Darvill were recruited and established the Complex Carbohydrate Research Center (CCRC). The CCRC and the Center for Metalloenzyme Studies have been especially important for my research on metalloenzymes involved in acetogenesis and on enzymes for the hydrolysis of cellulose and hemicelluloses.

An additional boost of biosciences came with the formation of the Georgia Research Alliance (GRA). It was organized in 1990 and includes six research universities, the state of Georgia, and some major industries. GRA promotes research and technology by furnishing funding for endowed chairs, major equipment, and cooperation between universities and industries. I had the privilege to serve on GRA during its early years to promote biosciences. Essential for my research was setting up the X-ray crystallography facility and recruiting B.-C. Wang to lead it. The crystal structures of several enzymes I worked with have now been obtained. I feel fortunate to have been a part of building the biochemistry department, which is now called the department of biochemistry and molecular biology (BMB). Harry Peck, John Wampler, and David Puett, who as chairmen of BMB during my tenure at UGA, deserve much praise. Their philosophy has been to serve the faculty members, allowing them the time and freedom to conduct the best research and to teach to the best of their abilities. Stephen Hajduk now leads BMB.

The Acetyl-CoA Pathway: Autotrophic Fixation of Carbon Dioxide

My first task at UGA was to apply to NIH for a grant to support work on folic acid and corrinoid-dependent enzymes in the total synthesis of acetate from CO_2. The application was successful, and in 1968 I received my first NIH grant. I had continuing support from NIH until 1996. My second task was to write a review about total synthesis of acetate from CO_2 in heterotrophic bacteria. We were still cautious about considering the acetate synthesis from CO_2 an autotrophic process (52).

The first acetogenic bacterium capable of the synthesis of acetate from CO_2 was isolated by Wieringa in 1935 (80). Unfortunately, not much work was done with this bacterium, which was lost. Thus, *M. thermoacetica*, isolated by Fontaine et al. in 1942 (28), was the only acetogen available to Wood and me for our studies of acetogenesis. But in 1967 El-Ghazzawi (23) isolated an acetogenic mesophilic bacterium which in 1970 was named *Clostridium formicoaceticum* (2). It ferments one mol of fructose, but not glucose, almost stoichiometrically to 3 mol of acetate via the Embden-Meyerhof-Parnas pathway. Hans Schlegel, who had spent some time at CWRU and knew Wood, sent me a culture of *C. formicoaceticum*. I now had the opportunity to study both mesophilic and thermophilic acetogens.

In the spring of 1968 my laboratory was ready. My first graduate student was William E. O'Brien, who had a MS degree from Mississippi State University. Shortly thereafter Rajinder Ghambeer from Australian University, Sue Neece from Duke University, and Jan Andreesen, codiscoverer of *C. formicoaceticum*, from the University of Göttingen, Germany, joined the laboratory. Also to my big surprise and delight, Wood decided to spend a sabbatical with me; Marvin Schulman and Paul Kucera, a technician, accompanied him. We were now an impressive group of people studying acetogenesis. One of our experiments was to establish, with $^{13}CO_2$ and mass analysis of the acetate produced, whether one third of the acetate produced with *C. formicoaceticum* was totally synthesized from CO_2 in a manner similar to that shown for *M. thermoaceticum* (69). This work was extended to two purine-fermenting bacteria, *Clostridium acidiurici* and *Clostridium cylindrosporum*, which had been shown by Rabinowitz and coworkers to use THF derivatives for synthesis of acetate from CO_2. The results showed that in fermentations of fructose with $^{13}CO_2$, *C. formicoaceticum* synthesizes

one third of the acetate totally from CO_2 and that two thirds are formed from the fructose, suggesting that it uses the same pathway as *M. thermoacetica*. Although they incorporate CO_2 in acetate, the purine-fermenting clostridia use another pathway (20).

Formate dehydrogenase, 10-formyltetrahydrofolate synthetase, 5,10-methenyltetrahydrofolate cyclohydrolase, 5,10-methylenetetrahydrofolate dehydrogenase, and 5,10-methylenetetrahydrofolate reductase are required for the conversion of CO_2 to 5-methyltetrahydrofolate. The methyl group of which is then transferred to the Co atom of a corrinoid to form a Co-methylcorrinoid, of which the methyl group is the methyl precursor of acetate. These enzymes are present in both *C. formicoaceticum* (57) and *M. thermoacetica* (4). Over the next 10 years, O'Brien and Neece, and two additional students, Michael R. Moore and Joan Clarke, purified all the THF enzymes from these two acetogens. Some differences were found between corresponding enzymes from the two acetogens. Most noticeable was that the enzymes from *M. thermoacetica* function better and are more stable at higher temperatures than the corresponding enzymes from the mesophilic *C. formicoaceticum.* These features are discussed below in connection with thermophily. In *M. thermoacetica* the activities of 5,10-methenyltetrahydrofolate cyclohydrolase and 5,10-methylenetetrahydrofolate dehydrogenase reside in the same protein, whereas in *C. formicoaceticum* these activities occur in separate proteins. In *Saccharomyces cerevisiae* the THF-dependent synthetase, cyclohydrolase, and dehydrogenase are combined in a trifunctional enzyme (70). At the time the prevalent idea was the one gene–one enzyme concept. We were surprised to find the bifunctional cyclohydrolase-dehydrogenase in *C. formicoaceticum*.

In growth studies using *M. thermoacetica*, we observed that adding metals, including Fe, Se, Mo, Co, and Ni, to the growth medium greatly increased cell yield, formate dehydrogenase activity, and incorporation of the methyl group of 5-methyltetrahydrofolate into acetate (4). According to postulated pathways for the fermentation of glucose and synthesis of acetate from CO_2 by *M. thermoacetica*, 3 mol of ATP are available as energy for growth. A bacterial growth yield (Y) in grams per mol of ATP determined experimentally is 10.5 (60). The Y(ATP) we obtained was 18, which indicated that additional energy was available, and we postulated generation of ATP by electron transport during the synthesis of acetate. In support of this were findings of hydrogenase in *M. thermoacetica* (17) and of H_2/CO_2 or CO as an energy source (35). The idea of electron transport generation of energy in acetogens excited us, and it led my graduate student Shiow-Shong Yang and postdoctoral associate James Elliot, in cooperation with Jean LeGall and Daniel DerVartanian, to look for electron-transfer proteins in *M. thermoaceticum*. This work resulted in the isolation of two ferredoxins and two rubredoxins. In addition, we found cytochromes and menaquinone, which according to textbooks of that day should be absent in heterotrophic bacteria because they did not carry out electron transport generation of energy. When back at the University of Göttingen, Germany, Andreesen was finding cytochromes and menaquinone in *C. formicoaceticum*, and together we published our findings (30). Jeroen Hugenholtz from Rijksuniversiteit in Haren, The Netherlands, and Amaresh Das from Bose Institute in Calcutta, India, joined the laboratory as postdoctoral associates. Together with David Mack Ivey, a graduate student, they isolated and sequenced F_1F_0-ATPases from *M. thermoacetica* and *Moorella thermoautotrophica* (*Clostridium thermoautotrophicum*), isolated by Wiegel et al. (75). By using hydrogen and carbon monoxide as electron donors, they established an anaerobic electron transport chain in these acetogens that generated a proton motive force that drove the synthesis of ATP and uptake of amino acids (32).

Carbon monoxide dehydrogenase in *M. thermoacetica* was purified in my laboratory by Ragsdale and colleagues (65). It contains two subunits, with the dimeric enzyme having 2 nickel, 1 zinc, and 11 iron atoms. Together

with Daniel DerVartanian, we demonstrated using EPR the presence of a nickel (III)-carbon species when the enzyme was exposed to CO (66). Another contribution to CODH/ACS from my laboratory was the sequencing of the enzyme by Morton and colleagues (55). As mentioned above, the final proof that carbon monoxide was the condensing enzyme for the methyl group of a methylcorrinoid, carbon monoxide, and CoA was done in Wood's laboratory by Ragsdale and Drake. As a result of their work, the pathway of acetate synthesis was called the acetyl-CoA pathway, which makes sense because acetyl-CoA is the first two-carbon unit of CO_2 fixation.

When he was a postdoctoral student in my laboratory, Andreesen discovered that the formate dehydrogenase was a tungsten-selenium-nonheme iron protein. This finding was the first evidence of tungsten as a biologically active metal. This discovery came about as follows: Pinsent (62) found that selenite and molybdate were needed for formate dehydrogenase activity in *Escherichia coli*. Tungstate was known as an antagonist of molybdate and it was added to the medium. The idea was to inhibit formate dehydrogenase activity, which would indicate it to be a molybdenum enzyme. Surprisingly, the bacterial cells grown with tungstate had 12 times higher activity than did cells grown with molybdate.

During the academic year of 1974–1975 I was awarded a Senior U.S. Scientist Award for Research and Teaching from the Alexander von Humboldt Stiftung, in Bonn, Germany. This award, which was sponsored by Professors Hans Schlegel and Gerhard Gottschalk at the Institute for Microbiology at the University of Göttingen, allowed me to work with Andreesen, who now led a research group at the Institute. We continued our studies of the tungsten-selenium formate dehydrogenase. With the use of [^{75}Se]-selenite and [^{185}W]-tungstate we showed that selenium and tungsten were incorporated into protein fractions containing the active enzyme. We made several attempts to purify the formate dehydrogenase, but they were only partially successful (47). Successful purification of the enzyme was achieved by Takashi Saiki, Shiu-Mei Liu, and Isamu Yamamoto (87). The enzyme consists of two subunits with the composition $\alpha_2\beta_2$, two selenocysteins residing in the α-subunits, two tungstens released by denaturation of the enzyme as a fluorescent tungsten cofactor similar to the molybdenum cofactor of several molybdenum enzymes, and potential binding sites for 48 iron-forming [4Fe-4S] and [2Fe-2S] clusters. Tungsten has now been found in many mainly thermophilic microorganisms, and the biological importance of tungsten has recently been reviewed (3). With the purification of the formate dehydrogenase all enzymes of the acetyl-CoA pathway for acetate synthesis from CO_2 had been purified. A simplified pathway is shown in **Figure 4**.

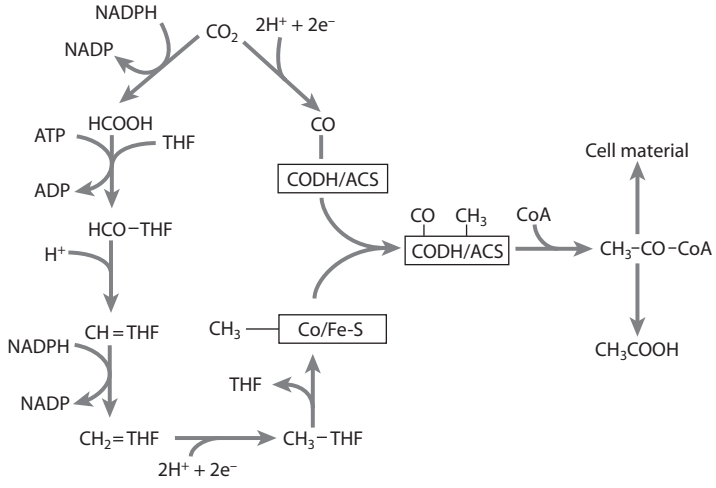


Figure 4

The acetyl-CoA pathway functioning in *Moorella thermoacetica*. Abbreviations: CODH/ACS, carbon dioxide dehydrogenase/acetyl-CoA synthase; Co/Fe-S, corrinoid/iron-sulfur protein; THF, tetrahydrofolate.

The presence of tungsten as an active metal in enzymes was somewhat controversial. At a biochemistry meeting in San Francisco, I met Fujio Egami, head of the Mitsubishi Kasei Institute of Life Sciences. He simply told me, "I do not believe that tungsten is a biologically active metal, come and visit me in Japan and give a seminar." The reason for his skepticism was that tungsten's concentration in oceans is

much lower than that of molybdenum. Egami had advanced theories that trace elements, especially transition metals, are involved in the genesis and evolution of life. It intrigues me that enzymes catalyzing relatively simple reactions of one-carbon compounds require several kinds of metals and also are complicated proteins. Perhaps at first they were metal complexes that later evolved by adsorbing amino acids and eventually gained their specificities and catalytic activities. Egami greatly increased my interest in the origin of life. I later got the chance to express that in a contribution to a book honoring Egami on his seventieth birthday (38).

Recently, largely through the efforts of Ragsdale and Das, the complete genome sequence of *M. thermoacetica* was published (61). To know the DNA sequence of *M. thermoacetica* was a long-standing wish for me. This information has confirmed and increased the significance of the work already done at the enzyme level. More importantly, I anticipate that knowing the complete DNA sequence will guide future studies on mechanisms of electron transport and energy generation in anaerobes. Its completion will point toward possibilities of metabolic capabilities not yet recognized, and it may explain how acetogens can interact with other bacteria in bacterial consortiums. Working in cooperation with Donald Kurtz and B.-C. Wang on the *M. thermoacetica* sequence, Das recognized genes involved in oxidative stress and oxygen tolerance and in methanol metabolism (13, 14). I am very excited about all the possibilities for research that have resulted from knowing the sequences of microorganisms, and I feel somewhat excited for and envious of younger investigators who may continue the work on acetogens.

During our first 15–20 years collaborating, Wood and I felt pretty lonely in our pursuit of the pathway of acetate synthesis in *M. thermoacetica*. With the isolation of additional acetogens such as *C. formicoaceticum*, *Acetobacterium woodii*, *Thermoanaerobacter kivui*, the rediscovery of *Clostridium aceticum*, and the discovery that acetogens can grow autotrophically, the interest in acetogens increased tremendously. Wood and I could not have foreseen the importance and distribution of acetogens or the importance of the autotrophic acetyl-CoA pathway and its distribution among microorganisms. We were so busy establishing the pathway that we did not see the forest for the trees. Now more than 100 acetogens have been isolated, and the pathway or its variations are found in other types of bacteria. They may fix or recycle CO_2 to the extent of 10^{12} kg per year, and they are found in many environments such as aquatic plants and the intestines of animals and insects. A most intriguing and exciting idea is that the acetyl-CoA pathway may have developed early and may be the first biochemical pathway (68). It seems that we are just beginning to understand the importance of bacterial activities of life on Earth. Perhaps more importantly, microorganisms are essential for maintaining Earth and keeping it livable. Drake et al. (18) have written a marvelous article covering the history, physiology, and ecology of acetogens.

In 1986, I reviewed the acetyl-CoA pathway and suggested that it be named the Wood acetyl-CoA pathway, but to my surprise it is now called the Wood-Ljungdahl pathway. This is the greatest honor one may receive. I am not sure I deserve it. There are many who have contributed to the elucidation of the pathway and developed an understanding of its importance. I take it as a recognition that I have influenced some wonderful people during my life, as I in turn have been influenced by them.

Back to Thermophilic and Anaerobic Cellulolytic Bacteria and Fungi

My interest in thermophilic bacteria developed when I was working on cellulases from *C. thermocellulaseum* and barley malt with Enebo in 1949 at the Stockholm Brewery Company. But it was while working with *M. thermoacetica* that I observed that proteins from thermophilic bacteria in general have higher thermostability than similar proteins from mesophiles. In reviewing the literature not much was known

about the thermostability of enzymes from thermophiles and their influence on growth at high temperatures. In addition, most studies (reviewed in Reference 1) utilized aerobic spore-forming bacteria. I believe this was because they were easier to culture than anaerobic bacteria. With the isolation of several pairs of enzymes and other proteins from the thermophile *M. thermoacetica* and the mesophile *C. formicoaceticum*, the enzymes and proteins could be compared. The project was done in cooperation with John Brewer, whom I consider a biophysicist, and several students. The enzymes and proteins studied were those of the THF-dependent enzymes of the acetyl-CoA pathway, formate dehydrogenase, and ferredoxins.

From the first part of our work, we found that all proteins purified from the thermophile had higher thermostability than the corresponding proteins from the mesophile. This higher thermostability was in agreement with the growth temperature of the bacterium the proteins were isolated from. The next observation was that the physical properties, such as molecular weights, numbers of subunits, Stokes radii, specific volumes, and sedimentation constants of similar proteins, did not differ much. Sequences for the proteins are currently available, and on the basis of these and several other properties the higher thermostability of proteins from thermophiles is intrinsic and depends on subtle differences in amino acid compositions and sequences between homologous proteins. Several investigators have observed that a single amino acid replacement in a protein may change its thermostability. Thermophiles and mesophiles have a common origin, as indicated by their homologous proteins. This is even more convincingly supported by findings from several investigators that enzyme systems such as ribosomes and ATPases are also similar. This and some of my reflections about thermophiles and the development of bacterial life are discussed in References 44 and 51.

While spending 1974–1975 at the University of Göttingen I got to know Juergen Wiegel, a graduate student in Hans Schlegel's lab, and Frank Maier, a professor at the Institute with a special interest in electron microscopy. Both joined my laboratory for periods of time and were essential for physiological studies of thermophilic bacteria involved in the degradation of cellulose and hemicellulases. When Thomas Brock and Hudson Freeze (9) discovered the isolation of *Thermus aquaticus*, an aerobic, nonsporulating extreme thermophile, a lot of interest was spurred mainly because it could grow at 79°C, it did not form spores, and it was found in a thermal spring in Yellowstone National Park. It certainly was different from spore-forming bacilli, at that time the most studied thermophiles. It encouraged Neil Welker and me to organize a seminar session at the ASM meeting in Miami in 1973. Speakers were Thomas Brock, Neil Welker, Richard Himes, John Brewer, Remi Amelunxen, and Robert Becker. Brock challenged us all to look for additional thermophiles in springs and other extreme condition environments.

When Wiegel joined my group in 1977, we decided to look for new anaerobic microorganisms, especially for those fermenting cellulose and other easily available substrates that could be converted to feedstock chemicals and fuels that could substitute for gasoline. We also would look for extreme anaerobic thermophiles and consortia of thermophiles (48). Our efforts were successful and led to the isolation of several strains of the spore-forming *Clostridium thermohydrosulfuricum*, which we found widely distributed in the environment (78), and to the finding of the nonsporing anaerobic *Thermoanaerobacter ethanolicus* in hot springs in Yellowstone National Park (77). When grown between 38 and 78°C, both bacteria ferment hexoses, pentoses, and cellobiose to ethanol and CO_2 as main products, and acetate and lactate as minor products. In addition, Wiegel isolated several strains of thermophilic sulfate reducers, methanogens, and cellulolytic bacteria. One of the cellulolytic bacteria was strain JW20 of *Clostridium thermocellum*, which was used for our future work related to the cellulosome (76). When several of the thermophilic isolates were combined, cellulose was converted to methane and CO_2. One idea we had was to

carry out a continuous fermentation of glucose with *T. ethanolicus* at 78°C, which is the eutectic boiling point of an ethanol-water mixture. A slow stream of nitrogen gas passing through the fermentation carried the gaseous ethanol-water mixture through a condenser. The distillate contained about 4% ethanol. I still feel a continuous fermentation is possible, but it will require some engineering to make it practical.

At an international microbiology meeting in 1978 I met Karl-Erik Eriksson, a professor at the Swedish Forest Products Research Laboratory in Stockholm. At the meeting Eriksson gave an interesting talk about the cellulolytic enzyme system of the white-rot fungus *Sporotrichum pulverulentum* (also called *Phanerochaete chrysosporium*). At the lunch afterward I inquired about the possibility to spend a year in his laboratory to study cellulases from *C. thermocellum* and to compare them to cellulases from white-rot fungi. Eriksson agreed and he arranged for me support from the Swedish Board of Energy Conservation during the academic year 1982–1983 as a Guest Researcher. It was thrilling to be back to work in Stockholm after 25 years. I had previous knowledge about the laboratory and was very happy with the arrangements.

During my stay in Eriksson's laboratory we found that the cellulolytic active proteins produced by *C. thermocellum* could easily be purified by using affinity chromatography using columns of cellulose powder. In a 100 mM buffer at pH 7 the cellulase adsorbed to the cellulose and it could easily be recovered after washing the column with the buffer using distilled water. At this time Lamed et al. (36) published their finding of the cellulosome in *C. thermocellum*, which they characterized as a large exocellular organelle containing more than 26 different polypeptides. Later, when back at UGA, Frank Mayer, Michael Coughlan, and Yutaka Mori joined my laboratory. We understood that what I had isolated in Eriksson's laboratory were cellulosomes as described by Lamed et al. (36). Using electron microscopy, we examined the purified cellulase fraction and found that it contained polycellulosomes with masses up to 80×10^6 Da and smaller cellulosomes of about 2×10^6 Da, both of which consisted of approximately 50 polypeptides ranging in size from 20 to 200 kDa. The polypeptides, most of which had cellulolytic activity, were bound together in rows; they could be visualized as cellulosomal subunits simultaneously binding to a cellulose chain, cutting it at several places at the same time (54). This picture of the cellulosome's structure is in agreement with work by several investigators and is discussed and summarized in several reviews (6, 15).

We continued our work on the cellulosome from *C. thermocellum* with some of the enzymatically active subunits. All subunits of the cellulosome are modular, having in addition to the catalytic module at least a dockerin module, which attaches the enzyme to a cohesin of the scaffolding protein. The subunits may also have carbohydrate binding modules, immunoglobulin-like modules (Ig), and fibronectin type 3-like (Fn3) or X modules. After a year of postdoctoral experience in the laboratory of Pierre Béguin at the Institut Pasteur in Paris, Irina Kataeva joined my laboratory in 1997 and started work on the importance of modules in glycoside hydrolases of the cellulosomes (90). She cloned and sequenced the gene encoding CelK, a 98-kDa major component of *C. thermocellum*. Surprisingly, the sequence of *CelK* has high homology, 84.3%, to the sequence of *CbhA*, a 138-kDa subunit of the cellulosome (88). This homology was indicative of gene duplication among the cellulases of the cellulosome. Kataeva prepared the modules separately and in combination with each other and with the catalytic module. We determined their effects on catalytic activity, thermostability, and cellulose binding. The most prominent finding was the interaction between the catalytic module and Ig-like module, which was important for cellulolytic activity. This was confirmed by X-ray crystallography (34).

The discovery of the cellulosome by the Lamed and Bayer research groups inspired much work not only on individual cellulases and other polypeptides with different enzyme properties, but also on how cellulosomal

protein complexes are assembled, which involves scaffolding polypeptides with cohesins that bind dockerins of enzymatic subunits and carbohydrate-binding modules (6). Bayer and Lamed's discovery of the cellulosome resulted in a new field of research with fundamental implications for protein structure, the environment, and industrial applications. It was a discovery worthy of a Nobel Prize. It also made clear that by producing cellulosomes the cellulase systems of anaerobic bacteria differ from those of white-rot and other aerobic fungi, which secrete several free cellulases (49).

While writing the review with Eriksson I learned about anaerobic fungi, which inhabit with bacteria and protozoa the gastrointestinal tract of herbivorous animals. In 1975 Orpin (58) discovered anaerobic fungi. At present, 17 morphologically distinct anaerobic fungi belonging to five genera have been isolated from 50 different herbivorous animals. Although morphologically different, the fungi form a distinct group, as evidenced by analysis of 18S ribosomal RNA and a low GC content ranging from 13% to 22%. They are oxygen sensitive and they have hydrogenosomes instead of mitochondria. They effectively hydrolyze plant carbohydrates, are considered the initial colonizers of lignocellulosic feed, and are essential for fiber digestion.

My interest in anaerobic fungi arose because they are anaerobic and strongly inhibited by oxygen and because they effectively digest plant materials. I wondered if they produced cellulosomes like anaerobic bacteria or free glycolytic enzymes like aerobic microorganisms. Initially, Eriksson did not believe in anaerobic fungi. He maintained they could be common fungi, which could grow anaerobically. Well, that was a challenge for me. I decided to pursue the matter. Back at UGA, after my stay with Eriksson in Stockholm, I approached Danny Akin at the Richard B. Russell Agricultural Research Center in Athens, Georgia. Akin already had some experience with anaerobic fungi. He became enthusiastic and suggested we work together. He had access to a fistulated cow and a technician, Bill Borneman, who wished to pursue graduate studies and was willing to consider the anaerobic fungi as his project. Five morphologically different fungi were isolated from the cow. Two of them, MC-1 and MC-2, were monocentric and resembled *Piromyces* and *Neocallimastix* species, respectively. Three were polycentric, PC-1, PC-2, and PC-3. PC-2 was identified as an *Orpinomyces* species and it was later chosen for most of our work (8). The five species fermented glucose and plant cell walls from Coastal Bermuda grass (CBG) to formate, acetate, ethanol, lactate, carbon dioxide, and hydrogen. All isolates produced enzymes to hydrolyze plant cell walls. When the fungi were grown on CBG, the xylanase activity was five to seven times higher than the cellulase activity.

To investigate the carbohydrate-hydrolyzing enzymes from anaerobic fungi, I obtained support from the U.S. Department of Energy and from a small company named Aureozyme, Inc. This funding made it possible to support an enthusiastic group of postdoctoral and graduate students, including David Blum, Huizhong Chen, Timothy Davies, Yi He, and Xin-Liang Li. Professor Carlos Felix and Eduardo Ximenes, a student, from the University of Brazil joined us. We first established that the anaerobic fungi produced cellulosomes with structures similar to those of the cellulosome from *C. thermocellum* and that they produced some free glycolytic enzyme in the growth medium. The fungal cellulosomes and polycellulosomes found with electron microscopy were the same size as those of *C. thermocellum*. They were clustered mostly at the growing tips of the fungal mycelium, where their hydrolytic enzymes would allow the fungal mycelium to penetrate the hemicellulose/lignin layer of the plant tissue and reach into the cellulose. The construction of a cDNA library of *Orpinomyces* strain PC-2 made it possible to use PCR and clone several glycolytic enzymes and obtain their sequences. These enzymes included 10 different cellulases and one each of β-glucosidase, xylanase, lichenase, mannanase, acetyl-xylan esterase, and feruloyl esterase. Most of these enzymes contain two copies of a 40-amino-acid cysteine-rich

noncatalytic domain that functions as a dockerin domain equivalent to the dockerin domain of *C. thermoaceticum*. A similar dockerin domain was found in enzymes from *Piromyces* species and other anaerobic fungi. The fungal dockerin domain has an amino acid composition and sequence different from the dockerin domains of anaerobic bacteria and is designated NCDD (noncatalytic docking domain) or FDD (fungal dockerin domain) (71).

Comparisons of catalytic module sequences of enzymes from anaerobic fungi with aerobic fungal and bacterial enzyme modules indicate that some of the anaerobic fungal enzyme modules have high homology to those of aerobic fungi, whereas others have high homology to the active sites of enzymes from bacteria. This observation indicates horizontal gene transfer from different sources to the anaerobic fungi (**Figure 5**). It also demonstrates that in anaerobic fungi modules from different sources are combined to form complete modular enzymes. Gene duplication also seems to be common in anaerobic fungi. Microorganisms living in close contact, as they do in the intestines of animals, might exchange genetic material representing modules and combine to form functioning enzymes (46). A recent book describing cellulosomes from different bacteria and anaerobic fungi is available (73).

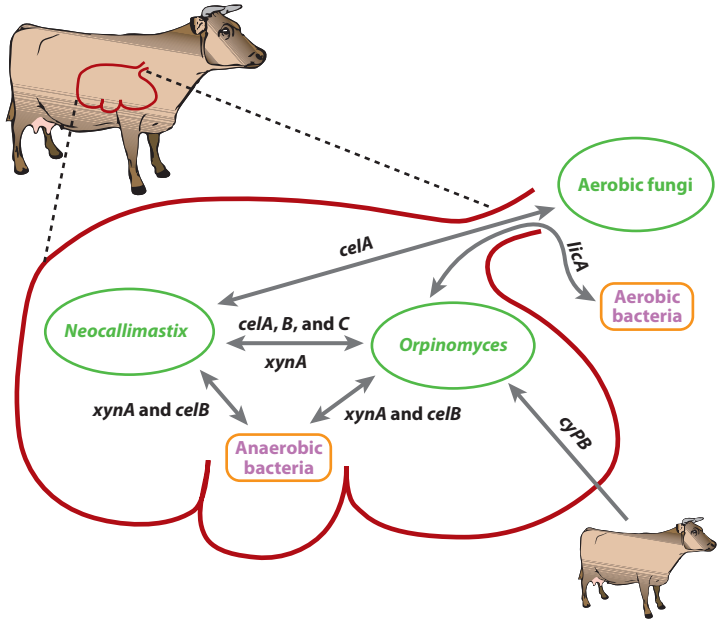


Figure 5

Horizontal transfer of genes between the host cow, anaerobic fungi, and bacteria present in the rumen, and aerobic fungi and bacteria introduced into the rumen from the outside environment. Gene designations are *celA*, *B*, *and C*, cellulases; *xynA*, xylanase; *licA*, lichenase; and *cyPB*, cyclophilin. Reprinted from *Cellulosome*, Cellulosome of anaerobic fungi, pp. 147–87, copyright 2006, LG Ljungdahl, HJM Op den Camp, HJ Gilbert, HR Harhangi, et al., with permission from Nova Science Publishers, Inc.

SOME REFLECTIONS

In writing this chapter I have concentrated on my education and on work performed in my laboratory. I have not reflected much on the importance or consequences of what we did and discovered. The world is in turmoil; we are using our resources at an accelerated rate to feed and sustain an increasing population, and we are wasting our resources on warfare. We are destroying the earth and something ought to be done to cure the cancer of it. Can microbiology be a positive force and help us? I remember not too long ago some prominent scientists almost abandoned microbiology by saying "*Escherichia coli* is the only bacterium we need to research to understand microbiology."

How wrong they were! We now have a new kingdom, the Archaea, that includes methanogenes, halophiles, and extreme thermophiles (81). The amount of bacterial biomass is larger than all other biomass on Earth (74), and new archaea and bacteria are found every day. We have isolated only the most obvious. There are still an untold number to be isolated. The acetogenic group of bacteria, to which *M. thermoacetica* belongs, is a good example of recent isolates. Only 7 acetogenic bacteria were isolated before 1980, 32 were described between 1980 and 1990, 53 between 1990 and 2000, and 11 after 2000 (18). How many are left to be found? The amount of CO_2 fixed autotrophically via the acetyl-CoA pathway is large. It has been estimated that 12×10^{10} kg of CO_2 are fixed by acetogenic bacteria per year in the anaerobic environment, an equal amount is fixed in the hindgut of termites, and

3.9×10^9 kg of CO_2 is converted to acetate per year in the guts of the world's human population. It is possible that acetogens fix and recycle as much as 20% of the earth's total fixation of CO_2 per year (85). The acetyl-CoA pathway is considered the main autotrophic pathway of CO_2 fixation by bacteria, but it is not the only pathway. Thauer (72) pointed out the existence of five known pathways. Microbial CO_2 fixation is a significant process and ought to be considered for sequestering CO_2 to solve global warming.

The pursuit of the pathway of acetate synthesis from CO_2 was undertaken for the sake of knowledge, with no practical goal. I remember, after having given a lecture for undergraduate students, being asked, "How can you find it interesting to work with one bacterial species throughout your life?" That is a legitimate question. I believe my answer was similar to what Sandegren told me when I started work at the Stockholm Brewery Company: "You will learn a lot about life by studying one species until you really understand it." I have enjoyed working with *M. thermoacetica* tremendously and I have learned a lot of microbiology, cell biology, and biochemistry. As shown here, the acetyl-CoA pathway depends on many metal enzymes, forcing me to learn about metal biochemistry. The enzymes of the pathway depend on several vitamins and cofactors, such as corrinoids, and I found them fascinating. Life at high temperatures was another aspect of *M. thermoacetica* that has furthered my knowledge about the stability of proteins, cell membranes, and organelles. Finally, as I proceeded with my research I applied new methods of genetics, molecular biology, biophysics, and bioinformatics. What a wonderful experience!

The acetyl-CoA pathway has garnered speculations about the earliest form(s) of life (53). The autotrophic fixation of CO_2 forming acetate is the most direct pathway for forming acetyl-CoA, which may be the primary building block of life. It is a simpler and more direct pathway than the reductive pentose cycle. The acetyl-CoA pathway is used in anaerobes and could have been utilized before the advent of O_2 on Earth. H_2, CO_2, CO, and metals such as cobalt, nickel, iron, tungsten, molybdenum, and selenium, which are involved in the catalysis of the acetyl-CoA pathway, were present on Earth's crust. Finally, the pathway is prominent in thermophilic and anaerobic bacteria, which developed early. All things considered, it makes sense to postulate that the acetyl-CoA pathway is a primordial biochemical pathway (85).

My work on cellulolytic anaerobic bacteria and fungi was triggered first during my time at the Stockholm Brewery Company and later with Wiegel during the oil crisis of the 1970s. I thought that cellulose could be used as a source of fuel and industrial feedstock. It requires efficient enzymes to hydrolyze plant carbohydrates to yield fermentable sugars, which can then be fermented by other bacteria to desired products. The work was also considered intriguing because it involved thermophiles. Work on cellulases then became interesting following the discovery of the cellulosome by Lamed and Bayer (36). The finding that cellulases include exocellular organelles such as cellulosomes and polycellulosomes led to a new research area involving modular proteins with dockerins and scaffoldins with cohesins. The cellulosome is a treasure trove for biotechnology (7), and anaerobic cellulolytic bacteria will with high certainty be used industrially (15).

During my 10-year editorship for the ASM journal *Applied and Environmental Microbiology*, I learned how well humankind is served by various microorganisms. Unfortunately, most people are afraid of microorganisms; it is true there are some bad ones, but most of them are our best friends. They help us clean and sustain Earth. Many microorganisms will be used for the production of food, medicine, alternative fuels, energy, and industrial chemicals. We need to conduct more research to understand what important benefits microorganisms have for us and life on Earth.

I have been honored with two symposiums during my life: The first, arranged by Harold Drake, Stephen Ragsdale, and Juergen Wiegel, was called "The Arts of Anaerobes" and

coincided with my seventieth birthday. The second was arranged by Juergen Wiegel, Robert Maier, and Michael Adams and was called "Incredible Anaerobes: From Physiology to Genomics to Fuel." It was in connection with my eightieth birthday and my retirement. The speakers were invited to publish their lectures (79). My appreciation and thanks go to those who arranged the symposiums, the presenters, and to all that participated. They were fantastic occasions. To be so honored while still alive and able to fully participate provided emotional experiences that words cannot begin to express. All I can say is thanks.

I would like to express my appreciation for all that have collaborated with me in my laboratory and for all I have interacted with. I also want to express my appreciation for the wonderful hospitality I experienced visiting my friends around the world in Japan, Germany, The Netherlands, Switzerland, Brazil, Taiwan, Italy, and of course the United States. One honor I am particularly proud of was my election to the Swedish Royal Academy of Engineering Sciences. I want to thank the two wonderful women in my life who greatly contributed to my ability to focus on my science: Britten, who died in 1995, and Despy, who I married in 1998.

DISCLOSURE STATEMENT

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

ACKNOWLEDGMENTS

I would like to acknowledge the many sources of financial support, in particular the Georgia Power Company for supporting me as Georgia Power Distinguished Professor in Biotechnology at the University of Georgia, the National Institutes of Health, and the U.S. Department of Energy. I wish to express my thanks to Kathleen Robichaud at Georgia Research Alliance; my daughter, Ann-Sofie Rhedin, and my son, Per O. Ljungdahl for their help in preparing this manuscript. This paper is dedicated to the memory of Britten and to my loving wife, Despy.

LITERATURE CITED

1. Allen MB. 1953. The thermophilic aerobic sporeforming bacteria. *Bacteriol. Rev.* 17:125–73
2. Andreesen JR, Gottschalk G, Schlegel HG. 1970. *Clostridium formicoaceticum* nov. spec. isolation, description and distinction from *C. aceticum* and *C. thermoaceticum*. *Arch. Mikrobiol.* 72:154–74
3. Andreesen JR, Makdessi K. 2008. Tungsten, the surprisingly positively acting heavy metal element for prokaryotes. *Ann. N. Y. Acad. Sci.* 1125:215–29
4. Andreesen JR, Schaupp A, Neurauter C, Brown A, Ljungdahl LG. 1973. Fermentation of glucose, fructose, and xylose by *Clostridium thermoaceticum*: effect of metals on growth yield, enzymes, and the synthesis of acetate from CO_2. *J. Bacteriol.* 114:743–51
5. Barker HA, Kamen MD. 1945. Carbon dioxide utilization in the synthesis of acetic acid by *Clostridium thermoaceticum*. *Proc. Natl. Acad. Sci. USA* 31:219–25
6. Bayer EA, Belaich JP, Shoham Y, Lamed R. 2004. The cellulosomes: multienzyme machines for degradation of plant cell wall polysaccharides. *Annu. Rev. Microbiol.* 58:521–54
7. Bayer EA, Morag E, Lamed R. 1994. The cellulosome—a treasure-trove for biotechnology. *Trends Biotechnol.* 12:379–86
8. Borneman WS, Akin DE, Ljungdahl LG. 1989. Fermentation products and plant cell wall-degrading enzymes produced by monocentric and polycentric anaerobic ruminal fungi. *Appl. Environ. Microbiol.* 55:1066–73
9. Brock TD, Freeze H. 1969. *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. *J. Bacteriol.* 98:289–97

10. Collins MD, Lawson PA, Willems A, Cordoba JJ, Fernandez-Garayzabal J, et al. 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int. J. Syst. Bacteriol.* 44:812–26
11. Consden R, Gordon AH, Martin AJ. 1944. Qualitative analysis of proteins: a partition chromatographic method using paper. *Biochem. J.* 38:224–32
12. Cormier MJ. 2007. *My Journey Into, Through and Beyond the Ivory Tower*. Chandler, AZ: OPA Publ. 99 pp.
13. Das A, Fu ZQ, Tempel W, Liu ZJ, Chang J, et al. 2007. Characterization of a corrinoid protein involved in the C1 metabolism of strict anaerobic bacterium *Moorella thermoacetica*. *Proteins* 67:167–76
14. Das A, Silaghi-Dumitrescu R, Ljungdahl LG, Kurtz DM Jr. 2005. Cytochrome bd oxidase, oxidative stress, and dioxygen tolerance of the strictly anaerobic bacterium *Moorella thermoacetica*. *J. Bacteriol.* 187:2020–29
15. Demain AL, Newcomb M, Wu JHD. 2005. Cellulose, clostridia, and ethanol. *Microbiol. Mol. Biol. Rev.* 69:124–54
16. Diekert GB, Thauer RK. 1978. Carbon monoxide oxidation by *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. *J. Bacteriol.* 136:597–606
17. Drake HL. 1982. Demonstration of hydrogenase in extracts of the homoacetate-fermenting bacterium *Clostridium thermoaceticum*. *J. Bacteriol.* 150:702–9
18. Drake HL, Gössner AS, Daniel SL. 2008. Old acetogens, new light. *Ann. N. Y. Acad. Sci.* 1125:100–28
19. Drake HL, Hu SI, Wood HG. 1981. Purification of five components from *Clostridium thermoaceticum* which catalyze synthesis of acetate from pyruvate and methyltetrahydrofolate. Properties of phosphotransacetylase. *J. Biol. Chem.* 256:11137–44
20. Dürre P, Andreesen JR. 1983. Purine and glycine metabolism by purinolytic clostridia. *J. Bacteriol.* 154:192–99
21. Edman P. 1945. On the purification and chemical composition of hypertensin (angiotonin). *Arkiv. Kem. Mineral. Geol.* 22A:1–52
22. Edman P. 1949. A method for the determination of amino acid sequence in peptides. *Arch. Biochem.* 22:475
23. El-Ghazzawi E. 1967. New isolation of *Clostridium aceticum* Wieringa and studies on the metabolic physiology. *Arch. Mikrobiol.* 57:1–19
24. Elsden SR. 1962. Photosynthesis and lithotophic carbon dioxide fixation. In *The Bacteria*, ed. IC Gunsalus, RY Stainer, pp. 1–40. New York: Academic
25. Enebo L. 1943. Om termofil cellulosajäsning I. *Svensk Kem. Tidskrift* 5:144–51
26. Enebo L. 1951. On three bacteria connected with thermophilic cellulose fermentation. *Physiol. Plant.* 4:652–66
27. Enebo L, Sandegren E, Ljungdahl LG. 1953. Cell wall decomposing enzymes of barley and malt. II. Cellulase increase during germination and influence of sugars on cellulase activity. *J. Inst. Brew.* 59:205–11
28. Fontaine FE, Peterson WH, McCoy E, Johnson MJ, Ritter GJ. 1942. A new type of glucose fermentation by *Clostridium thermoaceticum*. *J. Bacteriol.* 43:701–15
29. Friedrich W, Bernhauer K. 1956. Chemistry and biochemistry of cobalamines. 1. Alkylation of vitamin B12-factor III and vitamin B12. *Chem. Ber.* 89:2030–44
30. Gottwald M, Andreesen JR, LeGall J, Ljungdahl LG. 1975. Presence of cytochrome and menaquinone in *Clostridium formicoaceticum* and *Clostridium thermoaceticum*. *J. Bacteriol.* 122:325–28
31. Guest JR, Friedman S, Woods DD, Smith EL. 1962. A methyl analogue of cobamide coenzyme in relation to methionine synthesis by bacteria. *Nature* 195:340–42
32. Hugenholtz J, Ljungdahl LG. 1990. Metabolism and energy generation in homoacetogenic clostridia. *FEMS Microbiol. Rev.* 7:383–89
33. Irion E, Ljungdahl L. 1965. Isolation of factor 3m coenzyme and cobyric acid coenzyme plus other B12 factors from *Clostridium thermoaceticum*. *Biochemistry* 4:2780–90
34. Kataeva IA, Uversky VN, Brewer JM, Schubot F, Rose JP, et al. 2004. Interactions between immunoglobulin-like and catalytic modules in *Clostridium thermocellum* cellulosomal cellobiohydrolase CbhA. *Protein Eng. Des. Select.* 17:759–69
35. Kerby R, Zeikus JG. 1983. Growth of *Clostridium thermoaceticum* on H_2/CO_2 or CO as energy source. *Curr. Microbiol.* 8:27–30

36. Lamed R, Setter E, Bayer EA. 1983. Characterization of a cellulose-binding, cellulase-containing complex in *Clostridium thermocellum*. *J. Bacteriol.* 156:828–36
37. Lentz K, Wood HG. 1955. Synthesis of acetate from formate and carbon dioxide by *Clostridium thermoaceticum*. *J. Biol. Chem.* 215:645–54
38. Ljungdahl L. 1981. Trace elements and the synthesis of acetate by *Clostridium thermoaceticum*. In *Science and Scientists: Essays by Biochemists, Biologists and Chemists*, ed. M Kageyama, K Nakamura, T Oshima, T Uchida, pp. 89–96. Tokyo: Jpn. Sci. Soc. Press
39. Ljungdahl L, Irion E, Wood HG. 1965. Total synthesis of acetate from CO_2. I. Co-methylcobyric acid and CO-(methyl)-5-methoxybenzimidazolylcobamide as intermediates with *Clostridium thermoaceticum*. *Biochemistry* 4:2771–80
40. Ljungdahl L, Irion E, Wood HG. 1966. Role of corrinoids in the total synthesis of acetate from CO_2 by *Clostridium thermoaceticum*. *Fed. Proc.* 25:1642–48
41. Ljungdahl L, Sandegren E. 1950. Free amino acid in brewing materials. *Acta Chem. Scand.* 4:1150
42. Ljungdahl L, Wood HG. 1963. Two thermophilic exchange reactions between pyruvate-CO_2 and formate-CO_2 in *Clostridium thermoaceticum*. *Bact. Proc.*: 109
43. Ljungdahl L, Wood HG, Racker E, Couri D. 1961. Formation of unequally labeled fructose 6-phosphate by an exchange reaction catalyzed by transaldolase. *J. Biol. Chem.* 236:1622–25
44. Ljungdahl LG. 1979. Physiology of thermophilic bacteria. *Adv. Microb. Physiol.* 19:149–243
45. Ljungdahl LG. 1986. The autotrophic pathway of acetate synthesis in acetogenic bacteria. *Annu. Rev. Microbiol.* 40:415–50
46. Ljungdahl LG. 2008. The cellulase/hemicellulase system of the anaerobic fungus *Orpinomyces* PC-2 and aspects of its applied use. *Ann. N. Y. Acad. Sci.* 1125:308–21
47. Ljungdahl LG, Andreesen JR. 1976. Reduction of CO_2 to acetate in homo acetate fermenting clostridia and the involvement of tungsten in formate dehydrogenase. In *Microbial Production and Utilization of Gases*, ed. HG Schlegel, G Gottschalk, N Pfennig, pp. 163–72. Göttingen: E. Goltze KG
48. Ljungdahl LG, Bryant F, Carreira L, Saiki T, Wiegel J. 1981. Some aspects of thermophilic and extreme thermophilic anaerobic microorganisms. *Basic Life Sci.* 18:397–419
49. Ljungdahl LG, Eriksson K-E. 1985. Ecology of microbial cellulose degradation. *Adv. Microb. Ecol.* 8:237–99
50. Ljungdahl LG, Op den Camp HJM, Gilbert HJ, Harhangi HR, Steenbakkers PJM, Li X-L. 2006. Cellulosomes of anaerobic fungi. In *Cellulosome*, ed. V Uversky, IA Kataeva, pp. 271–303. New York: Novo Sci. Publ.
51. Ljungdahl LG, Sherod D. 1974. Proteins from thermophilic microorganisms. In *Extreme Environments. Mechanisms of Microbial Adaptation*, ed. MR Heinrich, pp. 147–87. New York: Academic
52. Ljungdahl LG, Wood HG. 1969. Total synthesis of acetate from CO_2 by heterotrophic bacteria. *Annu. Rev. Microbiol.* 23:515–38
53. Martin W, Russell MJ. 2007. On the origin of biochemistry at an alkaline hydrothermal vent. *Philos. Trans. R. Soc. London B Biol. Sci.* 362:1887–925
54. Mayer F, Coughlan MP, Mori Y, Ljungdahl LG. 1987. Macromolecular organization of the cellulolytic enzyme complex of *Clostridium thermocellum* as revealed by electron microscopy. *Appl. Environ. Microbiol.* 53:2785–92
55. Morton TA, Runquist JA, Ragsdale SW, Shanmugasundaram T, Wood HG, Ljungdahl LG. 1991. The primary structure of the subunits of carbon monoxide dehydrogenase/acetyl-CoA synthase from *Clostridium thermoaceticum*. *J. Biol. Chem.* 266:23824–28
56. Noble EP, Stjernohlm R, Ljungdahl L. 1961. Carbohydrate metabolism in leucocytes. III. Carbon dioxide incorporation in the rabbit polymorphonuclear leukocyte. *Biochim. Biophys. Acta* 49:593–95
57. O'Brien WE, Ljungdahl LG. 1972. Fermentation of fructose and synthesis of acetate from carbon dioxide by *Clostridium formicoaceticum*. *J. Bacteriol.* 109:626–32
58. Orpin CG. 1975. Studies on the rumen flagellate *Neocallimastix frontalis*. *J. Gen. Microbiol.* 91:249–62
59. Partridge SM, Blombäck B. 1979. Pehr Victor Edman, 14 April 1916–19 March 1977. *Biogr. Mem. Fellows R. Soc.* 25:241–65
60. Payne WJ. 1970. Energy yields and growth of heterotrophs. *Annu. Rev. Microbiol.* 24:17–52

61. Pierce E, Xie G, Barabote RD, Saunders E, Han CS, et al. 2008. The complete genome sequence of *Moorella thermoacetica* (f. *Clostridium thermoaceticum*). *Environ. Microbiol.* 10:2550–73
62. Pinsent J. 1954. The need for selenite and molybdate in the formation of formic dehydrogenase by members of the coli-aerogenes group of bacteria. *Biochem. J.* 57:10–16
63. Poston JM, Kuratomi K, Stadtman ER. 1964. Methyl-vitamin B12 as a source of methyl groups for the synthesis of acetate by cell-free extracts of *Clostridium thermoaceticum*. *Ann. N. Y. Acad. Sci.* 112:804–6
64. Ragsdale SW. 2007. Nickel and the carbon cycle. *J. Inorg. Biochem.* 101:1657–66
65. Ragsdale SW, Clark JE, Ljungdahl LG, Lundie LL, Drake HL. 1983. Properties of purified carbon monoxide dehydrogenase from *Clostridium thermoaceticum*, a nickel, iron-sulfur protein. *J. Biol. Chem.* 258:2364–69
66. Ragsdale SW, Ljungdahl LG, DerVartanian DV. 1983. 13C and 61Ni isotope substitutions confirm the presence of a nickel (III)-carbon species in acetogenic CO dehydrogenases. *Biochem. Biophys. Res. Commun.* 115:658–65
67. Ragsdale SW, Wood HG. 1985. Acetate biosynthesis by acetogenic bacteria. Evidence that carbon monoxide dehydrogenase is the condensing enzyme that catalyzes the final steps of the synthesis. *J. Biol. Chem.* 260:3970–77
68. Russell MJ, Martin W. 2004. The rocky roots of the acetyl-CoA pathway. *Trends Biochem. Sci.* 29:358–63
69. Schulman M, Parker D, Ljungdahl LG, Wood HG. 1972. Total synthesis of acetate from CO_2. V. Determination by mass analysis of the different types of acetate formed from $^{13}CO_2$ by heterotrophic bacteria. *J. Bacteriol.* 109:633–44
70. Shannon KW, Rabinowitz JC. 1988. Isolation and characterization of the *Saccharomyces cerevisiae* MIS1 gene encoding mitochondrial C1-tetrahydrofolate synthase. *J. Biol. Chem.* 263:7717–25
71. Steenbakkers PJ, Li XL, Ximenes EA, Arts JG, Chen H, et al. 2001. Noncatalytic docking domains of cellulosomes of anaerobic fungi. *J. Bacteriol.* 183:5325–33
72. Thauer RK. 2007. Microbiology. A fifth pathway of carbon fixation. *Science* 318:1732–33
73. Uversky V, Kataeva IA, eds. 2006. *Cellulosome*. New York: Nova Sci. Publ. 317 pp.
74. Whitman WB, Coleman DC, Wiebe WJ. 1998. Prokaryotes: the unseen majority. *Proc. Natl. Acad. Sci. USA* 95:6578–83
75. Wiegel J, Braun M, Gottschalk G. 1981. *Clostridium thermoautotrophicum* species novum, a thermophile producing acetate from molecular hydrogen and carbon dioxide. *Curr. Microbiol.* 5:255–60
76. Wiegel J, Dykstra M. 1984. *Clostridium thermocellum*: adhesion and sporulation while adhered to cellulose and hemicellulose. *Appl. Microbiol. Biotechnol.* 20:59–65
77. Wiegel J, Ljungdahl LG. 1981. *Thermoanaerobacter ethanolicus* gen. nov., spec. nov., a new, extreme thermophilic, anaerobic bacterium. *Arch. Microbiol.* 128:343–48
78. Wiegel J, Ljungdahl LG, Rawson JR. 1979. Isolation from soil and properties of the extreme thermophile *Clostridium thermohydrosulfuricum*. *J. Bacteriol.* 139:800–10
79. Wiegel J, Maier RJ, Adams MWW, eds. 2008. *Incredible Anaerobes: From Physiology to Genomics to Fuels*, Vol. 1125. Boston: Blackwell Publ./NY Acad. Sci. 373 pp.
80. Wieringa KT. 1936. Over het verdwijnen van waterstof en koolzuur onder anaerobe voorwaarden. *Antonie Leeuwenhoek* 3:263–73
81. Woese CR, Kandler O, Wheelis ML. 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci. USA* 87:4576–79
82. Wood HG. 1952. A study of carbon dioxide fixation by mass determination of the types of C13-acetate. *J. Biol. Chem.* 194:905–31
83. Wood HG. 1952. Fermentation of 3, 4–^{14}C-and 1–^{14}C-labeled glucose by *Clostridium thermoaceticum*. *J. Biol. Chem.* 199:579–83
84. Wood HG, Katz J. 1958. The distribution of ^{14}C in the hexose phosphates and the effect of recycling in the pentose cycle. *J. Biol. Chem.* 233:1279–82
85. Wood HG, Ljungdahl LG. 1991. Autotrophic character of the acetogenic bacteria. In *Variations in Autotrophic Life*, ed. JM Shively, LL Barton, pp. 201–50. San Diego: Academic
86. Wood HG, Stjernholm R. 1962. Assimilation of carbon dioxide by heterotrophic organisms. In *The Bacteria*, ed. IC Gunsalus, RY Stainer, pp. 41–117. New York: Academic

87. Yamamoto I, Saiki T, Liu SM, Ljungdahl LG. 1983. Purification and properties of NADP-dependent formate dehydrogenase from *Clostridium thermoaceticum*, a tungsten-selenium-iron protein. *J. Biol. Chem.* 258:1826–32
88. Zverlov VV, Velikodvorskaya GV, Schwarz WH, Bronnenmeier K, Kellermann J, Staudenbauer WL. 1998. Multidomain structure and cellulosomal localization of the *Clostridium thermocellum* cellobiohydrolase CbhA. *J. Bacteriol.* 180:3091–99
89. Ljungdahl L, Wood HG. 1965. Incorporation of C^{14} from carbon dioxide into sugar phosphates, carboxylic acids, and amino acids by *Clostridium thermoaceticum*. *J. Bacteriol.* 89:1055–64
90. Kataeva IA, Blum DL, Li XL, Ljungdahl LG. 2001. Do domain interactions of glycosyl hydrolases from *Clostridium thermocellum* contribute to protein stability? *Protein Eng.* 14:167–72