ANNUAL REVIEW OF MICROBIOLOGY

VOL. 45 1991

ANNUAL REVIEW OF MICROBIOLOGY

VOLUME 45, 1991

L. NICHOLAS ORNSTON, *Editor*
Yale University

ALBERT BALOWS, *Associate Editor*
Centers for Disease Control, Atlanta

E. PETER GREENBERG, *Associate Editor*
University of Iowa, Iowa City

ANNUAL REVIEWS INC. 4139 EL CAMINO WAY P.O. BOX 10139 PALO ALTO, CALIFORNIA 94303-0897

ANNUAL REVIEWS INC.
Palo Alto, California, USA

International Standard Serial Number: 0066-4227
International Standard Book Number: 0-8243-1145-0
Library of Congress Catalog Card Number: 49-432

Typesetting by Kachina Typesetting Inc., Tempe, Arizona; John Olson, President
Typesetting Coordinator, Janis Hoffman

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA

PREFACE

There is no dearth of information in our age. Indeed, the abundance of accessible data could be regarded as a protective covering that hides knowledge from our view. Should one have any doubts about this, ask any student what represents the greatest obstacle to learning. What knowledge is likely to be most important? How do we bring it forth without leaving behind something that is even more important? The driving force in this quest must be curiosity. The quality of science, as it is learned or as it is taught, is determined by the quality of the questions posed.

A good scientist, nascent or established, asks bold questions and tests them rigorously. In this context, existing information is called upon and new information gained. Perhaps the most difficult lesson for students is that present knowledge is not absolute. After years of taking multiple choice tests, students are not prepared to recognize that an insight challenging established knowledge is a source of delight. One way to trigger their curiosity is to introduce a simple concept with myriad consequences. For example, creatures with small size and limited genome are likely to be specialists that enter into consortia with other organisms.

One can ask the student, as an act of imagination, to become the microorganism: From the perspective of its niche, review the creature's requirements and opportunities, and allocate resources to optimize survival. Abandon preconceptions. A microorganism may behave very badly (when viewed from its host's perspective), but the imaginary microorganism's role should be acted with zeal. Remember that anyone who has attempted to lead a good and decent life is entitled to a few hours playing the villain. Furthermore, a visit to the soul of a villain may give valuable insight into the cause of villainous behavior. There are no recorded instances of microorganisms possessing souls, but a visit to the world-view of a microorganism may prove enlightening to the microbiologist. Such visits make vivid the diversity of microorganisms and their essential role in the balance of Nature.

The sources of delight within this volume were selected by the members of the Editorial Committee of the *Annual Review of Microbiology*. Good actors all, they have visited distant places and returned to tell the tale. They had questions that demanded the information provided in the review articles, and this information was shaped into knowledge by questions posed by both the

(continued)

reviewers and the authors of the reviewed articles. All of these individuals deserve our gratitude.

Guests of the editorial board in the planning of this volume were Edward Katz, Gary Sayler, and Simon Silver. Their contributions are deeply appreciated. Production of this volume remains in the highly capable hands of Amanda Suver. Rosemarie Hansen has assumed the difficult task of organizing the editor, and her successes are testament to remarkable skill.

L. NICHOLAS ORNSTON
EDITOR

Annual Review of Microbiology
Volume 45, 1991

CONTENTS

(continued)

Annu. Rev. Microbiol. 1991. 45:1–35

MY KIND OF BIOLOGY

Ralph S. Wolfe

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

KEY WORDS: methanogenic bacteria, ferredoxin, Archaebacteria, coenzymes of methanogenesis, interspecies hydrogen transfer

CONTENTS

Because the rigid format of scientific publishing renders the published product nearly sterile of the fun of doing science, I have attempted to provide here some background and perspective on the contributions to science in which my laboratory played a part.

Toward Microbiology

I was 25 years old before I saw a living bacterial cell. As an undergraduate, I had majored in biology at Bridgewater College in Virginia, a small liberal arts college. Undergraduate biology at that time was mostly descriptive, but "Doc" Jopson in the biology department insisted that I take organic chemistry

0066-4227/91/1001-0001$02.00

and minor in chemistry; this advice was sound. I had been raised on the campus of a small college where my father taught religion and philosophy. He would go to the campus, present a lecture, and return home to work in his shop or garden. His summers were free of college obligations. To me in my late teens, this seemed an ideal way to spend one's life. The pay was poor, but the freedom was fantastic—especially with a three-month summer. After I had taught high school for a brief period, a friend who had just received his Master's degree in history at the University of Pennsylvania recommended Penn as a "good university"; so I hitchhiked to Philadelphia intent on getting a MS degree and then teaching in a small college. (I never reached the small college.)

In my youth, I had been fascinated by petrified bones and had thought that working in a museum or digging up petrified skeletons would be exciting. In Philadelphia, I went to the Academy of Natural Sciences, visited the paleontology research area of the museum, and talked to a curator. The experience was rather sobering. Here was an investigator in a dimly lit area working under a light bulb, surrounded by what seemed to be acres of petrified bones. I remember thinking on my way out that I would have to find something more alive than this.

A fixture at many universities are frustrated individuals who enjoy keeping graduate students in line. When I inquired at the graduate college office of the university about the possibility of doing graduate work in biology, I was told, "We don't have a department of biology. We have a department of botany and a department of zoology; now which will it be?" Off the top of my head I blurted out, "botany." "Well in that case, you must go to McFarlane Hall and talk with Dr. Schramm." Professor Schramm was a white-haired kindly man who seemed interested in me. Instead of asking what courses I had taken, he inquired as to what I knew about various subjects. One question in particular stands out in my memory: "Do you know anything about bacteriology?" I replied that I knew nothing, but that I had often thought that might be an interesting subject. So, he signed me up for general bacteriology. This was the only course I could take because I needed to find employment to support myself, and I asked him if any jobs were available in the department. A job in the herbarium involved mounting pressed flowers on sheets of paper; would I be interested? A job was a job, and since I was to be a student in botany this seemed to be an ideal way to learn the names of flowers. Besides, I could set my own hours. So my career as a graduate student in botany was launched.

I was enthusiastic, and on my way to the first class in bacteriology I wondered if I would get to see a living bacterial cell. I did; I was fascinated. As the laboratory course unfolded, the fact that one could start to grow these living cells in the afternoon and the next morning could read the results seemed incredible to me. This was my kind of biology. The course was taught

by W. G. Hutchinson, and he turned me on to bacteriology. I did well—after all I was only taking one course—and when an opening occurred for a teaching assistant in bacteriology, Professor Hutchinson invited me to assist him. I was elated and did not hesitate to leave the job of mounting flowers. It had become painfully obvious to me that something was wrong; I could not remember the names of the plants! In contrast, I had no trouble remembering the names of bacteria.

So as my second year in graduate school began, I moved to the old public health laboratory on 34th Street where Professor Hutchinson introduced me to a young assistant professor, D. J. O'Kane, who had just arrived from Cornell and was setting up his laboratory. I was to be a teaching assistant in general bacteriology and would take a new course being developed by O'Kane to give students some exposure to biochemical activities of microbes. I never had a formal course in biochemistry, and sometimes it shows.

As a teaching assistant, I soon found that a successful laboratory course was based on thorough preparation. The degree of success depended on attention to details; everything must be checked: the cultures, the media, the glassware, the incubator. Assume nothing! It was great when everything worked. I especially enjoyed being a teaching assistant with W. G. Hutchinson. However, his interests were not in the area of bacterial physiology and metabolism, an area that was on the forefront at the time and that seemed attractive to me. So O'Kane accepted me as a graduate student, one intent on getting a terminal Master of Science degree. I worked on the enzyme hippicurase, from *Streptococcus*, a hydrolytic enzyme that cleaved a peptide-like bond and was considered of interest because the mechanism of peptide bond synthesis was unknown at the time. My initial attempts at research were rather painful for my professor, I'm afraid, especially when I didn't even know how to plot the data I generated. I needed to be spoonfed. Eventually, I found that doing the experiment on my own without letting my professor know was fun. When I thought I had established a scientific fact, we would talk. I liked this system; the thrill of discovery on one's own is the best motivating force.

After receiving a MS degree, I decided to take a year off from graduate school. Graduate students were paid $950 for nine months, so it was necessary to save enough money during that period to live during the summer. I wanted to see what nonacademic life was like and try to shore up my financial condition. I accepted a position as a technician in Ruth Patrick's laboratory at the Academy of Natural Sciences. She had become interested in devising ways for assaying the toxicity of stream pollutants. This work broadened my appreciation of biology. My professors at Penn encouraged me to continue graduate studies toward a PhD degree, but before returning to the university, my life became complete; Gretka Young, who worked for the American Friends Service Committee, and I were married in September, 1950. My goal

of teaching in a small college would become untenable in the next three years as my professors made me aware that the PhD degree was a research degree, that to do research one needed proper equipment, and that adequate facilities were rarely found in a small college. I eventually would realize that I had been scientifically seduced.

Professor O'Kane offered me a choice of two thesis topics involving a new factor, the pyruvate oxidation factor, which he had worked on in the laboratory of I. C. Gunsalus at Cornell. This factor was later identified as lipoic acid. He wanted to know whether lipoic acid was involved in the oxidation of pyruvate by *Escherichia coli* and *Clostridium butyricum*. I instantly chose the clostridial system because it seemed challenging and unknown. I was able to show with treated extracts that diphosphothiamin, coenzyme A, and ferrous ions were required for the oxidation of pyruvate, but I could find no role for lipoic acid (166). Various dyes could be used as electron acceptors to bypass hydrogenase; however, I could not make progress on the natural electron acceptor. Exchange of $^{14}CO_2$ with the carboxyl group of pyruvate occurred readily (167). These experiments introduced me to the use of radioisotopes. I completed my thesis work in June 1953, a few months after our first child, Danny, was born.

The event that encouraged me to consider the possibility of an academic position in a research environment involved the preparation of my first manuscript from part of my thesis. I was apprehensive about submitting a manuscript to the *Journal of Biological Chemistry* because I lacked confidence in writing. However, I carefully patterned the manuscript in the style of the journal and gave it to my professor. He returned it and announced, "I think this is fine, Ralph; let's send it in." We did, and it was accepted! This was the first time anyone had ever expressed approval of my writing and gave me confidence that perhaps I could become successful at scientific writing. I owe much to D. J. O'Kane, who made me appreciate the importance of hard data in nailing down a concept as well as the importance of freedom in exploring and making discoveries. Later, I would use this same philosophy in running my own laboratory.

Very few academic jobs were available in the summer of 1953. I had two interviews, one at a small college, which had only a case-full of student microscopes as equipment, and one at the University of Illinois. I was hired as an instructor at Urbana by H. O. Halvorson. My case had been presented in a sufficiently positive way through the efforts of my scientific grandfather, I. C. Gunsalus (known to everyone as Gunny). The Department of Bacteriology was an exciting place; with recent appointments of Halvorson, Spiegleman, Luria, Gunsalus, and Juni, the department was considered one of the best in the country, and I was fortunate to join it. A heavy teaching load didn't leave much time for research, and my program was rather slow in evolving. Before

I started anything, I was determined to prepare a manuscript for publication from the last part of my thesis. So I closed my office door whenever I had a chance and worked on the manuscript.

This action was interpreted as inaction but led to a revelation of one of the truisms of academic life. One day there was a knock on my office door. Professor Halvorson entered, sat down, and in a very concerned manner said, "I just want to tell you one thing—you are paid to teach; you get promotions for doing research." He departed immediately, and I pondered these words of wisdom. They are as true today as they were 38 years ago.

The faculty wanted to augment their specialties with someone who had a real interest in diverse organisms and who would want to teach a van Niel–type course. Largely through persuasion by Gunny, van Niel accepted me as an observer in his course at Pacific Grove for the summer of 1954. The class was a fantastic experience that opened my eyes to a microbial world of unfamiliar organisms and made it possible for me to attempt to fill the niche for which I had been hired at Urbana. I returned to Illinois with many ideas from van Niel that, together with some from Gunny, Luria, Sherman, and myself, became an organisms course that would be taught for nearly three decades.

When I left Penn, O'Kane generously allowed me to take the pyruvate clastic system with me to serve as a basis for my research program. In response to an inquiry about equipment that I would need to get started, I had suggested to Professor Halvorson a colorimeter, a vacuum pump (with which to freeze dry cell extracts) and a Warburg apparatus. He seemed a little dismayed by this, and I thought his response was a bit curious, for the request seemed modest to me. A year later, I was told, "We have enough biochemists around here; I hired you because I thought you weren't one." The message was clear—I had better begin visibly studying unusual organisms or I did not have a future at Illinois. Having a mandate to study such organisms was great, but I also instinctively knew that to gain the respect of the scientific community I must be involved in an in-depth study of a biochemical phenomenon. Isolation and cultivation of "funny bugs" (although challenging and rewarding) alone was not enough. I knew my abilities would limit how far I could go, but I was determined to become a respectable microbiologist.

Ferredoxin

As a graduate student I poured ferredoxin down the sink for three years. Fortunately, much later one of my own graduate students was involved in its discovery. At the time of my studies, the research community knew that clostridia did not possess cytochromes. Because no other protein electron carriers were known, we assumed that the unknown electron acceptor for pyruvate oxidation would be a soluble cofactor. Formation of carbon dioxide

and acetyl phosphate from pyruvate could readily be followed in treated cell extracts upon addition of dyes, but I could find no evidence for a coenzyme that could play a role similar to nicotinamide adenine dinucleotide (NAD) or flavin adenine dinucleotide (FAD). When I accepted my first graduate student, Robert Mortlock, at Urbana, I suggested that he identify the electron acceptor and study the reversal of the reaction. He obtained convincing evidence for synthesis of pyruvate from acetate and carbon dioxide (99, 100). The nature of the electron acceptor remained obscure, however.

In the summer of 1957, an undergraduate student, Raymond Valentine, expressed an interest in learning about research with bacteria, and I suggested that he begin by helping Mortlock with studies on the pyruvate clastic reaction. Valentine was highly motivated, and the two developed an isopropanol precipitate of crude cell extract that actively decarboxylated pyruvate with methyl-viologen as electron acceptor. He realized the significance of this resolved extract and wanted to pursue study of the natural electron acceptor for a thesis.

One of the most difficult decisions in science is deciding when to quit one line of research and start a new one. There is always the possibility that one more cast might do it. I had been casting for a strike on the electron acceptor for about eight years and was eager to try something new. So, much to Valentine's disappointment, we dropped the clastic reaction and began to study anaerobic allantoin degradation. To augment Valentine's training, arrangements were made for him to spend a summer working on organic synthesis with Roe Bloom, an organic chemist with whom I was acquainted at the DuPont Company in Wilmington, Delaware. In the meantime, a paper by the DuPont nitrogen fixation group headed by Carnahan appeared. Mortenson had found that the addition of pyruvate to extracts of *Clostridium pasteurianum* greatly stimulated nitrogen fixation. We realized that the electron acceptor from pyruvate oxidation for which we had an assay might be the electron donor for nitrogenase, and Valentine communicated this to Gunny, who was a consultant to DuPont at the time. Gunny picked up the phone and arranged for Valentine to join the nitrogen fixation group for the summer. He applied the isopropanol precipitation procedure developed in Urbana to extracts of *C. pasteurianum* and produced a methyl-viologen-dependent pyruvate clastic reaction. With this assay, he began to test various nitrogenase preparations and fractions. Mortenson suggested that he should test a brown preparation stored in a certain refrigerator, a fraction prepared by Ralph Hardy before he left the company. When he opened the refrigerator, he found two bottles, each of which contained a brown fluid. The first one tested instantly denatured the proteins of the assay mixture; it turned out to be chromate cleaning solution. A sample from the second complemented the resolved enzyme preparation; the electron carrier had been located and could

donate electrons to hydrogenase. (The first name considered for the carrier was "co-hydrogenase.") Under Mortenson's leadership, the protein was fractionated, purified, and shown to be an iron-sulfur protein, a new type of electron carrier. The naming group at the DuPont Company named this new product ferredoxin.

Upon returning to Urbana, Valentine wanted to pursue ferredoxin as a thesis, but I felt that the discovery had been made in DuPont laboratories, and to continue to study the ferredoxin of a saccharolytic *Clostridium* in my laboratory would be inappropriate. However, looking for ferredoxin in other anaerobes would not be a problem. We used the finding of ferredoxin in *Micrococcus lactilyticus* as a lever with which to pry the original proprietary data on *C. pasteurianum* into publication, and the papers were published back-to-back (98, 139). In the next 10 years, hundreds of papers on this new type of iron sulfur protein electron carrier, ferredoxin, appeared. We explored several ferredoxin-dependent reactions (18, 19, 136, 137, 140, 145).

I wished to know if the pyruvate reactions in *Bacillus macerans*, one of the sugar-fermenting members of the genus *Bacillus*, were similar to those of *C. butyricum*. Raymond Hamilton (65) studied the exchange reactions of CO_2 and of formate with the carboxyl group of pyruvate and found that the CO_2 exchange reaction was similar to that in *C. butyricum*, but that a separate formate-pyruvate exchange reaction also occurred.

Methanobacillus omelianskii

In 1960, I arranged to spend my first sabbatical leave in the laboratory of Sidney Elsden at Sheffield, England. He had been a student of Marjorie Stephenson and C. B. van Niel and was interested in microbial physiology. I thought he would be an ideal sounding board for the major purpose of my sabbatical, which was to write a guide to the isolation of unusual organisms from nature, for which I had received a Guggenheim Fellowship. After about a month in the library, I realized that this project wasn't much fun, so I threw down the pencil and moved to a lab bench. We decided that methane bacteria would be a good project. H. A. Barker kindly sent us a culture of *Methanobacillus omelianskii*, which I was able to culture. Our purpose was to study the synthesis of amino acids from [^{14}C]-labeled CO_2 and acetate by this unusual anaerobe. We got off to a good start; the labeling patterns were definitive on several amino acids, and Martin Knight continued the project for his thesis (85).

I returned to Urbana convinced that *M. omelianskii* could be mass cultured, and that the time was ripe to go after the biochemistry of methanogenesis. My colleague, M. J. Wolin, in the Department of Dairy Science, was interested in this subject, so I invited him to collaborate. We hired Eileen Wolin to initiate cultures and scale them to the 3-liter–florence flask stage. They were then

brought to my laboratory where carboys were inoculated. We developed a production line so that we could harvest a carboy of cells at least three times a week, each carboy yielding enough cells for one experiment. Cells were immediately broken in a Hughes press, and a dark brown cell extract was prepared. Norman Ryckman played an essential role in growing and harvesting cells and in preparing cell extracts. Old Warburg flasks fitted with serum stoppers served as reaction vessels, and samples of the flask's atmosphere were transferred by syringe to a gas chromatograph to test for methane formation. The first extract was tested in October 1961, and for the next five months the recorder pen never moved from the baseline except to respond to a standard injection of methane. Extracts refused to oxidize ethanol or acetaldehyde and reduce CO_2 to methane under any condition. In March, out of desperation, I tipped my old friend, pyruvate, into the extract. I shall never forget the zing of the recorder as the pen soared to the top of the chart and back precisely at the time methane should elute from the column. The first formation of methane by a cell-free extract had occurred (171).

This assay allowed us to optimize the system and to show that the role of pyruvate was to provide electrons, carbon dioxide, and ATP. The discovery by Blaylock & T. Stadtman (11) that the methyl group of methylcobalamin could be reduced to methane by extracts of *Methanosarcina* in the presence of pyruvate was a major breakthrough. M. J. Wolin synthesized methylcobalamin, and we showed the ATP-dependent reduction of the methyl group to methane by extracts of *M. omelianskii* (172) and that B_{12r} was the product (173). John Wood (178) studied the reaction and showed that the cobamide derivatives, methyl-Factor B, and methyl-Factor III also were effective methyl donors for methanogenesis (180). The ferredoxin-dependent conversion of formate or acetaldehyde was worked out by Winston Brill (18, 19), and Wood (176) showed that the methyl group of methyl-tetrahydrofolate was reduced to methane. Additional studies suggested that carbon-3 of serine was a precursor of methane via conventional C_1-tetrahydrofolate intermediates (175). Wood also obtained evidence for alkylation (177) and propylation (179) of a cobamide enzyme involved in methanogenesis. Wolin (170) found that viologen dyes were potent inhibitors of methanogenesis. These experiments were exciting as we groped to figure out how extracts of *"M. omelianskii"* made methane from carbon dioxide. The laboratory of T. Stadtman was the only other group actively working on this project.

Interspecies Hydrogen Transfer

In the summer of 1965, I asked Marvin Bryant, who had recently joined the Department of Dairy Science, if he would teach me the Hungate technique. For the roll tube experiment, he suggested that we carry out an agar dilution series using *M. omelianskii* because one of his colleagues was not satisfied

that the culture met the criteria of a pure culture. We decided to use a rich medium that contained rumen fluid to encourage the growth of any contaminants. In one series of roll tubes, we added H_2 and CO_2. We picked isolated colonies back into the ethanol carbonate medium of Barker, but nothing grew—not an unusual observation when working with methanogenic bacteria at that time. Soon the summer was over, and we returned to other duties. About seven months later, Bryant found a rack of roll tubes from our experiments in the incubator. One tube contained a large isolated colony, and, when he removed the rubber stopper, he found a strong negative pressure. The organism oxidized hydrogen, and a serial dilution in agar roll tubes established that the culture was pure and that it made methane. The organism was labeled strain M.o.H. and would not grow in the ethanol medium of Barker, so Hungate suggested to Bryant that the original culture must contain a companion organism that used ethanol as a substrate, and he should go after it. After many difficulties, Bryant succeeded in isolating the S organism, which was inhibited by the hydrogen that it produced.

M. J. Wolin realized what was going on. He blew the dust from his physical chemistry book and calculated the free energy change for the oxidation of ethanol to acetate and hydrogen vs the partial pressure of hydrogen. *M. omelianskii* was a symbiotic association of two organisms. The S organism oxidized ethanol to acetate and hydrogen; the methanogen, *Methanobacterium* strain M.o.H., lowered the partial pressure of hydrogen by oxidizing it to reduce CO_2 to CH_4; this allowed the anaerobic oxidation of ethanol by the S organism to become thermodynamically favorable. Thus, interspecies hydrogen transfer (the importance of the partial pressure of hydrogen in anaerobic biodegradation), one of the first principles of anaerobic microbial ecology, had been discovered. We thought this paper would be a suitable way to honor C. B. van Niel (22).

With the discovery that *M. omelianskii* was a mixed culture, the roof of my research program more or less collapsed. Much of Winston Brill's thesis could not be published because we did not know which enzymes came from which organism. During this work, I could not figure out why such a bright, dedicated student was having so much difficulty with variability of cell extracts. I have always regretted this and feel that I should have been more astute about the culture. Much of Abdel Allam's thesis could not be published, and Richard Jackson's work with Lovenberg on the amino acid sequence of ferredoxin was in doubt concerning which organism produced the iron sulfur protein. Five years of work on methanogenesis by extracts of *M. omelianskii* needed to be reinterpreted.

The real challenge now was to develop a mass culture technique and a method for growing cells on H_2 and CO_2. I accepted this challenge, but all attempts to culture strain M.o.H. by sparging gas through a liquid medium

failed. In desperation, I developed a closed system in which a diaphram pump recirculated the gas atmosphere over heated copper and back into the culture vessel. I named this gadget the gaspirator. (Spiegleman referred to it as Wolfe's last gaspirator.) It provided a few good runs, but its performance was erratic, and it was abandoned. However, I developed a system for slowly shaking 200 ml of medium under a gas atmosphere of H_2 and CO_2 in a flask with a small continuous addition of gas (21). I was delighted that the inoculum grew when transferred to a 12-liter fermentor and could then be harvested or used to inoculate a 200-liter fermentor. Soon we had kilogram quantities of cells. This work could not have been done without Marvin Bryant, who patiently provided us with inocula of strain M.o.H. Langenberg (87) documented the electron microscopy of strain M.o.H. Anthony Roberton (109) studied the ATP requirement for methanogenesis from methylcobalamin by cell extracts of strain M.o.H. He showed that intracellular pools of ATP increased when cells were oxidizing hydrogen and carrying out methanogenesis, but decreased when hydrogen was removed (110).

Coenzyme M, the Terminal Methyl Carrier

Barry McBride arrived from the University of British Columbia, Vancouver, at a propitious time. A technology for growing cells on H_2 and CO_2 had been developed; he was able to contribute to the mass culture of cells at the 12-liter stage and was the first one to have the courage to grow a 200-liter batch of strain M.o.H on H_2 and CO_2. He discovered a new cofactor that was required for the formation of methane from the methyl group of methylcobalamin by cell extracts. Evidence suggested that this cofactor was involved in methyl transfer, so we named it coenzyme M (CoM) (94). He found a curious inhibition of methanogenesis by DDT (95). In testing various buffers to optimize the assay for CoM, he noticed that a strong garlic-like odor was produced in arsenate buffer, and we (96) documented the synthesis of dimethylarsine by cell extracts from methylcobalamin and arsenate. His work opened up a new era in the biochemistry of methanogenesis, one that we would follow for 20 years. We owe much to Barry McBride, who later pioneered use of the Frêter chamber for handling methanogens, the growth of methanogens on Petri dishes, the fluorescence of methanogen colonies, the use of the epifluorescence microscope to detect individual cells of methanogens, and the bright fluorescence of protozoa. Unfortunately, he did not receive proper acknowledgment for the discovery that individual cells of methanogens fluoresce.

The study of CoM was taken up by Craig Taylor, who over a five-year period purified the factor to homogeneity and determined its structure as 2-mercaptoethanesulfonic acid (128). He showed that the coenzyme was methylated on the reduced sulfur atom to form 2-(methylthio)ethanesulfonic