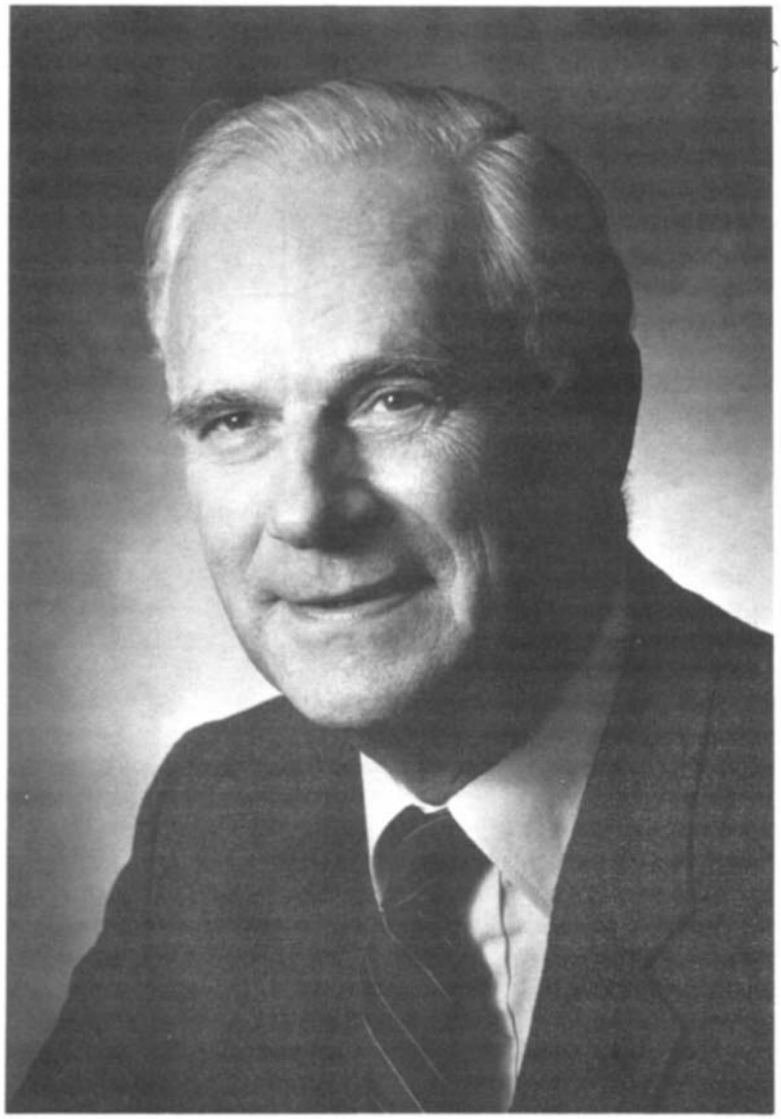

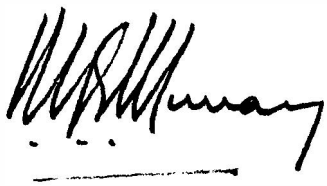

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A STRUCTURED LIFE

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> One of the profound lessons to be learned from science is not what it has done, but the way it goes about doing it.
>
> Hanbury Brown: *The Wisdom of Science*

I may not have been introduced to thinking about microbes at birth, but this was a likely event soon afterwards. I count myself lucky that bacteriology, its history, and science in general lived for me because my father, E. G. D. Murray (1890–1964) (88), was a bacteriologist whose teachers were young contemporaries of the giants of the early days. A few of that earlier generation were still teaching at Cambridge when I was there as a student, which emphasized the bridge between scientific generations. They were long past

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retirement but had valuable things to say about science and the early days. This gives me a good feeling about being on postretirement appointment, as I am now, and still having contact with dedicated students. Between us, my father and I have enjoyed devotion to bacteriology for almost three quarters of this century.

It is hard to decide from biographies whether or not a career in science is assisted by having a parent a scientist. At the least, it gives a more defined set of prejudices as a basis for deciding what to do in life, or what to avoid. At best, it provides a strong set of interests, standards, stimuli and concerns which shape the inevitable explorations of the world around us and direct the neophyte's progress insensibly and in subtle ways from tentative to defined courses of action. A wealth of understanding gained informally provides the "gilt on the gingerbread" that amplifies capabilities.

BEGINNINGS

The Murrays who were my forebears lived in South Africa from 1835 and for the most part were dedicated to the land; my mother's family was English and of mercantile bent. My grandfather, G. A. E. Murray, departed from those norms by qualifying in medicine, and he set up for surgical practice and family life in the infant city of Johannesburg in 1888. My father followed in his footsteps and studied medicine at St. Bartholomew's Hospital, London. He did a degree at Cambridge (Christ's College, 1912), with a special interest in zoology, which I was to profit from in years ahead. While still a student at St. Bartholomew's he was much influenced by Dr. Mervyn Gordon, and the two worked together on studies of meningococcal meningitis. This interest continued during World War I after my father's qualification, marriage, and army service. He was in the Royal Army Medical College Vaccine Laboratories and was concerned with the making of vaccines when I was born in 1919 in Ruislip, a London suburb. The war was over and the bacteriological and epidemiological emergencies of wartime were displaced by the influenza pandemic with all its alarming complications. Soon after I arrived on the scene the family moved to Cambridge, where my father was appointed a research bacteriologist for the Medical Research Council (1920–1926). He revived his wartime interests in the meningococcus while supervising the production of therapeutic antisera. My very early memories include attending the bleeding of large and elegant horses at the Field Laboratories outside Cambridge.

My father was soon back to Cambridge academic life in Christ's College and in the Department of Pathology, where he became a lecturer. His chief, and later my teacher, was the redoubtable Professor H. R. Dean. We shared energetic summer vacations with his family for several years. Professor Dean

was a pathologist with interests in bacteriology and immunology. (He introduced the concept of optimal proportions into serology, and he also studied complement.) It was my father's task to help him develop an honors course in pathology, which included medical microbiology in those days and still does. The first class graduated in 1925, and the nine students, now very distinguished British medical microbiologists, included A. A. Miles, C. H. Andrewes, and two who taught me later on, E. T. C. Spooner, who later returned to Cambridge, and Frederick Smith, who was to join my father at McGill. They were forerunners of a considerable company of medical scientists, and I was to join them myself.

My childhood was that of a homebody, but life changed considerably in 1927 when I was sent to Summer Fields, a boarding school at Oxford devoted to helping as many pupils as possible gain scholarships to enter one or another of the great "public" schools. It was a tough time because I had remarkably little experience of children my own age. It was a good school and there were miseries; but we worked hard and played hard as required by the school's motto, "*Mens sana in corpore sano.*" I have every reason to bless the school for the basics (in everything but music, alas) that have served me well.

In 1930 my father became professor and head of the Department of Bacteriology and Immunology in the Faculty of Medicine of McGill University, Montreal. It was an exciting transition for all of us and one we embraced enthusiastically, even if I never managed more than minor modifications to an English accent. I was old enough to be more aware, and being at home for much of the year, I could now hear what was going on in academic life. So Montreal became my second home. As a great metropolis encompassing two cultures, the city provided an ambience of great importance to my development. The change in school systems was a traumatic experience, but happily I went to Lower Canada College, an English-type boy's school that offered a suitable program and games familiar to me, such as soccer and cricket. What helped me most in becoming Canadian without too many tears was the enthusiasm with which my parents took to the country and its pace, space, and natural beauties.

By 1936 I was ready for McGill University and completed the matriculation examinations, which then still required Latin. There was no science requirement, and indeed I did only a minimal combined chemistry and physics course at school. So McGill provided the grand and eagerly awaited start in the nuts and bolts of science and the opportunity to learn under senior and very capable teachers whom, in many instances, I already knew and admired. Largely because of my father's early interest in and retentive memory for zoological information, I set out with an intention to do zoology. However, all courses were interesting, and I performed well in the examinations for the two years 1936–1938.

There were omens of war in Europe, and evidently it would be now or never if I were to go to Cambridge University, which meant so much to my father and which I wanted with all my heart. Arrangements were made for me to enter Christ's College in October 1938. However, there was a summer to fill in, and I had the opportunity to go to a marine biological station for a course in invertebrate zoology at Salisbury Cove, near Bar Harbor in Maine, under Professor Ulric Dahlgren of Princeton University. The course was fun and influential; I was later to marry (in 1944) one of my classmates (Doris Marchand, also from a scientific family). Also, the scientific community provided much informal discourse with first class people such as Homer Smith and Gairdner Moment. After this very educational interlude I set off for England by ship during the Munich Crisis in September 1938.

Cambridge was a revelation to me, not just because of the quality of teaching but because of what was expected of the students. It was a matter of attitude and preparation. The attitude was expressed in a way by my tutor, C. P. Snow, who said (and he didn't say much more), " . . . you realize you have to present yourself for examination in May, two years hence." We had to pace ourselves both in and out of term, keep up, and do a wide range of independent reading, quite apart from lectures and laboratories. In this we were assisted by a supervisor of studies (I was assigned to John Yudkin, later a distinguished nutritionist, who then had interests in *Escherichia coli* physiology). We were expected to have the basics or quickly acquire them; I thought myself inordinately well prepared, but to my chagrin, my scholarly classmates just out of school had a remarkably well-honed understanding of basic physics and chemistry. The teaching also reflected attitude. For instance, the lectures and laboratories in introductory biochemistry were given by Ernest Baldwin. The first-term course was on comparative biochemistry, given in a fascinating fashion. Baldwin later went on to systematic biochemistry and the outlines of what was to become a distinguished textbook. It was good stuff and we learned a lot.

I was enrolled in medical studies, which meant the inclusion of anatomy, biochemistry, physiology, pathology (including bacteriology), and pharmacology in the first two years. Despite a rather miserable performance in anatomy, I was able to go on to a third year (Part II of the Natural Sciences Tripos) spent entirely in one disciplinary area, which was in my case pathology and bacteriology, still under Professor Dean. The Part II course in pathology was a combined effort coordinated for the most part by G. Williamson (pathology), E. T. C. Spooner, and A. W. Downie (medical microbiology). Downie was then running the Emergency Medical Service Laboratory in Cambridge. He was dealing with problems ranging from streptococcal diseases to leptospirosis, which he and his colleagues shared with the five of us students in exemplary fashion. Likewise, A. M. Barrett saw that we got all the tissue and bacteriological specimens that we needed from the autopsies he

did for Addenbrooks Hospital. It was an extraordinarily thorough experience in the details and practice of medical bacteriology and pathology, full of interests to suit each of us. There were special additions: parasitology at the Molteno Institute and special series of lectures by senior (some very senior) colleagues such as G. S. Graham-Smith (toxins and venomous animals and insects), Louis Cobbett (tuberculosis), R. I. N. Greaves (immunology and the preservation of blood and sera), R. R. Race (blood grouping), and L. Faulds (cancer research). To me it was sad but not distressing that they excluded much general microbiology, which I heard a little about from a few fellow students in botany. The biochemistry department included some distinguished contributors to microbial biochemistry, and the group of us arranged an informal session or two with Marjory Stephenson and W. E. van Heynigen. As it turned out, I did not cover myself with glory and received a Class II overall, which was a bit humbling since my classmates John Stowers, Leo Wolman, and Alec Comfort all were Class I, as they have shown by their distinctions in later life. But after this year I understood what kind of advanced bacteriology course my father had developed at Cambridge (when Part II was initiated) and later introduced in refined form at McGill University.

Graduation in wartime brought the necessity of a clear track in medicine, in research, or in military service. My decision, encouraged by Alan Downie, came with the news of my acceptance at the McGill Medical School. The issuance of an exit permit and my assignment to a ship for the Atlantic crossing was slow, and I did not sail until late October 1941. I effectively spent the four-month interval on the wards of Addenbrooks Hospital learning physical diagnosis, which helped me considerably. I arrived back in Canada just before the invasion of Pearl Harbor changed the aspect of the war, after an exciting three-week voyage in convoy. We had few losses thanks to Canadian corvettes and the newly acquired lend-lease destroyer escorts supplied by the United States.

McGill had introduced an accelerated medical course with no vacations, and I settled down to a rather exhausting routine. Graduation came late in 1943, followed by internship at Royal Victoria Hospital. My internship was unconventional because it was based in the clinical laboratory of the Department of Bacteriology, which provided services for the hospital. This internship gave me clinical experience in most departments and laboratory experience in the fullest possible range of microbial diseases. The experience was all the better for the teaching of Fred Smith (who was holding the fort for my father while he was on war service) and for the practical clinical laboratory instruction of Gertrude Kalz, who really put the polish on my bacteriological training, for which I am ever grateful. Internship also brought me marriage and the start of family life.

The army called in 1944 with suggestions of using my laboratory com-

petence, but predictably, after the usual training courses I ended up as a medical officer in tank training regiments. The war was all but over when an offer came from the dean of medicine at the University of Western Ontario.

INFLUENCES

Small things, thoughtful people, and lucky happenstances enrich life, teach us in subtle ways, establish values and interests, prepare the mind, and without intention guide the footsteps. Some of these influences I identify in what follows, with the certainty that others of equal importance are left out or were never recognized.

Nature was revealed to me in my family's series of well-tended gardens and country walks. My mother's keen eye for birds, beasts, and natural wonders, and my father's habit of collecting interesting specimens and sharing his knowledge about them were a part of many days' outings. Fishing was important to my father and it became so to me; his interest in the sport and his competence were infectious, and we fished together for many years. I acquired a great deal from the opportunity for gentle but serious conversation and for airing what we knew or thought we knew about life around us.

Microscopes had a pervasive, persuasive, and recurring role in my life because of my father's interest in microscopes and microscopy. Certainly I must have seen them early on just as I admired them on later occasions when visiting laboratories. However, I have a clear memory (identifiable by circumstance to the time when I was three or four years old) of seeing Leeuwenhoekian animalcules down an old brass microscope; their source was an inverted bell-jar aquarium full of weeds, water beetles, copepods, and other small forms. That same microscope was in more regular summertime use when I was seven to ten years old, and I still have it.

I did no formal biology until I went to McGill. Two kindnesses were of particular importance: I was attending N. J. Berrill's invertebrate zoology course and he asked me, "Would you like to have six feet of bench?" For sure I would. He gave me space in a corner, the use of a microscope, and facilities for fixing, paraffin-embedding, sectioning (an old "Cambridge rocker"), and staining preparations for microscopy. I enjoyed myself, learned by doing, and produced good sections of a frog kidney adenoma among other things. My father's kindness was no less formative; it was the present of a Leitz binocular microscope with appropriate optics and slide boxes of the preparations of microscopic invertebrates that he had made for himself as a student in 1909–1912. Thus I became familiar with preparative techniques and with an instrument that I have and use to this day, and which has provided all of my light micrographs to date.

I cannot remember life without books and discourse about books. My father

was an inveterate book-buyer and reader of all sorts of subjects from Middle English to evolution. Books were treasured. Even the bad books were kept because the argument and the horrible example might be needed; they were annotated with marginalia, incisive comments on the title page if they were bad enough, and a personal index to interesting titbits noted on a back flyleaf. The home and the laboratory contained eclectic collections of books, journals, and reprints; as a result I became a card-carrying, second-generation pack rat and omnivorous reader. Fortunately few near disasters resulted, but a few there were, from such dilettantism. As long as I can remember the dinner table was a place to talk, to try out outrageous arguments, to remember interesting facts, and to embroider a good story. My mother had to contend with an impossibly elastic timetable for the course of a dinner.

I was lucky indeed that at home, at secondary school, and at the universities I was blessed with mentors who cared enormously about how we expressed ourselves in speech and in writing. At Summer Fields I took classes from L. A. G. Strong (a substantial novelist) and Cecil Day-Lewis (a major poet). At school in Montreal I was helped enormously by Hugh MacLennan (a major Canadian novelist and academic), D. S. Penton (a fine teacher), and V. C. Wansbrough (a scholarly headmaster), who were as much friends as teachers. Behind-the-scenes work on the school magazine, which was a rather elaborate annual, introduced me to the life of an editor. The interest must have been latent because I was to spend much time on the *McGill Medical Journal,* then a substantial quarterly, and one year as its editor. My involvement with the journal provided experience every step of the way from soliciting of contributions, to copy editing, proofreading, and production, to review writing. Only seven years later I was to be on the Editorial Board of the *Journal of Bacteriology,* enjoying the first of a number of appointments in scientific editing.

I was most fortunate to have done half of my schooling and half of my university training at first-class institutions on each side of the Atlantic. These opportunities were given at no little expense to my kind, generous parents, who gave me the best that they could of both worlds and all their support. I can only imagine a certain anguished concern at sending an only son as a student, not a serviceman, in the direction of crisis and war in 1938.

The final good fortune was to get started in teaching and research at a small medical school in a small university that had only one way to go and that was up, fast. There was infectious energy and ability at the top in the person of G. E. Hall, a fine scientist turned administrator, who knew what he wanted, usually got it, and made good decisions about people more of the time than do most administrators. He brought in able young professors to guide into the bright future the small (two–faculty member) departments, such as R. J. Rossiter (biochemistry) and A. C. Burton (biophysics), who became my very

good friends and colleagues, as well as even younger faculty (of whom I was one) to back them up. His successor as dean of Medicine, J. B. Collip, a most distinguished endocrinologist, was even more influential and also pushed hard for productive involvement in basic research. Collip strengthened the school, with the help of Hall, then the university president, to a level to be proud of. There was no temptation to look for another job; promotion came fast, and the school was, and still is, an exciting place to be. As a result I lost no time along the way due to relocation and I received every possible encouragement. We prospered intellectually; we had to cooperate in all things because there were so few of us (in 1950 there were 18 full-time faculty in the Medical School), and we enjoyed each other's company.

A FLYING START IN ACADEME

The invitation to the University of Western Ontario Medical School was urgent because the professor of bacteriology and immunology, I. N. Asheshov, was in the hospital and was likely to be there for a few weeks. It was September 1945, and the course for 44 medical students had just started. Dean Hall, himself recently appointed after a senior posting in the Royal Canadian Air Force, knew how to pull strings. A brief weekend visit was enough for me to say yes to the post of lecturer in the department with duties to include teaching medical students, assisting in clinical bacteriology for the hospital, and involvement in research. Two weeks later I was seconded to the Wolsey Barracks, London, Ontario, for "special duties." My wife and infant daughter, with the help of friends, found us a temporary living place. Within a week of our being together again I was embarked on a 12-week stint of four lectures and four labs a week, in the midst of which I was demobilized. Many of this first class were ex-servicemen, and not a few were older than I was. We all survived the experience and I learned more from and with them in a shorter time than ever before or since then. Three of that class are senior colleagues still in this faculty.

Asheshov was out of the hospital before the end of the term, in time for a few lectures in his remarkable style, and he was marvelously helpful and supportive to me. He was a lively minded scientist, then engaged in seeking antibiotics, but at heart he was interested in bacteriophages, an area in which he had worked productively since 1922. He and I taught hard for five months of the year. It was a good old-fashioned bacteriology course topped off with minor dollops of virology, parasitology, and mycology. In many ways it was the oral examination rather than written papers that taught me the most about my subject, our teaching, and students. Whatever may be said about subjectivity in examinations, the hard work involved in these oral examinations was repaid in knowledge of the student and understanding of the strengths and

weaknesses in teaching. Even more importantly, after the war we had mature veteran students who were remarkable people: dedicated, serious, accomplished, and worth every effort. They taught me a lot.

I had to get started on research in 1946 because there was time and I wanted to do it. Dr. Asheshov gave me every encouragement, without demanding any service or direct assistance in his own work, which was mighty kind of him. I was equally encouraged by Dean Hall and by Dean Collip (who succeeded Hall in 1948), who provided me with an initial grant for a phase microscope. Funds were very hard to get, so I had to make use of the sorts of things we worked with every day in the clinical laboratory. Each of my first four independent forays into research were important to my life and work in the laboratory. I write about them with that in mind.

The first project was prompted by the isolation of a mucoid Group A *Streptococcus pyogenes* from a chronic lung infection. This reminded me that when I was an intern I had seen similar mucoid colonies collapse flat in the neighborhood of some colonies of staphylococci and pneumococci. Knowing then that the capsular polysaccharide was hyaluronic acid provided a method for ready detection of hyaluronidase ("spreading factor") and also a diffusion assay for the enzyme, which was of great interest at that time as a component of virulence targeted on the intercellular matrix of connective tissue. Fortunately, R. H. Pearce, an equally young biochemist in the Department of Pathological Chemistry, was interested in acid mucopolysaccharides. The resulting joint paper was good experience and was accepted without demur by the *Canadian Journal of Research* (64). So I started out in collaborative research, and I am grateful to Pearce because I learned from him some of the many things I should have known had I had doctoral training in science. Entering PhD studies was an option, but I decided to get on with research because there was time and opportunity.

The next project arose from observing the remarkable motile colonies shown by a fortuitous isolation of *Bacillus circulans,* and it initiated a lifelong interest in swarming and motility. Both the rotating colonies and the curving paths of the bullet-shaped colonies showed an exact 2:1 ratio of counterclockwise to clockwise motion, which is still unexplained. For the first time I had the assistance, in the summers of 1947 and 1948, of a veteran medical student with laboratory experience, R. H. Elder, now a senior and respected clinical microbiologist in Ottawa. We wrote a neat paper which was accepted by the *Journal of Bacteriology* (60), and we made a short 16-mm movie, using a haywire rig, to illustrate a paper given at the 1948 ASM Meeting in Minneapolis. Most importantly, Carl Robinow introduced himself after that presentation, and we discussed swarming but also his observations on bacterial nuclei and endospores. So this project introduced me to a fascinating phenomenon which is still in mind, and to a dear colleague whose association with me and our students has been nothing but rewarding and a pleasure for 40 years.

Igor Asheshov taught me about phages and how to handle them just at the time the papers on the T-phages were coming out. The excitement of following the fate of the phage and the host components in these papers induced thoughts about research projects. Asheshov was advising Fred Heagy (an MD interested in science), a PhD candidate studying T2 phage infection, and suggested a parallel study of changes in the host cell in advance of lysis. Indeed, there were indications that these changes should be observable by microscopy (46). We tried Robinow's cytological techniques because I had been reading his papers and his "Addendum," which was a remarkable seminal chapter on bacterial cytology appended to *The Bacterial Cell* by René Dubos (75). We had hardly started on this in 1948 when Asheshov and his technical forces left to work on a March of Dimes project seeking antiviral agents at the Bronx Botanical Garden. So I was left willy-nilly to elaborate my own expertise and to supervise a doctoral candidate my own age. It was another learning experience, to use the language of educators, to become acting head of the department.

That summer and the next, Heagy and I were helped by D. H. Gillen, a medical student, and we made lots of preparations at timed intervals following infection of *Escherichia coli* B with phage T2. Our preparations were terrible but encouraging. The situation improved dramatically after Robinow paid us a visit and showed us in his inimitable fashion delightfully simple technical stratagems, which paid off in the quality of the preparations and the photomicrographs. There was a progression of cytological events involving a shift in the distribution of chromatin with eventual dissolution (host-cell DNA), gradual depletion of the cytoplasmic basophilia (host-cell RNA), and developing granularity in the last two thirds of the cycle due to synthesis of phage DNA. We published a good descriptive paper (62), and we were only slightly sorry to have been scooped by Luria & Human (45), who compared cytological events due to several of the T-phages. It was evident that the effect of phages on host cells was visible and was determined by the virus rather than the host. Our paper was appreciated and brought me into touch with S. E. Luria and his exciting group, paved the way for a collaboration with G. Bertani, and gained me an introduction to their colleagues at the University of Illinois, where laboratory visits were exhaustingly educational, night and day. The project was also the beginning of real cytological studies in our department because Dean Collip's unstinting support allowed me to invite Carl Robinow to join me in the department, which he did in 1949.

The fourth seminal research project followed the invitation by Charles Evans for me to spend the summer of 1949 in the Department of Microbiology, University of Washington, Seattle, as a sessional lecturer. There were fascinating people to learn from such as Erling Ordal and Evans. More to the point, I was able to put my newfound cytological competence to good use in

helping Howard Douglas study the growth and division of an interesting budding and phototrophic bacterium, *Rhodomicrobium vanielii* (59). It was fun to find out that nuclear division took place in the mother cell and that one of the nuclei so formed was exported to the new bud through the long, 0.3-μm–diameter hyphal tube, which had the newly budded cell at its end. We spent happy hours observing fixed and stained preparations and taking time-lapse photomicrographs of living cells under phase contrast. I learned much that was useful from Douglas and his colleagues as well as from my teaching assistants, Wesley Volk and Quentin Myrvik. While I was there a telegram came from Dean Collip telling me I had been appointed professor and head of the department. This meant, I suppose, that the invitation from Seattle was a stimulus to decision. There was considerable rejoicing; I was just 30 years old and an expression of trust had been given by the university. As I see it, I was still drying behind the ears, as the saying goes.

RESPONSIBILITIES

I was lucky to be given from the outset as much responsibility as I could manage, such as the provision of a clinical microbiology service to the Children's Hospital and Victoria Hospital, our main teaching hospital, in which the department was then situated. I was responsible for this service from 1948 until 1965, when the department moved to the university campus and broadened its horizons. The clinical work kept us busy, and the major clinical problems came to me, day and night, in those days before we had physicians who specialized in infectious diseases. Antibiotics were with us and so were the developing problems concerning policy in treatment, spread of resistant strains within the hospitals, and infection control. This meant that research was a source of enjoyment and recreation in the midst of a busy life. Rightly or wrongly, the research projects grew more and more general and drifted further than might have seemed proper from the medical applications which were our clinical and teaching preoccupations.

Relief came in the late 1950s and early 1960s, when increased professional and technical forces were appointed to cope with the burgeoning use of clinical microbiology due to the introduction of a hospital insurance program in Ontario.

In those days there was much greater interdepartmental cooperation and we often had to teach what we could with any help we could get. I note with some astonishment that I gave courses in epidemiology (1948–1950), human genetics (1955–1958), and history and methods of medical science (for graduate students, 1958–1964). Luckily we were mostly young and energetic as well as communicative with each other owing to a lively lunchroom. This spirit and

the efficiency and some of the enjoyment that went with it disappeared in a very few years with the increasing size and complexity of departments.

The move to the university campus in 1965 brought with it new faculty members and the integration of an honors course in microbiology and immunology into the biology programs. We were interlopers, and the introduction of six new programs by the basic medical sciences group was seen as a threat to the biologists and was not happily received. Time has resolved most of the problems, but increasing devotion of energy to administrative matters was required, which brought with it devotion to university affairs. I sat on the senate and many committees and spent a year as acting dean of science. So it was a degree of relief to give up being head of the department in 1974, after 25 years at it, and to administer thereafter nothing bigger than my own research group.

Another change came because of vastly increased faculty forces in medical microbiology, which drifted my teaching from medical microbiology to general bacteriology and systematics. It is not a bad thing to have such a shift in the midstream of a career and to be able to step away from administration before it becomes tedious or all-consuming. Too many good people regret losing their foothold in their academic discipline, and I have seen them get tired before their time. Life has to be lived with some care for what is best for the individual. Fortunately some good administrators and some good researchers and teachers find their appropriate niches and avoid the pitfalls of academic life; others are not as lucky as I was.

EXPLORING BACTERIAL CYTOLOGY

My feet were well set on cytological trails, but the diversity of my studies through the 1950s was needed to fix the major direction of my work. Observations on the cytology of phage infections continued with J. F. Whitfield and were extended by collaboration with G. Bertani to follow the cytological events during the establishment of a lysogenic state for P1 and P2 phages (94). Interesting as these phenomena were, perhaps the most fruitful derivative of questions about why changes in nuclear form accompanied phage infections turned out to be a study of the effect of the influx or efflux of cations on the conformation of the nucleoids of bacteria (95). As we and Kellenberger's group were to remember years later (35), an understanding of ionic effects on charged polymers is crucial to assembly and conformation of working structures and to lifelike preservation during cytological fixation of specimens. In fact, the crucial events involved in cellular structures and processes are nature's physicochemical experiments. So it was natural that my interest turned to trying to learn more about the structure of the host cell and

that my studies broadened to the general cytology of bacteria. In this effort the daily discussions, suggestions, and joint efforts with Carl Robinow were determinative.

We started out in two directions, based much more on Robinow's experience than on mine. One was to do the best possible light microscopy of the components of what would now be called the cell envelope, i.e. the cytoplasmic membrane/cell wall complex (76). This was a necessary step because electron-microscope preparations of the time did not yet provide the contrast and differential staining in sections needed to demonstrate such membranes, although they soon would do so. The other approach, which was undertaken by P. C. Fitz-James (29), was to initiate studies of the biochemistry of a distinct cytological entity, the endospore of a *Bacillus* species. These studies of spores were influential then because they broke new ground and because they contributed to bacterial cytology (29). They indicated to us how powerful combined structural/biochemical research was in providing seminal data even with the restriction of resolution due to the wavelength of light. At this point it became obvious, despite our considerable pride at being able to attain excellent photomicrographs, that the potential of electron microscopy was developing rapidly and that high resolution was essential.

It is hard to realize now what we did not know about the structure of bacterial cells in 1950–1953. The microscopy of the day was giving a new dimension to understanding of cellular components and their functions by correlation with biochemical studies of many kinds of higher cells, and this was a stimulus to finding out what was hidden behind the somewhat undramatic facade of bacteria. The technical and intellectual essentials for effective electron microscopy were coming into focus. Metal shadowing (96) had been available for a few years and showed the topography of bacterial surfaces (36) and external structures such as flagella. Fractionation and chemical analysis of walls following disintegration of the cell was giving exciting data on the nature of bacterial walls in the capable hands of Salton & Horne (79) and could be monitored by electron microscopy even if the techniques were poorly developed. Sections thin enough for effective electron microscopy were obtainable from embeddings in methacrylate (70), and cytological fixatives such as osmium tetroxide (73) were being recognized as suitable preliminaries to embedding and sectioning tissues and bacteria for electron microscopy. I remember still my pleasure and excitement on viewing the first good micrographs of sections of a bacterial cell in the paper by Chapman & Hillier (16) submitted to me as an editorial board member for the *Journal of Bacteriology*. Importantly, the paper demonstrated that the images were recognizable in terms of the light-microscope cytological preparations that we and others were studying and relating to the biochemistry of fractions. Establishing the technical basis and accumulating the experience necessary for adequate prepara-

tion of bacterial cells and cell fractions for electron microscopy took more than a decade, and the techniques are still being refined.

Although we recognized the electron microscope as the coming instrument of cytology, Carl Robinow and I firmly believed then, as we do now, that the light microscope is here to stay. I remember brashly trying to persuade G. E. Palade that light microscopy could still do good things for understanding the insides of bacteria. Certainly, light microscopy gave us useful first approximations to the arrangement of the elements of the cell surface in studies of *Bacillus cereus* and *Bacillus megaterium*. A chance but very useful observation (65) showed us that when fixed cells were crushed on the cover slip and then stained, the walls were a rigid sleeve containing septa and the poles were hemispherical caps. The next step was based on Robinow's observation that these cells could be fixed in the plasmolyzed state to retain separation of the surface of the protoplast and the enveloping wall structures. Interpretation of the nature of the protoplasmic interface was greatly assisted by the partition of polar dyes (notably the Victoria blues) into an infinitely thin layer at the protoplasmic interface. This convinced us that this interface was an osmotic barrier, as had been known since 1895, and also was a differentiated structure equivalent to the cytoplasmic membrane of higher cells; the membrane profile was detected rather than resolved by light microscopy (76). We thus had an anatomical concept but were disappointed that the Chapman & Hillier (16) electron microscopic image and others of the time did not show a distinct cytoplasmic membrane at the surface of the protoplast. But most of the elements were there even if their topological features might be dim or distorted owing to inadequacies of fixation and processing; we could see a wall, a remarkably dense cytoplasm with some possible inclusions or spaces for them, cell wall septa accomplishing division, and something that could represent the Feulgen-positive nucleoids.

Salvation was around the corner. Robinow took his spore structure questions to K. R. Porter's laboratory at the Rockefeller Institute and found that sectioning was rewarding because it gave access to the hitherto inaccessible structure of the spore and revealed the cortex beneath the coats (75a). I took my interest in making preparations of infected host cells to R. W. G. Wyckoff's laboratory at the National Institutes of Health in Bethesda, Maryland, and found that whole-cell preparations were not going to take us far in phage or host-cell studies. These first experiences in electron microscopy and visits to James Hillier at the RCA Laboratories at Princeton, New Jersey, convinced us to seek funds for an electron microscope as an essential tool. A Philips EM-100 arrived early in 1954, which we shared for some years with our histologist friend and colleague, R. C. Buck. Learning was fun, but getting the very best out of that instrument (our only electron microscope until 1965) would have been slow indeed without the skill and experience of Aksel

Birch-Andersen (State Serum Institute, Copenhagen), who spent six months with us in 1955 and taught us more than the basics. Many tricks were needed then, including knowledge of when and where to kick an EM!

Carl Robinow's research interests turned to fungal cytology after 1953, and P. C. Fitz-James, now a department member, continued to utilize light and electron microscopy as a monitor and guide to recognizing the steps in spore morphogenesis and fractionation for biochemical studies. From then on each of us developed our own lines of inquiry, but they were close enough for mutual stimulus.

For my part, my graduate students and I explored the anatomy of a variety of bacteria including "blue-green algae." There was thought of taxonomic returns, then, as well as general insights into bacterial structure because J. P. Truant, J. W. Costerton, and I looked at a far wider range of physiological groups than was strictly necessary for the purposes of their theses. We worked toward better cellular descriptions for some bacterial genera such as *Moraxella* (67) and *Vitreoscilla* (19), with comments that applied to taxonomy. Obviously these experiences induced some broad intentions, because I wrote in 1960 (51): " . . . we can now glimpse the bare outline of what can be done to bring our knowledge of bacterial structure to a point where it can be incorporated into the whole picture we should like to have of the kingdoms of living things. . . . All the approaches to the unravelling of structure are bringing to light unique properties of bacteria that will stimulate more effective research." These forays were to be continued thereafter by increasingly focused inspections of *Lampropedia* (17), *Thioploca* (48), nitrifying bacteria (68), *Listeria* (31), and *E. coli* (66), each of which turned our thoughts to details of the cell wall, membranes, and protoplasmic structure.

At the end of all this work I had an unparalleled chance to assess the state of a large part of bacterial cytology by writing a chapter (51) for *The Bacteria,* a benchmark series edited by I. C. Gunsalus and R. Y. Stanier and published in 1960. I believe that volume, devoted entirely to structure and part of a still-significant series, put structural studies into the thinking of many microbiologists.

The major factors impeding the revelation and interpretation of cellular ultrastructure involved preparative techniques. Bacterial cells, particularly the nuclei, were sensitive to the ionic environment during fixation, and most fixation schedules gave unpredictable results. A suitable routine was not available until Ryter & Kellenberger (78) published in 1958 their still-useful method for using osmium tetroxide followed by uranyl acetate in a defined veronal buffer system. This method was not improved upon until dialdehydes, e.g. glutaraldehyde, were introduced in the mid 1960s and used in a prefixation step before osmium tetroxide postfixation. Embedding in a synthetic

resin evaded the disturbing artefacts generated by differential polymerization rates in the methacrylates used up to that time.

The sections that we cut were good enough, but the structural contrast due to scattering of electrons by the embedded cell substance was inadequate and could be even less than that of the embedding plastic. Many microscopists realized gradually that scattering could be increased by treating the sections with heavy metal salts to be taken up by ligands on structural polymers; for some time we favored lanthanum or uranyl salts rather than lead, which tended to precipitate. What was so surprising at the time was the discrimination of layers and substructure that became possible with these staining methods. An early outcome due to applying metal salts was our recognition in 1957 that a double-track unit membrane did indeed enclose the protoplast of the bacterial cell (50); not less important as a lesson was our then finding that earlier papers had shown such a membrane but it was not recognized. Generally we saw what we were prepared to see at each stage of our understanding of ultrastructure. We applied the technique usefully, for example in my study with Francombe and Mayall (61) of the remarkably direct effect of penicillin poisoning on the structure and integrity of staphylococcal cell walls, which helped to focus attention on the cell wall and peptidoglycan as the target of that remarkable antibiotic. Eventually in 1963 Reynolds (74) put into general use a method of combining lead citrate and uranyl acetate for staining sections, which became a standard treatment to give contrast.

We needed an effective method for following the fractionation of cells and cell walls and assessing the nature of the pieces of cells displayed after ballistic or sonic disruption. We used light microscopy to monitor cells and fractions negatively stained with nigrosin, a most useful technique then and now, but limited as to resolution; and we sectioned pellets to get profiles. Despite the amount of nigrosin that we used it never occurred to us that negative staining for electron microscopy was possible; the demonstration of this technique and practical applications by Brenner & Horne (7) was a scientific bombshell with repercussions continuing today. Brian Mayall and I tried it and got immediate results looking at a moiré pattern of an array on a wall fragment of a strange coccus, later identified as *Deinococcus (Micrococcus) radiodurans*. We described this in a review (52) and demonstrated it in a paper at the 1958 Microbiology Congress in Stockholm. It was exciting enough even if no formal publication resulted, but it did lead us into other applications and particularly studies of the nature of wall arrays in *Aquaspirillum serpens* (53), which have engaged us ever since.

These general and technical developments, which I hasten to say we participated in but did not originate, allowed us to enter into more detailed and complex studies. A worthwhile preliminary to our relation of structural studies and biochemical fractionation was determination of the location of the

peptidoglycan component in the sectioned wall profile of *E. coli* and some other gram-negative bacteria (66). The location correlated exactly with the order established in the elegant biochemical dissection of the *E. coli* wall initiated by Weidel and colleagues (93). Then to carry this further I studied what happened to these layers during cell wall septum formation and cell division with Pamela Steed-Glaister, then doing her PhD studies, and we established an effective baseline for the wall studies to come (86). This work allowed us to understand the various forms of septum that we see. Septa incorporate a peptidoglycan layer as the primary component, which intrudes either as a double loop of peptidoglycan or as a single layer that thickens and differentiates into a doublet (laughingly attributed to "zipperase") to allow intrusion of outer layers and the ultimate separation of the cells. However, the constrictive divisions of *E. coli* and all but a few mutant enteric bacteria were a puzzle until we were able to show (first serendipitously, by my having let a water bath get too hot) some 10 years later that an appropriate fixation regime revealed septum formation of the basic type involving the peptidoglycan layer. We had to be confident and persuasive, thanks to the clear experiments of Ian Burdett (14), that this was an image that could not possibly be an artefact; yet the way some of our colleagues refer to septation in *E. coli* makes one wonder whether or not they are convinced. However, the structural details of these events are now being resolved in great detail for both gram-negative and gram-positive models (34, 38, 47).

Electron microscopy, although limited for cytochemical analysis, was adept at revealing structures in search of a function. Among these were the membranous intrusions often found near the site of septum formation and at the poles of the dividing cell. These lamellated structures were named mesosomes by Fitz-James (29) and were recognized as having continuity with the cytoplasmic membrane. These may have been among the sites identified earlier as bacterial mitochondria, because the granules exhibited strong oxidation-reduction reactions (49). However, cell fractionation indicates that respiratory activity is a general property of the cytoplasmic membrane. The polemical arguments died and left behind the interesting problems raised by the variety of membranous structures becoming visible in the cytoplasm. Some of these structures were obviously functional, as was supported by biochemical data, providing for photosynthesis or for complex metabolic processes requiring coordinated energy transfers such as nitrification.

Stanley Watson and I (68) were excited by the elaborate membranes of nitrifying species of *Nitrosocystis, Nitrosomonas,* and *Nitrobacter*. It was clear from these and other examples that there were membranous organelles, but in most cases they arose from and were still continuous with the cytoplasmic membrane. The exceptions, where the internal membranes separated from the peripheral membrane, still originated from the cytoplasmic

membrane, however specialized the final function. Thylakoid membranes in cyanobacteria may yet prove to be the only bacterial membranes independent of the cytoplasmic membrane. In general, a major demand for a membrane-based function results in more membrane, and space for it has to be gained by internal projection, which may be structurally differentiated for whatever energy-linked process the cell may require. We now realize that not all membrane functions can be everywhere in the periphery and that a degree of area specialization is a necessity.

What is really involved in the formation of mesosomes is still far from certain; they may be artefacts of fixation because they are absent in freeze-cleaved preparations not exposed to a fixative (99). Yet they occur in persuasive sites, and the bits of membrane that give rise to them, even as artefacts, may be associated with some specific function. One concept that may not be too farfetched is that their function derives from their association with nucleoids (77) and the probability that DNA replication and nucleoid segregation requires an association with membrane sites.

VENTURING INTO MACROMOLECULAR ASSEMBLIES

An important outcome of the introduction of electron microscopy has been our ability to recognize specific cellular components and, by their structural characteristics, to recognize them also in the fractions generated after cell disintegration. This discriminatory function has been as essential to many cell biology structure/function studies as it has been to virology. Discrimination was made immensely more effective when negative staining with appropriate heavy metals (notably phosphotungstates, molybdates, and uranyl salts) was added to the techniques of metal shadowing of specimens and freeze-cleaved and etched preparations (7). What then became possible was the outlining of the shape and form of some kinds of macromolecules, mainly proteinaceous, to a level of detail limited by the resolution attainable on that type of specimen and the potential of that electron microscope. So an effective resolution of about 2.5 nm became possible on biological specimens, which cannot be infinitely thin. Negative staining was applied in many fields, but in microbiology there was a rapid evolution of structural studies on viruses, membranes, ribosomes, and in our case bacterial surface arrays.

I was very impressed by the images we obtained early in the 1960s from negatively stained specimens of cell wall fragments of *Deinococcus (Micrococcus) radiodurans* (52), *Lampropedia hyalina* (17), and *Aquaspirillum serpens* (53). The fragments showed as two-dimensional, hexagonal arrays of linked subunits with paracrystalline regularity that enveloped the external surface of the cells. The arrays had every possibility of being amenable to fractionation and analysis after the fashion of the techniques applied to *E. coli*

by Weidel's group (93). Other possible arrays were assessed; M. V. Nermut, who was visiting in 1966, and I found that *B. polymyxa* possessed a tetragonal array (69). We explored the stability of this array on exposure to various chaotropes (e.g. types of detergents), which we thought might be of use in attempting isolation of the components. We found, as did Baddiley's group (32), that the fraction containing the array was mostly protein. We also took a first step toward image processing by enlisting the help of Klug's group at Cambridge. They found that each unit forming the tetragonal array was formed by four centers of mass arranged in p4 symmetry with the whole array (28), in which the lattice frequency was 10 nm. The units are now better resolved (15).

It was obvious that these arrays, or S-layers as they are now termed, were a common feature of bacteria in nature and, as a major protein component of the cell, would be retained on these cells for reasons of selective advantage. They needed serious study, and we chose to study the S-layer of *A. serpens* because it was a single layer and seemed to have properties useful for the manipulations required for isolation, as Pamela Steed-Glaister's studies of stability during growth in fluid media had shown (87). Francis Buckmire and I (11–13) isolated the *A. serpens* VHA S-layer protein and explored its properties, and these studies formed the basis of a study that I still continue in association with Susan Koval (42). The protein proved to be a large, 140-kd, acidic protein that can self-assemble into a lifelike array with the help of Ca^{2+} and the template provided by the outer membrane of the organism. This protein has been subjected recently to further biochemical and assembly studies (39–41), and its properties help to provide a basic description of many of the S-layer proteins (81, 82). I explored the variety of structures possible on the walls of a number of species of *Aquaspirillum* with T. J. Beveridge. Among them *A. putridiconchylium* showed p2 symmetry (4, 89), and several had two or more layers. *A. serpens* MW5 had a double layer and, in similar fashion, was subjected to image analysis in a collaboration with Murray Stewart (90). The MW5 array showed in freeze-etched preparations as a linear structure and in negative stains as a linear moiré. The image analysis resolved this moiré as two hexagonal arrays with similarly sized units superimposed but slipped a half interval along one three-fold axis. I studied the proteins with Marion Kist (37), and we found that the inner layer protein (150 kd) would self-assemble in vitro but would also form a directly linked array on the outer membrane. But the outer layer protein (125 kd) would assemble only on the formed inner layer, and these assemblies (like that of strain VHA) required either Ca^{2+} or Sr^{2+} even if the inner layer hexamer unit did not. The proteins have few or no sulfur-containing amino acids, and it is apparent in our model systems that divalent cations are required for both assembly and the conformation of the monomers in most cases (41). The structural studies of the

VHA S-layer were only two-dimensional or planar until recently; in 1986 some fortunate preparations allowed Robert Glaeser's group (24) to undertake filtered Fourier transform reconstructions from a tilt series of micrographs to give a three-dimensional model of the assembled units, each consisting of six monomers. However, other aspects of understanding are primitive or nonexistent, and these include molecular structure, transport, and regulation.

The other early examples of arrays that started us off have not been neglected: *D. radiodurans* turned out to have some considerable biochemical and taxonomic peculiarities, as I shall recount, and less attention was paid to the S-layers (43, 91, 100). However, Baumeister's group (2) has undertaken detailed structural analyses. *B. polymyxa* engaged me again in a comparative study with Stephen Burley (15), which served as an exercise in establishing our image-processing facility. Eventually, I have returned to the double S-layer that surrounds the cells of *Lampropedia hyalina* (1); this was much too complex a structure to consider before, and even now it is a challenge to work out the order of assembly and the identity of the multiple components of the outermost layer.

The considerable amount of information now available about S-layers is not susceptible to summation in this essay. They have been described as a component of the walls of about 200 species of bacteria from most major phylogenetic groups, and the phenomenon must be considered as a general attribute with varied functions. Fortunately there are several recent reviews (42, 81, 82) to provide the details. To a considerable extent my early work sparked an interest in these models of structural assembly of macromolecules, and the 25 years since then have involved me with 25 coworkers; sadly, it is not possible to give appropriate credit to all of them in this essay.

I had hoped at one time to be able to contribute to a structural and macromolecular description of the basal complex, the motor, of flagella in studies undertaken with James Coulton (20). It is more than a challenge to resolve components of this puzzling rotor-stator mechanism, which is an organelle not much more than 25 nm in total depth and diameter, effectively described in principle by DePamphilis & Adler (23). The closest we got to any macromolecular description of the operative part in the plasma membrane was to see that the central rod was made of triads forming a hole down the center, and to find a circlet of studs around the M-ring in the freeze-cleaved membrane. I remain sceptical, as are others (27), about the exact relationships of the structure to the plasma membrane, and I believe that we are missing the boundaries of a compartment and some part of the complex penetrating to the cytoplasm.

The bacterial wall is a remarkably complex and dynamic structure interposed between the cell and an often hostile environment. The export-import services and machinery such as flagella that traverse the wall put

special demands on the maintenance of integrity and the assembly-disassembly capabilities of the entire complex. Enormous effort has been given to understanding the governance of the murein covalently cross-linked network because it is a target for antibiotics. The entropy-driven assemblies of membranes and porins and the integral transport mechanisms have had well-deserved attention in recent years. The S-layers are equal participants in the dynamics of walls in growth and division, and go by a simpler but no less sophisticated set of rules. Furthermore, they draw attention to functions other than strength (42), and the more complex of them may act as generalized models for the assembly of cell structures.

TAXONOMY IN TRANSITION

There were compelling reasons for my getting involved in taxonomy: My father was a trustee of *Bergey's Manual of Determinative Bacteriology* (1936–1964) and we talked about the problems; my work involved diagnostic bacteriology and wrestling with identification, and the study of the structure of bacteria inevitably drew attention to the inadequacies of descriptions. An early direct contact with the *Bergey's Manual* trust was an invitation from R. S. Breed in 1955 to join in discussing the description of the "Schizomycetes" and how it might be revised; my advice was not taken.

Work on structural aspects of bacteria had brought both Carl Robinow and me into contact with Roger Y. Stanier, whose thoughts about the nature of bacteria and approaches to taxonomy, sharpened by his association with C. B. van Niel, were particularly penetrating. His papers shaped my views and my intentions toward the definition of unique features of bacteria. The opportunity to do something about them came hot on the heels of my completing the review of structure for *The Bacteria* (51) with the invitation to contribute to the 1962 *Symposium of the Society for General Microbiology*. This essay, "Fine structure and taxonomy of bacteria" (52), had some good ideas, but the conclusions were not as strong and definitive as those of the almost coincident essay, "The concept of a bacterium," by Stanier & van Niel (85). Neither essay developed a formal taxonomic proposal, but both were disposed to accept any nomenclatural arrangement that recognized the relationship of blue-green algae and bacteria, that incorporated them both into a grouping distinct from all other microbes and macrobes, and that recognized their unique features of organization. Stanier & van Niel referred to them consistently as "prokaryotic organisms," which reflected Stanier's decision the year before (84) to describe bacteria as cells and to use Chatton's vernacular terminology, introduced in 1937 (18), for the major divisions of cellular organization, eukaryotes and prokaryotes. I feel that I rode on the shoulders

of worthier colleagues because, almost inadvertently, I was the author of the formal name, the kingdom Procaryotae (54). I had something to do with establishing the generalizations that supported a position that became agreeable to most microbiologists.

It was clear to me that taxonomy was a worthwhile endeavor, even if it was still unpopular, because it required the use of all that one knew (or thought one knew) about organisms and because it drew attention to great gaps in basic knowledge. At that time objective means of checking the relationships in an arrangement of taxa or within any taxon were only just developing. Both the selection and the reliability of the phenotypic characters were largely a matter of faith. Mechanisms evolved over the past 30 years have given a scientific aspect to the assessment of characters and, far beyond the powers of serology, the definition of taxa. These mechanisms are now well known and came to include, successively, numerical (computer-assisted) taxonomy, murein (peptidoglycan) types, the G+C ratio in DNA, DNA/DNA homology, DNA/RNA hybridization, and the exploitation of the highly conserved RNA cistrons and the ribosomal RNA sequences. The power of these approaches to taxonomy has only been fully realized in the past decade, and the data supporting a phylogenetic assessment are still only partially complete.

I joined the *Bergey's Manual* Board of Trustees in 1964. The chairman, R. E. Buchanan, was strongly oriented to nomenclature and classical approaches. Most of the trustees were practical bacteriologists, but there was one grand heretic among them in the person of S. T. Cowan (22). A consensus in views was hard to accomplish, and arguments prolonged the gestation of the 8th edition, which did not appear until 1974. The turning point came in 1969, by which time R. Y. Stanier was also a member, with the decision to use vernacular names for the chapter headings of the 8th edition because the higher taxa were of dubious validity. However, our expert authors were by no means convinced, or if they were in principle, many traditional arrangements were nonetheless maintained.

We were by no means satisfied, scientifically, with what we had done, despite all the good intentions of N. E. Gibbons (the editor, following Buchanan's death) and the board of editor-trustees. I wrote at the time (55): "The future will have to bring a regrouping of higher taxa to express a more coordinate view. . . . Haste is unwise; all previous classifications seem to have suffered infinite rearrangement due to insufficient information. . . . The new insights are likely to come from a clear understanding, on a comparative level, of the components of the genome of procaryotic cells." That goal is closer now.

The ten years after 1974 saw some remarkable changes in the rules that govern the nomenclature that expresses the taxonomic conclusions of bacteriologists. The 1975 *International Code of Nomenclature of Bacteria* (44) allowed for clearing the records of the enormous list of synonyms and useless

names by declaring January 1, 1980 to be the new starting date for bacterial nomenclature and arranging for the *Approved Lists of Bacterial Names* (80) to be available from that date. We owe this most unusual form of taxonomic housekeeping (which generated envy in taxonomists devoted to plants and animals) to the international efforts of V. B. D. Skerman and P. H. A. Sneath, in particular, through the Judicial Commission of the International Committee for Systematic Bacteriology. It was time for an assessment of taxonomic problems in the groupings of bacteria, and the *Bergey's Manual* trustees resolved to produce a systematic manual.

A new view of bacterial taxonomy has arisen since Carl Woese and his group proposed (30) that the computer-assisted comparison of T_1 ribonuclease–resistant oligonucleotide sequence catalogs of the 16S ribosomal RNA of representative bacteria gave clear evidence for the phylogenetic lineages emerging from the main stem of the clonal evolution of bacteria and, in fact, all living things (97). They all share the ribosomal mechanism for constructing proteins. The ribosomal RNA molecules are up to now the most powerful, universal, and practical biological semantides or molecular sequences documenting evolutionary history in the sense of Zuckerkandl & Pauling (101). So the past decade has seen the accumulation of data on some 500 representative strains from many but not all taxa, and many old taxonomic assumptions can be tested. There are several distinct high-level phylogenetic taxa in both of the major phylogenetic divisions, the Archaebacteria (at least three) and the Eubacteria (at least 10). Some of the rRNA groupings of Eubacteria (98) appear to pose few problems of phylogenetic and phenotypic interpretation (e.g. the spirochetes and the cyanobacteria); some provide associations that are hard for traditional bacteriologists to assimilate (e.g. among gram-positive bacteria, *Micrococcus* closely associated with *Arthrobacter*); and others show such a remarkable diversity of morphology and physiology that there will be difficulty in developing phenotypic consistency [e.g., notably, the purple photosynthetic bacteria and their relatives (92)]. One can be optimistic, as Woese is (97), that the bacteria will eventually fall into naturally (i.e. phylogenetically) defined taxa and that an appropriate search will reveal unifying phenotypic characters. I hope that we will soon discover other cistrons determining nearly universal, complex, and essential cellular functions that are highly conserved. We must try to cross-check the phylogenetic conclusions, now based almost entirely on the RNA cistrons, especially in the diverse and rapidly evolved groups to detect anomalies or lateral transfer. This is the more important because transfer of heterologous ribosomal RNA genes (*Proteus vulgaris* to *E. coli*) has been attained with a plasmid vector (71) and as a result 25% of ribosomes contained the heterologous rRNA. Genomic integration has also been claimed, and if this is true, a phylogenetic chimera is possible. Who knows what strange experiments have succeeded in nature's laboratory?

With all this ferment, the development of *Bergey's Manual of Systematic Bacteriology* in four volumes appearing serially 1984–1988 presented unusually difficult problems, which came during my time as chairman of the trustees, 1976–1988. The data were compelling but patchy. Of course, it is sad that one cannot instantly and dramatically reform the whole of bacterial taxonomy, as was the fervent hope of the main laborers in the field of molecular phylogeny (98). It was possible to institute no more than cosmetic changes in many groups, major revisions in only a few, and phylogenetic consistency only in the *Archaebacteria*. Now it can be recognized that it is important to express the principle of *festina lente* (make haste slowly), as exemplified by the approach to the problems in the *Bacillaceae* (83), and keep the complexities in reserve until a more complete survey and decisions are accomplished. This attitude is helpful for the retention of a useful framework of classification for groups, including several genera threatened with major surgery.

I have been interested in applying modern forms of taxonomy to the *Deinococcus* group (9), seemingly gram-positive cocci with gram-negative characteristics, which proved (10) to be a distinctive and ancient lineage but phenotypically deceptive. If it is an ancient set of clones there should be relatives which might be hard to recognize. The superficially distinctive feature of radiation resistance is a mutable character, so the usual selective mechanism of using radiation is likely to reveal only clones similar to the extant species. The distinctive polar lipid profile (21) may aid recognition, in addition to the ribosomal RNA sequences and select signatures, which have already identified a gram-negative relative in *Deinobacter grandis* (72). I am prepared to follow with great interest the next steps in developing a new natural taxonomy of bacteria.

Fortunately, what appears to be accepted in classificatory schemes is not immutable. Taxonomy has to represent the best of science and for that reason is bound to change with new knowledge. I have speculated on the need for an academic taxonomy as opposed to a practical classification to serve the bench worker who identifies bacteria (57). However, there is need for only one taxonomy (92), soundly based in phylogenetic terms and phenotypically recognizable at all ranks, expressing a best view of a natural order. The bench worker does not need a complete hierarchy but operates within a framework of experience and needs only a number of identifiable vernacular groupings. No doubt, we must eventually develop new molecular and phenotypic markers for the recognition of all taxa. The tests used should be available and practical.

There will be changes in approaches to identification, but it is to be hoped that whatever systems are used, they will not inhibit the recognition of new taxa. Almost every ecological niche includes species that have not been

cultivated and recognized, which should dispel any feeling of diminishing returns in the study of natural populations or in taxonomy.

ODDS WITHOUT END

Research may owe its continuing support to high-profile projects, but the next generation of research and so-called innovation will owe a lot to the odds and ends undertaken out of sheer curiosity, to satisfy a hunch, to provide experience for a summer student, and for many other ostensibly trivial but important reasons. These items enliven what otherwise might be periods of diminishing returns and spawn unexpected new research; so they become "odds without end." All research programs should devote a proportion of their funds to free-wheeling exploration (What the X Foundation doesn't know isn't good for it), much as buildings need a proportion of cost devoted to art. Some experiments should be done with "controlled sloppiness," as S. E. Luria told me years ago in order to encourage the unexpected. (If you do the same old thing you will get the same old answer.)

When T. J. Beveridge was working with me on his doctorate and studying the wall structure of spirilla we had many an occasion to discuss the problem of revealing substructure by staining with metal salts. So we experimented with isolated *Bacillus subtilis* walls to see what sort of capabilities the durable cell-wall polymers and heteropolymers might have in capturing and sequestering metal ions from solutions of their salts. The results (5) showed that substantial amounts of many metals were taken out of solution (including those important to metal enzymes such as Fe^{3+}, Cu^{2+}, Mn^{2+}, and Zn^{2+}) but some were not absorbed (such as Li^{+}, Ba^{2+}, Co^{2+}, and Al^{3+}). Furthermore, if the walls were linked to a column and a series of metal-salt solutions was run through it, some metals were strongly bound (including Mg^{2+}, Ca^{2+}, Fe^{3+}, and Ni^{2+}) and others were displaced or replaced. We were interested in the effects of modifying ligands (6) or, for instance, of saturation with Mg^{2+} on the subsequent staining of structures with ruthenium (severe) or lead and uranyl acetate (minimal). But our geologist colleague, Professor W. S. Fyfe, was more interested in why many ore bodies have a high percentage of organic residues and particular selections of metals. The mediation of biopolymers provided a stimulating hypothetical mechanism. The observations were extended by collaboration on geological diagenesis (3) and have been explored further in recent years (26), as have the consequences for the physicochemical well-being of wall components (25). Of course, parallel work has been and is going on in many laboratories concerned with ore leaching and metal transport in waters, to which our observations have contributed.

A discussion with Carl Robinow, C. L. Hannay, and Philip Fitz-James

about why *Bacillus laterosporus* spores were eccentric in the cell led to the recognition of parasporal bodies and to the rediscovery of the spore-associated crystals in *Bacillus thuringiensis* (33). This is certainly a fertile field of research and practical application today.

On a lesser scale, an early study with Aksel Birch-Andersen (58) on the nature of flagella basal structures was diverted somewhat by the finding of a decoration on the inside of the plasma membrane of *Aquaspirillum serpens*, which we called polar membrane because of its position surrounding the polar tuft of flagella. An exactly similar structure is associated with many lophotrichous bacteria. We have now tried to understand this "structure in search of a function" and have found that the polar membrane of *Campylobacter jejuni* is a close-packed array of ATPase (8). What is consequential is that this particular ATPase turned out to be unusual by being specifically directed to ATP, activated by Mg^{2+} but inhibited by Ca^{2+}, made of a single subunit, and serologically distinct from *E. coli* ATPase; it is also likely to be a phylogenetically distinct lineage of that highly conserved enzyme.

As at the beginning of my time in science, the odds and ends still prove to be a stimulus. The current example is the lucky finding that high growth temperature stimulates cyst formation by *Azospirillum brasilense* (63). Now we know that a heat shock is equally effective for cyst formation at a growth temperature that is usually ineffective.

SOME SERVICES TO SCIENCE

I was brought up to believe that science knew no boundaries and that it was a duty of scientists to communicate freely. So from the outset I joined with the major societies in my orbit and took part in the meetings of the Laboratory Section of the Canadian Public Health Association and the Society of American Bacteriologists, as they were known then. The friendly winter meetings of the former, with extensive discussions of medical microbiology, and the comprehensive coverage of microbiology each springtime at the meetings of the latter were just what I needed as a stimulus to get started. But I was soon (1950–1951) in the midst of organizing a Canadian Society of Microbiologists, a much-needed catalyst and unifier for the diverse applied and basic microbiologists of the country. It took off owing in large part to the efforts of N. E. Gibbons, the founding secretary. I was put by my senior colleagues in the position of chairman of the organizing committee and the inaugural meeting in Ottawa in 1951. This was unforgettable for me, not just because it gave me a fine experience and the confidence of my colleagues, but because my headache on the last day of that meeting was due to encephalitis, which bedded me in that city for three weeks and took seven months out of my working life. The charter members elected me the founding president, per-

haps as an act of kindness. Societies and their journals have been important to me and my work ever since, particularly the Canadian and the American societies.

I had an interest in editing, but it is not clear to me why I was asked to join the editorial board of the *Journal of Bacteriology* by J. Roger Porter in 1951; the invitation was unexpected because I had published less than a handful of papers and only three in that journal. Membership on the editorial board was good experience for my appointment as founding editor of the *Canadian Journal of Microbiology* (1954–1960); this appointment was less mysterious because I had a role in persuading the National Research Council of Canada that the journal was justifiable. In these years I learned the basics of editing from experienced colleagues and by doing it. My most challenging assignment was as editor of *Bacteriological* (later *Microbiological*) *Reviews*, 1969–1979, when I learned properly that a scientific editor's crucial job was not just to adjudicate the reports of referees but was to help authors to do their best (56). It was no mean exercise in diplomacy to deal with the sensitivities of undoubted authorities in their fields, both authors and referees, who could produce not only an appalling text but also a distressing narrowness of view. This was one aspect of my education; the other was association with people of marvelous ability and judgment both on the editorial boards and on the Publications Board of the American Society for Microbiology, notably L. Leon Campbell and Robert A. Day, who really taught me about the management and production of scientific publications. My association with the International Committee for Systematic Bacteriology and the *International Journal of Systematic Bacteriology* has brought experience in the additional stringencies of monitoring the description of bacteria and the application of rules of bacterial nomenclature. There is a great need to help authors in a field unfamiliar to most of them. I feel strongly that an editor is not the savior of science; if the editor is any good, the role is more of a "friend in court" for the author, who is the only person really responsible for what is written. The editor is the author's most concerned critic and has the advantage of taking or refusing the comments of referees to formulate rational advice.

My involvement in *Microbiological Reviews* increased my interest in the affairs of the American Society for Microbiology and my interest in what societies do for science and society.

Being an editor of a first-rate journal has an enhancing effect on one's scientific profile. I was most honored to be a candidate and to be elected president of the American Society for Microbiology, 1972–1973, which was in all respects a remarkable experience. I am sure I owe this signal recognition by the membership as much to my activities as an editor as to my scientific contributions.

I regret no part of these varied experiences and recommend that all aspiring

young scientists owe a duty to scientific societies and their journals. We have to be prepared to communicate and to promote the highest standards in both the meetings of scientists, in the broadest arenas possible, and the journals of science. It is not good practice to speak only to a group of connoisseurs. We must play the roles of critic and of supporting actor in the interplay of science and society.

ENVOI

Science has shifted in the past 50 years from being the province of scientists to being a crucial part of our culture, permeating all conditions of life and living. Microbiology may have been ahead of most disciplines in this matter because of the sensitivity to matters affecting human health. The shift should be encouraging because one might hope that wisdom should accompany or be encouraged by understanding and technical competence. But there has been no more than a minimal attainment of that goal, and man's place in nature's world (and vice versa) seems ever more insecure, much as we would prefer to interpret it otherwise. My own contributions to the fostering of wisdom have been minimal, especially on the public and political fronts, which are so critical. At one time I scorned taking on public enlightenment, but I am convinced now that it is the only route to the heart of the political animal, which is effectively indifferent to anything beyond votes and short-term gains. I now believe that scientific societies and institutions should balance their concerns for communication within the house of science with an equal concern for public understanding as a stimulus to political action. I am not proud of my inaction in the public arena, nor am I impressed with what we have managed to do in our universities and colleges to inculcate a high level of understanding in the 15–25% of the new generations that transit these institutions seeking enlightenment.

A major factor in fostering my scientific activity and productivity has been the continuing support by research grants thoughtfully administered, and I owe thanks to the old Medical Committee of the National Research Council of Canada and its successor, the Medical Research Council of Canada. The former did me a great service for ten of my early years by awarding a block grant, which had no strings attached and only required a letter at year's end asking that it be continued, with a minimal accounting. It was a fixed sum and I had to abandon the award due to inflation of costs, but it did a great deal of good. Life is not so easy for even the most fortunate of young scientists today, who must spend 10% or more of their year in supplicating for funds. From the way such applications are treated one wonders whether or not anything should be done for the first time.

An unwitting scientific reward resulted from my study of bacterial structure

because understanding of the structure of cells was an essential accompaniment to the development of cell biology and modern microbiology. As I have pointed out, a part of the transformation of bacterial taxonomy involved the realization that the unique features of bacteria as cells are crucial to description, and this was equally true of the understanding of function. The microscopist was, therefore, in a fortunate position on the sidelines of the great game between the cell biologists and the molecular biologists, and had entry to the game in some of the interesting plays. I may not have contributed more than peripheral structure to the game, but some friends and colleagues in cytology were major players, such as Keith Porter and Edward Kellenberger, to each of whom we owe a lot of our understanding. The great importance of correlating structure and function will be no less tomorrow than it is today.

Many of my contemporaries joined in the great exploitation of *E. coli* and a few other "handmaidens of science," doing a great service to science and mankind in the doing. However, their lives had a hectic and unenviable pace compared to the life I led without the hot breath of competitors on my neck. My colleagues and I protected ourselves from that fate because, it may be noted, we usually worked on organisms that were not commonly exploited and we could work at our own rate. This attitude also allowed a diversity of research topics, from biochemical cytology to taxonomy, which could be pursued as and when competence allowed. There was opportunity for me to be helpful to colleagues and for them to be helpful to me, which provided scientific pleasures in good company.

Where is the bacteriology I once knew? It is changing in a technological fashion in the medical diagnostic laboratories where I worked in the past. I fancy I would be more comfortable now with the microbial ecologists and those interested in organisms and their associations; their studies give rise to vistas broader than the most direct route to diagnosis. Times change and attitudes must change with them, but our old bacteriologists have done their duty and assisted the birth and development of microbiology and a remarkable range of disciplines and subdisciplines of biology. Whatever happens to current developments, and despite all diminishing interdisciplinary distinctions, we must see that there is a strong disciplinary base of teaching about the life and interrelationships of microbes. Microbiology departments should not be indistinguishable from departments of biochemistry, however well we get on with each other. The biosphere has been explored only in part, and there is much that we need to learn about the life and nature of the microbes that are no small part of it.

It is obvious that I owe much to the people in my life, and I can mention only a few of them in the context of even fewer activities. The roles of some, such as my parents, have been made clear and must not be underestimated. However, my life would not have been half as productive without the

understanding of my first wife, Doris, who was my helpmate for just short of 40 years, or, in the past few years, the supportiveness of my second wife, Marion. My life has been enlivened and the work in the laboratory has prospered because of the talent, energy, and adventurousness of the graduate students and postdoctoral fellows who have spent their time with us. I am ever grateful to a passing parade of friends in and around the laboratory; but a few, who are all colleagues whatever their calling, have contributed more to me over the years than I can possibly acknowledge: Igor N. Asheshov†, Terrance J. Beveridge, Aksel Birch-Andersen, Myrtle Hall, Phyllis Hobson, Gertrude G. Kalz, Susan F. Koval, Marion I. Luney, John F. Marak, Dianne Moyles, Carl F. Robinow, Roger J. Rossiter†, and Gertrude Vaughan-Dragon.

Microbiology, in its own ways, tells us about life as it is reflected in the microbial cells, which have been in existence for longer than the imagination can appreciate. It tells us about how and where life can be lived, about the extremes of survival, and something about how life has evolved; but how it came about and where it came from is among the mysteries. Mankind must listen to the messages.

My dream is faded now, and I am through
With dreaming . . . yet I know
The iris still will keep its gorgeous hue.

Shushiki (Transl. C. H. Page)

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