ANNUAL REVIEW OF MICROBIOLOGY

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L. NICHOLAS ORNSTON, *Editor*
Yale University

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

PAUL BAUMANN, *Associate Editor*
University of California, Davis

ANNUAL REVIEWS INC. 4139 EL CAMINO WAY PALO ALTO, CALIFORNIA 94306 USA

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PREFACE

To be well rounded is to be equally limited in all directions, and microbiologists should never aspire to be well rounded. Microbiology applies an overlapping set of limitless disciplines to understanding of creatures covering a range so diverse that metazoa appear as a blip on the evolutionary scale. Microbiologists must always be on the move, seeking new directions and never forgetting their primary drive, curiosity. We strive for balance, rather than roundedness, as we select topics that bring readers to the perimeters of our science. With *Annual Review of Microbiology*, the difficult choice of topics is made possible by the advice of the editorial committee and guests who gather annually to plan each volume. Members of the editorial committee generally serve for five years; for this volume, terms of appointment were such that no retirements occurred. Unchanged, the committee renewed the vigorous discussion that shaped the preceding volume. The deliberations were enlivened by guests Peter Greenberg and Abdul Matin.

Primary responsibility for success of the volume rests with the authors. We are grateful to them for producing stimulating, well-prepared manuscripts. The most difficult editorial task, copyediting manuscripts with a keen eye for grammar and consistency of expression, was performed by the Production Editor, Dave DeRoche, who will be leaving *ARM* after this volume. We shall miss him greatly, and we wish him continued success in his future ventures. As always, operations in my own office depend entirely on the talents of my gifted administrative specialist, Anne Harrison, and it is a pleasure to express my thanks to her.

L. NICHOLAS ORNSTON
EDITOR

Annual Review of Microbiology
Volume 39, 1985

CONTENTS

(continued)

Ann. Rev. Microbiol. 1985. 39:1–20

MARINE MICROBIOLOGY FAR FROM THE SEA

Robert A. MacLeod

Department of Microbiology, Macdonald College of McGill University, Ste. Anne de Bellevue, PQ H9X 1C0, Canada

CONTENTS

INTRODUCTION

Most of my scientific career has been spent studying marine bacteria in a laboratory located approximately a thousand miles from the sea. The location has not lent itself to studies of the bacteria in their normal habitat but it has permitted me to concentrate on aspects of the biochemistry and physiology of selected organisms. The approach taken has provided insights into some of the ways in which the bacteria studied are similar to and different from bacteria from other habitats. I think it has also indicated how such studies can contribute to an understanding of the ways in which marine bacteria relate to and depend upon their environment for survival and growth.

I am honored by the invitation to contribute this chapter describing aspects of my work and career.

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EARLY YEARS

I was born in Athabasca, Alberta, a tiny town about 100 miles north of Edmonton on a bend in the Athabasca River, which flows north into lake and river systems draining into the Beaufort Sea. My father had come to the Great White North to homestead a half section of land. After returning from overseas at the end of World War I he had taken advantage of the opportunity made available to returning servicemen by the Canadian government to take a short course in agriculture at a Canadian university and to obtain a grant of land. He had elected to take his course at the University of British Columbia in Vancouver and while in Vancouver met and married my mother. My mother had emigrated from England to Canada and had found employment as a nurse and governess caring for the children of a prominent Vancouver family. My father lasted only three years in the north. He had been injured during the war and the hard work and rigorous climate led to complications requiring hospitalization and major surgery. He abandoned the farm and we moved first to Edmonton and then to Vancouver. Fortunately for us my mother's father and mother had emigrated to Canada to live with us. My grandfather had been a quite successful shopkeeper in Hull, England and had sufficient "means" to buy a house for our family. We settled in what was then a rural area near Vancouver on about an acre of land. My father set about to develop a chicken ranch on this property. The operation prospered until the crash of '29. Overnight the price of a dozen eggs fell from nearly a dollar to ten cents. It was cheaper to eat the eggs and the chickens than to sell them.

We were luckier than most of our neighbors. My father received a disability pension for his war injuries that provided a very small but regular income. These years were very hard on my parents, who sacrificed a great deal to ensure that my sister and I would be respectably turned out and able to participate in at least some of the amenities of school and community life. Although we, as children, were thus buffered from the worst of the Depression, we lived in an area of high unemployment. I can well remember the despair of able-bodied men unable to find employment. Heads of families as a last resort went "on relief," a primitive form of welfare whereby men dug ditches and repaired roads in return for just enough money to buy sufficient food to sustain life. I became convinced at an early age that the key to financial security was to obtain a good education and to specialize, and I was determined that this was the direction that I would go. Certain people strongly influenced me to make this decision. This was an important factor, because in our area few young people considered attending university.

One of these influences was the family that my mother had worked for before she was married. The head of the family, Dr. Brock, was a geologist and Dean of the Faculty of Applied Science at the University of British Columbia. Dr.

Brock, his wife, and their five boys had developed a great affection for my mother and remained in close touch with us throughout my childhood. As one can imagine, their example had a strong influence on my expectations for the future. Tragedy befell the Brock family when I was still quite young: Dean and Mrs. Brock were killed in a plane crash. The boys have remained among my closest friends.

My years in high school were undistinguished. The school was poorly equipped, especially for science courses, but the teachers were competent and dedicated. During my third year in high school I was directed by the principal to a person who had called at the school to find out if there were a boy who might be interested in doing some gardening part-time. Part-time jobs were hard to obtain in those Depression years, so I jumped at the opportunity. I could not have guessed at the time, however, how really lucky I was.

Dr. Blythe Eagles was Head of the Department of Dairying in the Faculty of Agriculture at the University of British Columbia. He and his wife, Violet, had purchased several acres of land on the side of a hill leading down to a lake. They had had a home built and were in the process of landscaping the property, converting it to the showplace it has now become. We worked in the garden together and talked of many things. Both Dr. and Mrs. Eagles had obtained PhD degrees in pathological chemistry at the University of Toronto, and my many discussions with them broadened my horizons appreciably.

UNDERGRADUATE AND GRADUATE STUDIES AT THE UNIVERSITY OF BRITISH COLUMBIA

I graduated from high school in June 1939. My accumulated savings from working through the summer in the garden, a small bursary from the university, and some help from a kindly maiden aunt in Boston allowed me to cover the cost of my first year's fees at the University of British Columbia. I lived at home and commuted by tram and streetcar or rode with Dr. Eagles to and from the campus.

In September 1939 Hitler marched into Poland, and Britain declared war on Germany. Within hours, Canada followed suit. This had very little immediate impact on us in Vancouver. The year following became the year of the "phoney war" when we all felt secure behind the Maginot Line. After a rather slow start during which I adjusted culturally and academically to university life, I registered in the honors program in chemistry. As I became involved in the program and learned how to study, my marks improved dramatically. My most outstanding chemistry teacher was Dr. E. H. Archibald, who taught us analytical chemistry. This remarkable man had recovered from polio but was still paralyzed from the waist down. He was brought to the lecture theater in a wheelchair. Two graduate students would strap hip-length steel braces to his legs and then prop him against the lecture demonstration table at the front of the room. Although Dr. Archibald used no visual aids and could not reach the blackboard to write, he was able to keep classes of 100 students spellbound for an hour. We discovered that if we followed his procedures to the letter, we were able to get good results. I can always remember my feelings of deep satisfaction when my duplicates in a quantitative analysis would agree to the fourth decimal place. I almost became an analytical chemist.

By now the war in Western Europe had been unleashed in all its fury. Hitler's panzer divisions had outflanked the Maginot Line, and France had fallen. Presented with an opportunity to continue my studies, I elected to do so, but it was a very difficult decision to make.

In my senior undergraduate years I was able to serve as a laboratory demonstrator. This assisted me financially and enabled me to become more knowledgeable in general chemistry. I also gained experience in other kinds of summer jobs. One summer I worked part-time packing express packages in baggage cars for the Canadian Pacific Railway and part-time as a taxi driver. Another summer I dumped lead in a lead refinery for Consolidated Mining and Smelting in Trail, British Columbia. Such jobs were interesting and different in the beginning but soon became repetitive and boring, and I was very glad to get back to my books in the fall.

I graduated in 1943, and because there was then no biochemistry program at U.B.C. I registered in a master's program in chemistry and biology. For my master's thesis I was assigned a war project in synthetic organic chemistry. This was one of a series of projects farmed out to university departments of chemistry by the Department of National Defence. One of the courses I took was a senior course in dairy microbiology given by my friend Dr. Blythe Eagles. Dr. Eagles had become very interested in the nutrition of the lactic acid bacteria, particularly in some of the findings of Orla-Jensen (21). It was really this course and my associations with Dr. Eagles that directed me to consider pursuing further graduate work on what I referred to then as the chemistry of bacteria. I was not alone in receiving inspiration from Dr. Eagles; he has inspired more young people to pursue careers in microbiology than perhaps any other professor in Canada.

I received my master's degree and accepted a one-year appointment as an instructor in the chemistry department, teaching general chemistry to the hundreds of returning soldiers who were registering at the university. The soldiers were in a hurry and the courses were crash courses. We lectured in the morning, repeating our lecture to groups of 100 students, and then ran laboratories every afternoon. The students were eager to learn and discipline was never a problem. If any student got out of line or appeared to be harassing the instructor he was immediately set upon by other members of the class. I learned I could teach and enjoyed the year immensely.

GRADUATE STUDIES AT WISCONSIN

I wrote to various schools about the prospects for graduate study. A reply received from the Department of Biochemistry at the University of Wisconsin offered me an opportunity to work in the laboratory of Dr. Esmond E. Snell, studying the nutrition of the lactic acid bacteria. Esmond Snell had recently received the Eli Lilly Award for his contributions in this area, but I did not know that at the time. I had, however, heard of the outstanding reputation of the biochemistry department at Wisconsin from my friend Dr. Eagles, and because I was also offered a teaching assistantship for financial support, I accepted with alacrity.

Within hours of landing in Madison I was launched on my research program. Esmond Snell and Orville Bentley (then a graduate student in Paul Phillips' laboratory) had developed a microbiological assay for Mn^{++} using *Lactobacillus casei*. The lactic acid bacteria are fastidious and require complex media for growth. Such media are contaminated with sufficient Mn^{++} to permit the near-maximum growth of Mn^{++}-requiring organisms. The complexity of the medium used made it impossible to remove the Mn^{++} by conventional extraction procedures. Esmond and Orville had discovered that if one prepared the growth medium double-strength, complete except for its normal complement of added Mn^{++}, and then inoculated it with the Mn^{++}-requiring organism, the cells would grow until they had used up the Mn^{++} present as a contaminant. One then needed only to remove the cells by centrifugation, adjust the pH, and add glucose and a vitamin supplement to have a medium in which the assay organism would grow but only if Mn^{++} were added. Esmond Snell recognized the potential of this procedure for learning more about the mineral requirements of lactic acid bacteria and assigned me to this project. Within days I was running responses of various lactic acid bacteria to metal ions using the microbiological assay technique. Esmond Snell was and is an incredibly perceptive man. I and the other graduate students under his supervision marveled at the way he analyzed problems and then designed experiments that would lead logically and systematically to their solution. I arrived in June and by September, under his supervision, had sufficient data to submit our first paper (29).

During the next three years we studied the Mn^{++}, Mg^{++}, Fe^{++}, K^{+}, and PO_4^{3-} requirements of the lactic acid bacteria. We observed that for some of the species, the amount of an inorganic ion required for growth could be spared but not replaced by related ions. We explained this on the basis that the required ion had multiple functions in the cell, some but not all of which could be carried out by the related ions. We also observed examples of ion antagonism that we were able to explain on nutritional grounds by assuming that an ion that suppresses growth does so by interfering with one or more of the essential roles played by an ion required for growth. Thus it became possible to show that the concepts of analog-metabolite antagonism that had been demonstrated with organic metabolites could be extended to include responses to inorganic ions as well (30).

The Department of Biochemistry at the University of Wisconsin was an exciting place. There were many distinguished professors and about 150 graduate students. Each of the many research groups in the department was in the process of making major contributions to knowledge in its area of research. There were enthusiasm, excitement, and competition even between groups within the department. I look back with pleasure on those days. The pace was hectic, the professors were demanding, but it was a fabulous learning experience.

While at the University of Wisconsin I met my wife, Patricia. She had come from the University of Alberta to be a graduate student with Henry Lardy. Pat was working on the role of biotin in CO_2 fixation in rats, while Harry Broquist in our group was studying the function of biotin in lactic acid bacteria. I had the feeling that both groups worried that as a result of my growing friendship with Pat I might be a security risk to both groups. Pat and I were married the year before we graduated with our PhDs. Henry and Annrita Lardy held the wedding reception at their home. For our honeymoon we took a week off and camped at a state park near Green Bay, Wisconsin. Because we were to write exams for our minor in organic chemistry at the end of the month, I felt obliged to take along some organic chemistry texts. Pat was an attractive (she still is), highly intelligent young lady with the potential to be an outstanding independent research worker. She elected, however, to marry and have a family, advising me initially that she would like to have 12 children. We did not have 12 children but we did have 6: 4 boys and 2 girls.

In 1949 business was booming and there were many job opportunities for Wisconsin biochemists, particularly in the pharmaceutical industry. I was, however, interested in an academic position, and both Pat and I were anxious to get back to Canada, not because we did not like the US but simply because we were homesick. I sent my curriculum vitae to every Canadian university and found only one with a tenure track position, an assistant professorship in the Department of Biochemistry at Queen's University in Kingston, Ontario. At the same time Esmond Snell asked me if I would like to be considered for a job in a new institute, the McCollum Pratt Institute, being set up at Johns Hopkins University for the study of the role of mineral elements in nutrition and metabolism. I was interviewed by Professor W. D. McElroy, who proudly demonstrated what he had just recently been able to show, that adding ATP to a suspension of crushed firefly tails caused luminescence. I also met the legendary E. V. McCollum, a former professor of biochemistry at Wisconsin. I was then offered a job as an assistant professor in the biology department with a cross appointment to the institute. Because I had also been offered the job at

Queen's, I had an agonizing decision to make. I finally opted for Canada. In a typically Canadian reaction, the administrators at Queen's were surprised by my decision.

A SOJOURN AT QUEEN'S

The Department of Biochemistry at Queen's was at that time very small, consisting of a chairman, an associate professor, and myself. The chairman, Dr. R. G. Sinclair, was an active research worker with an excellent reputation in his field of study, lipid biochemistry. His presence in the department was its main attraction for me from a scientific standpoint. We arrived in Kingston and I began settling into the department in early August, 1949. Unbelievably and tragically, Dr. Sinclair was drowned in a swimming accident three weeks later. We pulled ourselves together under the acting chairmanship of the associate professor. Our major teaching commitment was a course in general biochemistry for medical students. I also introduced a graduate course in biochemistry. With funds from an interim research grant, I hired a technician, bought some equipment, and began developing my research program, an extension of some of the observations I had made as a graduate student.

I applied for and received a research grant of $10,000 from the Medical Research Council of Canada. This was an almost unheard of amount in Canada in those days. Because the university did not charge overhead, and because the Canadian dollar was then worth $1.10 American, this represented a considerable sum of money. I later learned why I had been so fortunate. My grant proposal had included a study of the role of K^+ in the nutrition and metabolism of bacteria. The proposal had been reviewed by Professor E. G. D. Murray [R. G. E. (Bob) Murray's father], one of Canada's most respected senior scientists and chairman of the Department of Bacteriology and Immunology at McGill. He confided to me later that he had reviewed the proposal and had remembered years before having trouble growing some organisms he had isolated. Because he had discovered by accident that he could solve his problem by adding K^+ to the growth medium, he was intrigued by my proposal to study K^+ effects and approved the grant. And because he approved the grant, I was granted the money—which says a lot about the way research was funded in Canada in those days. At that time a very small group of men served as the scientific establishment in Canada. They sat on the research boards, ran the scientific establishments, and disbursed the grant money. Now we have peer review committees and more people involved in what appear to be decision-making processes. I have the feeling, however, that the really big decisions, those that determine how many millions for research should be allotted here or there, are still being made by a very small number of people and in just as arbitrary a fashion as ever.

Our first child arrived during our first year in Kingston and our second during our second. This raised a problem that at that time was bothering all the staff at Queen's. Salaries at Canadian universities had remained at pre-WWII levels while the cost of living had increased. My starting salary had been competitive with those offered in the US, but the long-term prospects seemed poor. I began to investigate other options. Professor G. B. Reed, Chairman of the Department of Bacteriology and Immunology at Queen's and also Chairman of the Fisheries Research Board of Canada advised me that there was a senior position available for a biochemist at the Fisheries Experimental Station of the Fisheries Research Board of Canada at Vancouver. He intimated that the station might need new blood but that the opportunities to develop a research program were good. Salaries had risen far more in the government and in industry than in universities. Although I was offered an associate professorship to stay at Queen's, the prospect of being able to return to the West Coast at an appreciably increased salary seemed too good to ignore, so we left.

BACK TO THE WEST COAST

Our first introduction to the Pacific Fisheries Experimental Station was disheartening. The station was located in an old Coca Cola plant in downtown Vancouver. The Coca Cola Company had moved to a new building of glass and steel in a more fashionable part of town. I was shown to my laboratory, an essentially empty room. The staff of the station was for the most part involved with short-term technological problems. Only one person, Dr. Hugh L. A. Tarr, a very able microbiologist, had managed to develop what I considered to be a viable long-term research program. One of the station's administrators took me aside and told me that although as a staff member at a university I might have felt obliged to publish one paper a year, at the station one had no need to feel under any such pressure.

I needed a complete set of equipment and compiled a list of requirements, the bill for which came to $30,000. I had some difficulty persuading the administrators at the station to submit the request to headquarters in Ottawa. The attitude had seemed to be that if one did not ask for much no one would notice if one did not do very much. I think the administration was even more upset when I received every penny of the money I had asked for as well as sufficient funds to employ some competent research assistants. I immediately imported my former research assistant at Queen's, Eve Onofrey, and we began to develop a research program. Because the mission of the station was to supply research support to the fishing industry, I felt obliged to develop a program with this in mind. I decided to have one part of the program concerned with more strictly applied projects and a second part that would involve long-term, more basic research. It proved to be extremely difficult to come up with relevant applied projects. The fishing industry was not a sophisticated one and had not taken much advantage of the backlog of very useful data on fish preservation and spoilage that Hugh Tarr had accumulated over the years. Nevertheless, we persevered with this aspect of our program. For our long-term research I decided to study the nutrition and metabolism of marine bacteria. My justification for this was that marine bacteria were involved in fish spoilage. Although the occurrence, importance, and classification of bacteria having the sea as their habitat had been the subject of many investigations, little was known of their nutritional requirements or metabolic activities. Our program was designed to supply some of the information that at that time was lacking.

I obtained cultures of marine bacteria from water samples that I collected one afternoon in Departure Bay, Vancouver Island from a rowboat borrowed from our sister station, the Pacific Biological Station. The cultures isolated from these samples, together with others from our station collection, were screened for their capacity to grow on a complex medium prepared with either seawater or distilled water. Those that grew on seawater but not on freshwater medium on initial isolation were classified according to Zobell's definition (45) as marine bacteria. Thirty-three different marine bacteria were isolated and their minimal nutritional requirements determined. In all cases, an artificial seawater, containing salts providing inorganic ions at concentrations similar to those in seawater, could be used in place of natural seawater for growth of the organisms. Because we now had chemically defined media for the organisms that could be prepared relatively free of contaminating traces of inorganic ions, we were in a position to study the inorganic ion requirements of the organisms. Selecting six that had required seawater for growth and six that had not, we observed that those that required seawater for growth required Na^+, K^+, and Mg^{++} and in some cases Ca^{++} also. Those that did not require seawater differed in that they either did not require the addition of Na^+ to the medium or required it in very small amounts. We proposed that marine bacteria might be distinguishable from terrestrial species not on the basis of a requirement for seawater but on a specific need for Na^+ for growth (28).

We began a study to determine the function of Na^+ in the growth of the cells. It quickly became apparent that to carry out these studies in depth we would need to restrict ourselves to a smaller number of organisms. We had begun with 33 and from these we first chose 6, then 3, and finally 1 that seemed to be reasonably representative of the others and grew very well. This organism, which Dr. Jim Shewan at the Torry Research Station in Aberdeen classified for us as a *Pseudomonas*, had been assigned the number B-16. It has been reclassified more recently as *Alteromonas haloplanktis* 214 by Dr. Paul Baumann, and we will refer to it subsequently by this name. This organism required Na^+ for respiration, the amount required varying with the substrate oxidized (23). An examination of the enzymes of the tricarboxylic acid cycle showed that none of these required Na^+ specifically for activity (26). I was greatly assisted in these studies by the presence in my laboratory of a number of outstandingly competent assistants and associates. Dr. Eagles had become Dean of the Faculty of Agriculture at U.B.C. and Dr. Jack J. R. Campbell had replaced him in the Department of Dairying. Jack, who was a U.B.C. graduate, had obtained his PhD at Cornell with Dr. I. C. Gunsalus and was now making a reputation for himself studying the intermediary metabolism of the pseudomonads. Jack had attracted some of the brightest young students at the university to his laboratory. Some of these, such as Margaret Norris, Aiko Hori, and Dr. Neil Tomlinson, became assistants or associates in my laboratory. Others worked there as summer students. With this pool of talent our work progressed quickly. Things were also looking up in other ways. Dr. Dave Idler, a graduate of Jack Campbell's lab, had gone on to take his PhD in biochemistry at Wisconsin and returned to take over the chemistry section of our station. He quickly developed a very vigorous program in chemistry that included a study of the distribution of sterols and steroids in marine animals. In the mid-1950s we moved into a new building on the campus of the University of British Columbia, and Dr. Hugh Tarr became station director.

To keep in touch with developments in our fields we had formed a biochemistry interest group to which all those living in the area who were interested in biochemistry were invited. We would meet very informally once a month, rotating from home to home. At these meetings we would discuss our research and consume home brew and cheese. One of the people who attended these meetings regularly was a young man who had just recently come to work at the British Columbia Research Council, Dr. Ghobind Khorana. I will never forget the evening he quietly announced that he had succeeded in synthesizing Coenzyme A. At the B. C. Research Council Ghobind began his studies on nucleotide and polynucleotide synthesis, which later at the University of Wisconsin led him to the first synthesis of a gene and a Nobel Prize. We were fortunate to have him and the outstanding young research associates he had attracted to his laboratory in our group for five years.

As the 1950s drew to a close the clouds began to gather again. Dr. Reed had retired as Chairman of the Fisheries Research Board and been replaced with a person much less tolerant toward basic research. I had endeavored to balance the efforts of my section so that approximately 50% of the personnel were engaged in applied research. It became evident that in the future this would not be considered enough. I decided to look elsewhere. Among other places, I sent my curriculum vitae to the Department of Biochemistry at McGill. I received a very kind reply from Dr. K. A. C. Elliott, who advised me that although he did not have a vacancy in his department he knew of one in the Department of Microbiology in the Faculty of Agriculture on the Macdonald Campus of McGill University. The new chairman of that department turned out to be an old friend from Wisconsin days, Dr. Clark Blackwood. He offered me an associate professorship, which I accepted. We left the West Coast not without regret. We had built a home there and were in the process of raising six children. The move was traumatic for Pat and me, but it did not bother the children a bit.

LIFE AND WORK AT MACDONALD COLLEGE OF MCGILL UNIVERSITY

Macdonald College of McGill University is located on a beautiful campus at the west end of the Island of Montreal, in a suburban area separated some 25 miles from the downtown campus of McGill in the City of Montreal. Newly arrived staff are able to rent a house on campus for a temporary period to enable them to become familiar with the community before settling in to more permanent accommodation. We were housed in a beautiful old brick home with five bedrooms, overlooking Lake St. Louis on the southern edge of the campus. This did much to ease the pain of leaving the West Coast.

There were only three of us in the department when I arrived: Clark Blackwood, Roger Knowles, and me. Although Clark and Roger had arrived only three years previously, the department had had by Canadian standards a long and venerable history. It had been established in 1907 with Dr. F. C. Harrison as chairman. Dr. Harrison later became the first Canadian to be elected President of the Society of American Bacteriologists (now the A.S.M.). The department awarded the first PhD in nonmedical bacteriology in Canada in 1923.

I had been assured that I could continue my program of research on the nutrition and metabolism of marine bacteria provided I could arrange with a granting agency to obtain funding. The National Research Council of Canada provided funds, I hired a technician and two graduate students, and we were soon able to get underway again. I also had teaching to do, this time in microbiology. Although I had worked with bacteria for many years, my formal training in bacteriology had been restricted to a limited number of undergraduate courses, and I was required to learn as I was teaching. It was a pleasure to be involved in teaching again.

My research program developed in two directions. I decided to pursue further the studies on the function of Na^+ in the metabolism of the cells and also to examine what appeared to be another characteristic of marine bacteria, their tendency to lyse at low salt concentration.

Our previous studies had shown that Na^+ was required specifically for growth and for the oxidation of various substrates by cell suspensions of *A. haloplanktis* but not by any of the enzymes in the cells that could be expected to be involved in the oxidation of the substrates. It thus seemed possible that Na^+ might be required for the transport of the substrates into the cells. Transport