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A CHARMED LIFE

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ABSTRACT

Boris Magasanik was born in Kharkoff, Ukraine, on December 19, 1919. He received his preliminary and secondary education in Vienna, Austria, and studied chemistry at the University of Vienna in 1937. He continued his studies at City College, New York (BS, 1941) and after one semester of graduate study at Pennsylvania State University, served in the US Army in England and France from 1942–1945. He obtained his PhD degree in biochemistry from Columbia University in 1948. A faculty member in the Department of Bacteriology and Immunology at Harvard Medical School from 1951 to 1960, he then continued his career as Professor of Microbiology at MIT where he served as head of the Department from 1967 to 1977. He became a member of the National Academy of Sciences USA in 1969 and received the Selman A Waksman

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Award in 1993. He is currently the Jacques Monod Professor Emeritus at MIT and is the author of over 240 publications dealing with microbial physiology and the regulation of gene expression in bacteria and yeast.

INTRODUCTION

Gathering my thoughts for the writing of a biographical chapter for the *Annual Review of Microbiology* forces me to come to grips with the obvious question of whether my life was sufficiently different from the life of other academic scientists to be of interest to potential readers. Perhaps where my career differs most from that of others is during the early stages. My life from birth to the age of 25 years was almost entirely determined by the political events of the period between the two world wars and by World War II. The beginning of my independent scientific career coincided with the beginning of large-scale government support of academic science. Somewhat later I had the opportunity to participate in the expansion of a biology department to a center for teaching and research in molecular and cellular biology.

VIENNA, NEW YORK, AND THE US ARMY

I was born in Kharkoff, which I thought was in Russia, but now turns out to be in Ukraine, on December 19, 1919. According to family accounts my birth coincided with the capture of Kharkoff by the communist forces in the civil war. My parents, belonging to the capitalist class, fled with me two years later to Vienna, Austria, where my mother had close relatives. I grew up in Vienna, where I attended the Akademisches Gymnasium, a secondary school that prided itself on having educated Schubert, Schnitzler, and other luminaries, graduating in 1937. The school was a Humanistisches Gymnasium with seven years of Latin, five years of Greek, excellent German literature and history, no modern languages, adequate mathematics, and poor science. I had private tutors for Hebrew, French, and English. Although history was my favorite subject, I decided to study chemistry, possibly because a younger brother of my father had obtained his doctorate in the field of physical chemistry in Germany and at that time was working at the agricultural research institute in Rehovot, Palestine; however, I knew this uncle only from his brief visits to Vienna.

The political and social situation in Austria during this period was bad. The government, a Fascist-Catholic dictatorship, succeeded in the bloody suppression of two revolts, one by the socialists and the other by the Nazis in 1934, and due to the world crisis, unemployment and poverty were extremely common. We, as refugees, did not have Austrian citizenship but rather a League of Nations (Nansen) passport, which made travel abroad difficult. I spent my

summer vacations mostly in the mountains near Vienna, but as a graduation present went to the World's Fair in Paris in the Summer of 1937.

I started to study chemistry in the fall of 1937. Almost all the time was devoted to laboratory work in qualitative analysis. I enjoyed the work, but at the beginning of the second semester, it all came to an end with the German occupation, which was accepted with great rejoicing by the majority of the population. All Jews were immediately excluded from the university.

Luckily, my older sister and brother-in-law had moved to New York two years earlier. He was a physician, well known for his research on diabetes and nutritional diseases, and had earlier been invited to give a series of lectures in the United States. I came to New York in the summer of 1938 and my parents followed in the fall.

I started my studies at City College where tuition was free. I got credit for some of the courses I had taken at the gymnasium, such as German, Latin, history, etc, and for analytical and inorganic chemistry. Switching between day, evening, and summer sessions and working as a technician in a research laboratory at Mt. Sinai hospital, I obtained my BS degree in June of 1941.

Because I had to take a combination of beginning courses in English composition, literature, and speech and advanced courses in chemistry, and because of my switching between day and evening sessions, I made no friends at City College, but was part of a circle of young people who like me had escaped from Vienna. In Vienna I had greatly enjoyed frequent attendance at the State opera at low cost, in the standing section of the fourth gallery. I was able to afford to attend outstanding performances at the Metropolitan Opera in New York in the same manner.

In the fall of 1941, I started my graduate education at Pennsylvania State College. This school had a high reputation for organic chemistry, was not too far from New York, and charged out-of-state graduate students the same tuition as residents of the state; in other words very little. It was at Penn State that I listened on the radio to the account of the Japanese attack on Pearl Harbor, and this event changed my life and career. All the other graduate chemistry students obtained defense jobs immediately upon our entering the war, but not having as yet been eligible for US citizenship I was excluded. I was drafted instead and found myself in the very cold February of 1942 in Camp Upton on Long Island near New York.

To my surprise, I was not shipped out of Camp Upton as quickly as the recruits who had come with me; it turned out that the army had a plan for me. In those days, civilian hospitals provided the officers, namely physicians and nurses, for military hospitals, while the army supplied the enlisted personnel. The army chose me to be the technician responsible for the blood chemistry laboratory of the 2nd General Hospital, whose officers had been supplied by the Presbyterian Hospital, associated with Columbia University in New York.

The hospital was being organized at Forth Monmouth between Baltimore and Washington. I was quickly promoted to corporal, and then to technician third grade, corresponding in rank to staff sergeant, and I retained this exalted position for the rest of the war. In June of 1942, when our hospital unit received orders to proceed to England, the proper authorities perceived that I still lacked US citizenship. Although under normal circumstances I would not become eligible for citizenship for approximately another year, I was taken by car to a judge in Baltimore where, with some emotion, I became a citizen of this country. Our hospital then embarked for England on a liner converted to troop transport. Our voyage was uneventful and I discovered only many years later that this June of 1942 was the month in which the allies sustained the greatest loss of ships to German submarines.

We set up our hospital in Oxford in new buildings, which now house the Churchill hospital. We operated this hospital until the spring of 1944, when we were chosen as one of the hospitals to function under tents in Normandy a few weeks after the landing. By the time we were set up, the front had moved farther east, and we followed after Thanksgiving of 1944 and set up in the buildings of a French army hospital in eastern France, in Nancy, the capital of Lorraine. The front by then having moved to Germany, some of us who knew some French participated in our spare time in courses the army had organized to teach English to interested citizens of Nancy. This experience led to pleasant associations and to some improvement in my knowledge of French. In October of 1945, our unit was demobilized, and we were sent home. I had served in the army for almost four years.

Although I was intent on resuming my studies I did not plan to start before the spring term in February. However, circumstances decreed otherwise. My sister and brother-in-law were friends of Erwin Chargaff and his wife Vera. He had been for several years Assistant Professor of Biochemistry at the College of Physicians and Surgeons of Columbia University, and I was encouraged to visit him to get advice for the studies I planned. Still in uniform, I appeared in his office within a few days after my discharge from the army. After some pleasant conversation, he introduced me to Professor Hans Thatcher Clarke, the head of the Department of Biochemistry, who subjected me to a brief oral examination in organic chemistry. Apparently I passed, probably because of the really excellent teaching of this subject at City College, and I was told that I was now a graduate student in biochemistry. As such, notwithstanding that the semester had already run for several weeks, I was to take advanced organic chemistry and thermodynamics in the Chemistry Department at the main campus at 116th Street, to take the biochemistry course at 168th Street, which for some reason started in December, and to start on some research problem. I tell this story in part as an example of how much simpler the admissions process was in those days. The only hurdle was the oral exam,

because as department head, Professor Clarke had full power to make decisions and he considered organic chemistry to be the foundation of biochemistry. Fortunately, having served in the army for almost four years, I could count on the GI Bill of Rights for providing the cost of tuition and of a stipend.

COLUMBIA

Since Chargaff had introduced me, everyone took it for granted that I would do the research for my doctoral degree under his supervision. Most readers of this essay will know Chargaff for his important analysis of the nucleotide composition of nucleic acids and for his biting criticism of molecular biology, but at this time, he had not yet begun his study of nucleic acids. He had wide-ranging interests including blood clotting, lipid membranes, and the metabolism of amino acids and sugars. He, alone in the Department of Biochemistry, owned a set of Warburg manometers, the classic tool for investigating fermentation and respiration. This apparatus, located in the large laboratory that housed all graduate students, had just become available, and Chargaff suggested that I study the oxidation of inositol isomers by the bacterium *Acetobacter suboxydans*. I discuss the outcome of these studies in a later section. I enjoyed my work, which profited greatly from frequent discussions with Chargaff. This experience committed me to a career of research in biology and set the style for all my subsequent studies. With luck, the work proceeded rapidly and I obtained my PhD in June of 1948.

In the meantime, Chargaff had begun his analysis of the base composition of nucleic acids. I stayed in his laboratory for another year, studying the chemical and enzymatic hydrolysis of RNA. I had fallen in love with a fellow graduate student, Adele Karp from Montreal, who was a student of DeWitt Stettens, had obtained her degree at the same time as me, and was continuing to work with Stetten at the Public Health Research Institute of the City of New York. Toward the end of this period, I was told by Professor Clarke that he had recommended me to Professor J Howard Mueller, the head of the Department of Bacteriology and Immunology at Harvard Medical School, who wanted to add a biochemist to his department. Although Adele had become so fond of New York that she looked with some misgiving at settling in Boston, making Mueller's acquaintance and visiting the department made us more receptive to the move. Since I had no specific training in bacteriology, Mueller offered me the departmental Ernst Fellowship. Adele accepted the position of Research Associate in the Department of Microbiology of the School of Public Health and began to study the metabolism of *Rickettsiae*.

We were married in Lake Placid in the summer of 1949 and then began our careers at Harvard.

HARVARD MEDICAL SCHOOL

A review I wrote for this publication paid tribute to Mueller's great scientific accomplishments in the field of microbial nutrition (20). When I joined the department, Mueller did his own experiments with the help of one long-time assistant every day between 6 and 10 AM. He then attended to his duties as department head and teacher. He expected everybody else in the department, including his graduate students, to carry out their research independently, but to keep him informed of their progress at monthly intervals. My role was to be available to provide advice on biochemical problems to other members of the department and to develop my own research program. From my old reprints, I can tell that I initially obtained support from funds belonging to Harvard University and perhaps beginning in 1951 from a grant from the NIH. I describe these studies, which initially were an extension of the research I had done for my doctoral dissertation, in a later section.

During my second year at Harvard Medical School, I had the good fortune to be offered a promotion to Associate, the lowest faculty rank, and to be nominated by the Medical School for a Markle Fellowship. The Markle Foundation had at that time decided to further medical education by offering medical schools support for a junior faculty member for a period of five years. Each medical school in the United States and, I think, in Canada had the right to nominate one young scientist each year, and the foundation selected approximately 20 on the basis of the proposal submitted by the medical school, followed by a weekend meeting of potential candidates with a committee of distinguished persons from walks of life not related to medicine or science. In my case, this somewhat stressful meeting of several candidates with the committee took place in a pleasant resort hotel at Hershey, Pennsylvania, and I was soon after notified that I had been chosen.

In addition to becoming a member of the faculty of the Department of Bacteriology & Immunology at the Medical School, I was also appointed a Tutor in Biochemical Sciences at Harvard College. This is an appointment I still hold and my pleasure in individual contact with bright undergraduate students has not diminished with the years. My teaching duties in the department were initially to participate in the laboratory instruction in the Bacteriology and Immunology fall semester course for second-year medical students and soon expanded to offering the introductory lectures on bacterial metabolism and the lectures on enteric and toxin-forming bacteria. I also taught a microbiology course for graduate students in the spring term. Teaching has remained an important and enjoyable activity throughout my career.

The funds I received allowed me to employ a technical assistant. In addition, two postdoctoral associates joined my laboratory, even though I was too young and unknown to attract postdoctoral students. These two scientists had come

to our department for postdoctoral training under the direction of Mueller, but as I mentioned earlier, he preferred to carry out his research with his own hands and therefore urged them to work with me. My research greatly profited from Mueller's trust in me. Then, two beginning graduate students decided to carry out the research for their doctoral dissertation under my direction. These associates enabled my research to expand in new directions. All the work of my laboratory since then has been the outgrowth of these studies.

I was promoted to Assistant Professor in 1953, before Muellers untimely death in 1954. His position as department head was filled three years later by Bernard D Davis from New York. Although I had no expectation of a permanent position at Harvard because the Department of Bacteriology & Immunology had traditionally only two tenure positions and both were filled, thanks to the efforts of the new department head I was appointed to a newly created tenure position as Associate Professor in 1958. Together with this promotion I was awarded an honorary AM degree by Harvard. This was the customary procedure for faculty members newly appointed to a tenure position who did not already have a Harvard degree. The President of Harvard, who came to a meeting of the medical school faculty to award me this degree remarked that this custom did not imply that Harvard recognized only its own degrees, but rather "the wish that you had already been one of us."

An unhappy event occurred soon after our arrival in Boston. As I mentioned earlier, Adele was working with *Rickettsiae,* the agent of typhus fever. Although she had been immunized against this disease, her immunity was apparently not yet sufficiently strong to protect her when the negligence of one of her associates created an aerosol of infected material. She became severely ill and probably survived only through the kindness of the late Dr. Max Finland, who provided aureomycin, then available only for research, for her treatment. After continuing to work for several years at the School of Public Health, she obtained a position at Children's Cancer Research Institute that enabled her to carry out independent research.

We lived in a very small rented house in Newton Center, a suburb of Boston. Most of our friends were biologists from Harvard and MIT, but Adele's interest in visual art brought us in contact with young painters and gallery owners. During this period, we started to buy original contemporary paintings and sculptures.

My work on the regulation of enzyme synthesis was strongly influenced by the studies of Jacques Monod on the *lac* system. I met Monod when he visited Boston, and I enjoyed his company and greatly admired his sharp, analytical mind. Therefore, the decision to spend a sabbatical period in his laboratory was easy. I obtained a Guggenheim Fellowship and Adele a period of leave from her position, and for the first time we were able to work together, at the Pasteur Institute from January to September of 1959. Although no publications

resulted from this work, I profited from being introduced to bacterial genetics. The most important event for our future also occurred at that time: I was offered the position of Professor of Microbiology in the Department of Biology at MIT, which I accepted.

MIT

It may be hard to understand why I chose to leave the prestigious Department of Bacteriology & Immunology of Harvard, where I had been comfortable and successful and had been awarded tenure, for the Department of Biology at MIT. There were several reasons, but the most important was the opportunity to develop with Salvador Luria a microbiology program in the Department of Biology. Luria, without question one of the greatest microbiologists of our time, had come on a sabbatical visit to MIT several years earlier and had then joined the department. The MIT administration, prodded by members of the physics department, had decided to make the Department of Biology a center for research and teaching in molecular biology. I was strongly attracted both by the prospect of working in close vicinity to an outstanding microbial geneticist such as Luria and by the plan to modernize together with him and other faculty members the undergraduate and graduate programs of the department.

In addition, at MIT Adele could work with me as she had done in Paris, a collaboration forbidden by the rules at Harvard. However, this collaboration soon came to an end. Adele's mother became gravely ill with cancer and Adele devoted several years of her life to taking care of her mother in Montreal, and then at our small house in Newton Centre. She decided not to return to work after her mother's death early in 1964. We had bought a house with a large neglected garden, and she found satisfaction in restoring the garden and in the visual art we had both begun to collect. We began to appreciate and collect Far Eastern, Precolumbian, as well as African and Oceanic tribal art. The pursuit of art objects has remained one of my greatest pleasures. Another pleasure was travel, generally in connection with my scientific work. We often escaped the harsh Boston winter by going to the Mediterranean or to Mexico. In both areas we particularly enjoyed visits to archaeological sites. We did not have children and consequently fell somewhat out of step with our contemporaries. Most of our friends were younger people connected with the visual arts.

Below, I describe the progress of the research in my laboratory on the regulation of gene expression, shifting from biochemistry through genetics to molecular biology, but always with a strong focus on microbial physiology. Initially, I taught a graduate course in microbial physiology, which evolved into an undergraduate microbial physiology course taught jointly with Luria

and later with Graham Walker. For several years, when the large number of students made it necessary to teach the undergraduate biochemistry course in both the fall and spring semesters, I taught the half of the spring semester course devoted to intermediary metabolism. I also taught for many years half of a graduate course dealing with the regulation of gene expression in microorganisms.

After serving as chairman of the departmental graduate committee for a few years, I was asked to become Head of the Department of Biology in 1967 and served in that capacity for 10 years. I was able to continue my research and teaching activities much as before thanks to Gene Brown, an excellent biochemist and extremely capable administrator, who joined me as Associate Head of the Department. Gene followed me as department head and then became Dean of the School of Science.

The period of my service as department head overlapped the period of student unrest caused by the war in Vietnam. At MIT, due to the good sense of the administration, the student leaders, and a large fraction of the faculty, there were no violent confrontations. The disenchantment of many students with high technology, because of its military use, and the rapid advances in molecular biology greatly increased the number of undergraduate majors in biology. This expansion of our teaching obligations, as well as the increase in support for research through grants from the NIH, allowed the MIT administration, which was already well disposed to biology, to underwrite a substantial increase in the size of the Department of Biology. It was and has remained our policy to recruit young scientists with a commitment to research and to the teaching of undergraduate and graduate students. These young assistant professors were carefully chosen for their ability to carry out research of the quality that would allow them to continue their career as tenured members of our department. I am very happy to say that this policy was successful. Many of the scientists who joined us during this period are now senior members of our department, widely recognized for their scientific contributions. Two of them, David Baltimore and Phillip Sharp, were honored with the Nobel Prize in medicine for the research they had carried out as junior members of this department.

The expansion of the faculty was greatly facilitated by the creation at MIT of a Center for Cancer Research supported by the NIH. Salvador Luria conceived the idea that MIT, although it did not have a medical school, would be an appropriate setting for such a center. The lack of a commitment to the traditional fields of zoology and botany had allowed us to create a department of biology unified by a common interest in molecular and cellular biology that offered instruction and facilities for research in biochemistry, genetics, microbiology, immunology, and later in developmental biology and neurobiology.

Luria, who became the first director of the Center for Cancer Research, believed that the members of the center should pursue their research on various aspects of the cancer problem as faculty members of the Department of Biology, participating fully in the teaching activities of the department. This arrangement has been completely successful.

Since 1977, when my service as department head ended, I have not carried a major administrative responsibility. I enjoyed being involved in the art acquisition and exhibition program at MIT as a member, and for several years as chairman, of the Committee for the Visual Arts, and I helped as a representative of the Department of Biology work out the rules governing the association between MIT and the newly founded Whitehead Institute. This association was modeled on the relation between the Center for Cancer Research and the Department of Biology, except that in contrast to the Center, which is an integral part of MIT, the Whitehead Institute is an independent institution. Nevertheless, the members of the Whitehead Institute are regular members of the faculty of the Department of Biology and play an important role in the teaching of both undergraduate and graduate students.

I became Professor Emeritus in 1990, having reached the age of seventy in the preceding December. A year earlier Adele had been diagnosed with lung cancer, and she died from this disease in the spring of 1991. I will not attempt to describe what this loss meant to me. Fortunately, I was not left without an occupation at this trying time. The MIT administration permitted me to continue my research and teaching activities as Senior Lecturer. Actually, I am currently looking forward, with some apprehension, to teaching a new course. Until now, all undergraduate MIT students were required to take one year of physics, one year of mathematics, and one half year of chemistry. To these science requirements, usually taken in the freshman year, one half year of biology has been added by decision of the MIT faculty. The Department of Biology, faced with this greatly increased teaching load, has decided to offer three courses that will fulfill the requirement, one in the fall and two in the spring semester, each with an estimated enrollment of 300 students. Each of these courses will present the fundamental aspects of biochemistry, genetics, and molecular biology, but then they diverge. One of the spring semester versions I plan to teach in collaboration with Professor Penny Chisholm, a microbial ecologist, will have as its special theme "microorganisms as geochemical agents responsible for the evolution and renewal of the biosphere and their role in human health and disease."

I can say, looking back over a period of 34 years, that I have never regretted my move to MIT. I have greatly enjoyed the association with my colleagues, postdoctoral associates, graduate and undergraduate students, as well as with the well informed and forward-looking members of the administration.

RESEARCH

Almost all of the research I describe in the following pages was carried out by a succession of graduate students and postdoctoral associates. I am very grateful to them for their efforts and for the intellectual stimulation and companionship they provided. In order to avoid the appearance of preference, I do not name them in my description of the research. The names of some appear in the literature cited section, but many who are coauthors of papers I could not cite because of space limitations made equally important contributions.

1945–1960: Inositol Metabolism, Purine and Histidine Biosynthesis, and Catabolite Repression

In an earlier section I described how I started to work in Chargaff's laboratory in the Department of Biochemistry at the College of Physicians and Surgeons, Columbia University in the late fall of 1945. Chargaff suggested that I should study the oxidation of isomers of inositol by *Acetobacter suboxydans.* Almost 50 years earlier, the French microbiologist Bertrand had derived the rules governing the oxidation of straight-chain polyhydroxy compounds by this organism. He found that they produced monoketones by oxidizing the secondary hydroxyl group next to the primary hydroxyl group, but only when the neighboring secondary hydroxyl group was in the *cis* position. Obviously, Bertrand's rule could not be directly applied to the inositols, hexahydroxy cyclohexanes that do not have a primary hydroxyl group. There are nine isomeric inositols, and it was known at that time only that the most commonly occurring isomer, *myo*-inositol, was oxidized by *A. suboxydans* to a monoketone.

I soon discovered that one other isomer was not oxidized at all, another one was oxidized to a monoketone, and two others were oxidized to diketones, and I identified the hydroxyl groups that had been oxidized. Contemplation of the planar projections of the inositol isomers provided no clue as to why particular hydroxyl groups had been chosen or rejected. The puzzle was solved when I read an article by Becket et al (3) elucidating the structure of dimethylcyclohexanes. These authors pointed out that the conformation of the cyclohexanes was the so-called chair form and that two chair forms were possible that were interconvertible through the so-called boat form. In the chair form, six of the substituents, hydrogens or hydroxyl groups in the case of the inositols, surround the ring of carbon atoms in what is now called an axial belt. The six others are perpendicular to the plane formed by the carbon ring, alternating above (north polar) and below (south polar) the ring. Furthermore, by the conversion of one chair form to the other, all axial substituents become polar and vice versa. Finally, the most strongly favored of the chair forms is the one with the fewest bulky substituents in polar positions. Graphic repre-

sentations of the conformations of the inositol isomers derived from these principles clearly showed that only polar hydroxyls were attacked (27); however, although this condition was necessary, it was not sufficient: additional experiments showed that the carbon in the *meta* position to the north polar hydroxyl group attacked, and the one in the *para* position must carry axial hydroxyl groups (18, 28).

I have described this work in some detail here because experiencing the thrill that comes from sudden insight gave me the will and the confidence to continue in scientific research.

When I was permitted to choose my own research problem at Harvard, I decided to continue the same line of research by studying inositol metabolism in an organism capable of using *myo*-inositol as sole source of energy and carbon. *Escherichia coli,* by that time already the favorite bacterium for biochemical and physiological studies, could not grow on inositol, but the related *Aerobacter* (now *Klebsiella*) *aerogenes* could. I soon found that *myo*-inositol's pathway of degradation differed completely from that of glucose and that the first step of the pathway was the same as in *Acetobacter suboxydans* (16). These studies were only completed many years later, revealing a pathway of six specific steps resulting in the conversion of *myo*-inositol to a mixture of CO_2, dihydroxyacetone phosphate, and acetyl-CoA (1). What had, however, aroused my interest at the beginning of this work was the fact that the organism acquired the ability to attack *myo*-inositol only after contacting this compound. Apparently the enzymes of inositol metabolism were inducible, and I was drawn to investigate this aspect of the problem.

An important new technique had been introduced a few years earlier by BD Davis and J Lederberg that allowed one to isolate bacterial mutants that required an amino acid or other building block for growth (auxotrophic mutants). We attempted to use this technique to examine whether or not the acquisition of the capacity to metabolize inositol involved protein synthesis. We therefore isolated several auxotrophic mutants of our strain of *K. aerogenes* and found that the lack of a required amino acid greatly impeded this acquisition, indicating that protein synthesis was involved (57). Monod and his collaborators subsequently proved this in their studies of the β-galactosidase of *E. coli.* We did not continue this work, wisely in retrospect, because we had not yet identified the enzymes responsible for the degradation of *myo*-inositol, which, as it turned out, are difficult to assay. Moreover, I was greatly intrigued by the novel and unusual properties of three of our auxotrophic mutants.

One of the mutants was a uracil auxotroph and found to accumulate the presumptive pyrimidine precursor orotic acid in the growth medium in an amount greatly exceeding that required for growth. Our experiments with intact cells showed that the addition of uracil prevented orotic acid formation by

enzymes present in the cells (5); however, since the enzymes responsible for the synthesis of orotic acid had not yet been identified we did not pursue this problem further. Arthur Pardee two years later identified the enzyme and the pyrimidine nucleotide responsible for the inhibition, and he is rightly honored, together with H Edwin Umbarger, as the discoverer of feedback inhibition.

Another mutant we isolated had a specific requirement for guanine and excreted xanthosine into the culture medium (26). This observation suggested that the hitherto unknown pathway of GMP synthesis involved the dehydrogenation of IMP to XMP, followed by the amination of XMP to GMP. We subsequently demonstrated these steps and showed that the mutant lacked the ability to convert XMP to GMP. Surprisingly, this mutant had not lost the ability to derive its adenine from the guanine provided in the medium.

We also became intrigued by the properties of another mutant, whose requirement for either adenine or guanine exceeded that of other mutants but which could be reduced by the addition of histidine. Exploring the suggested relationship of purine and histidine biosynthesis, we found that carbon-2 and nitrogen-1 of the imidazole ring of histidine were derived from carbon-2 and an attached nitrogen of guanine (19, 30). Although we initially thought that this transfer played a part in the conversion of GMP to AMP, we soon discovered that the actual donor of this C-N unit was ATP. We were able to show that the first specific step in histidine biosynthesis was the attachment of ribose-5-phosphate to nitrogen-1 of ATP or AMP, followed by a reaction with glutamine resulting in the formation of the histidine precursor imidazole glycerol phosphate and of 5-amino-1-ribosyl-4-imidazole-5′ phosphate, the immediate precursor of IMP in purine nucleotide synthesis (40, 41). Thus, a purine nucleotide cycle was shown to play an essential catalytic role in histidine biosynthesis (29).

We could also show that in the intact cell, feedback inhibition by histidine of the first step of the histidine biosynthesis is not only essential for proper regulation of histidine formation, but also for maintenance of the proper intracellular concentration of adenine nucleotides (52). Finally, we could show that a newly discovered enzyme, GMP reductase, catalyzed the reduction of GMP to IMP, an essential step in the conversion of exogenous guanine to AMP (33). Thus, we had proven the existence of a second cycle of irreversible reactions: IMP→XMP→GMP→IMP, which, however, never actually functions as a cycle since the first step is inhibited by GMP and the last step by ATP (29).

Another line of research I have followed until now had its origin in an unexpected property of another mutant of *K. aerogenes* that required histidine for growth. With glucose as the major source of energy, histidine was needed in an amount similar to that required by histidine auxotrophs of *E. coli*, but with inositol as the source of energy, approximately 25 times as much histidine was needed (57). An explanation for this unexpected result was the ability of

the organism to degrade histidine by a set of inducible enzymes whose synthesis was prevented by glucose, but not by inositol (17). This phenomenon had been previously observed in the case of other inducible enzymes and was called the glucose effect. An example of this effect was the phenomenon of diauxic growth observed by Monod in cells of *E. coli* cultured on mixtures of glucose and lactose (39). In the next few years, we found that *K. aerogenes* could use histidine as the sole source of carbon, energy, and nitrogen, and that this amino acid was degraded in four steps to a mixture of ammonia, glutamate, and formamide (25). The fact that glucose prevented the formation of these enzymes made it unlikely that the cells should be able to utilize histidine as sole source of nitrogen when glucose was the major source of carbon. Nevertheless, we found that the cells grew well in such a medium and contained the enzymes required for this histidine degradation pathway; however, addition of ammonia resulted in an immediate arrest of the synthesis of these enzymes (42).

The results of this study in conjunction with studies on the effect of glucose on the synthesis of inositol degrading enzymes led to the hypothesis that the effect of glucose on enzyme synthesis was due to the rapid metabolism of glucose resulting in the formation of catabolic products at a rate exceeding that at which they could be utilized by the biosynthetic reactions. The same catabolites could also be formed by the degradation of the substrates of the inducible enzymes subject to the glucose effect, but at a slower rate. The glucose effect, according to this view, was explained by feedback repression exerted by these catabolites on enzymes whose products were not required by the cells since they were already being provided by the rapid degradation of glucose. The fact that, in the case of histidine-degrading enzymes, omission of ammonia from the medium appeared to overcome the effect of glucose seemed to provide evidence for this hypothesis: since ammonia was produced by the catabolism of histidine, its presence, in addition to that of the other catabolites of glucose, should be required for the repression of the histidine-degrading enzyme. This reasoning led me to introduce the name catabolite repression for this phenomenon (21). Subsequent studies from many laboratories have generally confirmed the catabolite-repression hypothesis, although the detailed mechanism is still not understood. However, as I shall discuss in a later section, an entirely different mechanism accounts for the ability of *K. aerogenes* to produce the enzymes for histidine degradation in a glucose-containing medium only in the absence of ammonia.

The brilliant studies of Monod & Jacob on the *lac* system had clearly shown that success in the investigation of problems of enzyme synthesis required genetic methods. The lack of any system for the study of genetic recombination made *K. aerogenes* a poor choice for such an investigation. Fortunately, *Salmonella typhimurium* could use histidine as source of nitrogen. In the course

of my stay at the Pasteur Institute in 1959 I received appropriate strains, transducing phages, and instruction from Francois Jacob. Although the histidine-degrading enzymes of *S. typhimurium* corresponded to those of *K. aerogenes* and were subject to induction by histidine, unhappily, these organisms failed to grow on glucose with histidine as the sole source of nitrogen, even though they could utilize histidine as a source of nitrogen when succinate or glycerol was the source of carbon. Consequently, we could study the genetic basis of the induction, but not of the apparent escape from catabolite repression.

1960–1971: Physiology and Genetics of the hut (Histidine Utilization) and lac Systems

At MIT we continued to study the system of enzymes responsible for the degradation of histidine in *K. aerogenes* and extended our studies to the corresponding systems of *S. typhimurium* and *Bacillus subtilis.* Using mutants defective in the ability to use histidine as source of nitrogen or carbon, we found that the actual inducer in the enteric organisms was urocanate, the product of histidase, the first enzyme in the pathway (51). In mutants lacking histidase, the remaining three enzymes of the pathway could be induced by urocanate or its nonmetabolizable analogue imidazole propionate, but not by histidine. On the other hand, mutants lacking urocanase produced the other enzymes of the pathway in the absence of an exogenous inducer, and this apparent constitutivity was shown to result from induction by the endogenously generated histidine. Apparently, in cells growing in the absence of exogenously supplied histidine, urocanase at its low uninduced level prevents this internal induction. This observation suggested that the use of the product, rather than the substrate, of the first step in a degradation pathway for an endogenously produced metabolite is advantageous to the cell. However, such a mechanism does not appear to be required in all cases; in *B. subtilis,* we found that histidine rather than urocanate was the inducer (6).

Searching for nonmetabolizable inducers of the *hut* system in *K. aerogenes,* we discovered that the histidine analogue α-methyl histidine could induce histidase (50). This induction resulted from the ability of this analogue to bring about an increase in the levels of the enzymes responsible for histidine biosynthesis, the products of the genes of the *his* operon, which caused excretion of histidinc. We showed that this effect of α-methyl histidine resulted from its interference with the transfer of histidine to its specific tRNA. These results clearly absolved histidine from responsibility for the repression of the enzymes catalyzing its biosynthesis and suggested that the expression of the *his* operon was regulated in response to the level of charged *his*-tRNA. This role of charged tRNA was explained many years later by the discovery of the control of gene expression by attenuation.

Using genetic methods to elucidate the *hut* systems of *B. subtilis* and *S.*

typhimurium, we found the former to comprise a single operon with the genes arranged in the order of the biochemical reactions (14), and the latter to be composed of two separate but closely linked operons with the order *hutIG,* specifying the third and fourth step of the pathway and *hutUH,* coding, respectively, for the second and first steps (54). In the case of *B. subtilis,* mutations resulting in the inability to express the operon, insensitivity to catabolite repression, and constitutivity were all clustered at one end of the operon. In the case of *S. typhimurium,* we discovered that the gene for the repressor, *hutC,* located between the two operons was actually a member of the *hutIG* operon; the product of the *hutC* gene was not only responsible for exerting repression on the operator of the *hutUH* operon, but also on the operator of the *hutIGC* operon (55). This was the first example of autogenous regulation of gene expression. The low level of the repression in uninduced cells makes the system very sensitive to induction by urocanate; and the high level of repressor in induced cells results in a rapid shut-off of the expression of the *hut* genes when urocanate has been exhausted.

An investigation of the kinetics of induction of the histidase of *B. subtilis* and of β-galactosidase and the tryptophanase of *E. coli* revealed that the transcription of these genes is initiated immediately after addition of the inducer (10, 13). We showed that the lag of approximately 2 min that precedes the acquisition of the capacity to produce these enzymes reflects the time required for completion of the synthesis of the corresponding messenger RNA, and that translation of this RNA occurs during this time. In addition, these experiments provided evidence that glucose prevented the initiation of transcription and not later steps.

Our studies on the effects of glucose allowed us to differentiate the severe transient repression of β-galactosidase that occurs immediately after contact of the cells with glucose from the less severe repression seen after a short period of growth in the presence of glucose (56). We found that many compounds other than glucose exert transient repression; the cell must possess a permease permitting the rapid entry of the compound, but the compound need not be metabolized. On the other hand, the weak permanent repression depends on metabolism of glucose, or under condition of a reduction of the rate of biosynthetic reactions, on the metabolism of any source of energy. We identified a site closely linked to the promoter but distinct from the operator as the target of both transient and permanent catabolite repression (53). After the discovery of the ability of cyclic AMP to overcome the effects of glucose, this site was identified as the site to which the CAP protein charged with cyclic AMP binds to activate the initiation of transcription.

I did not continue the study of catabolite repression, although much remains to be done to elucidate the molecular mechanism of this important control system (31). One reason for my turning away from this problem may have

been disappointment in that I had failed to realize the importance of cyclic AMP at an early stage. A more important reason, however, was the fact that at about this time a transducing phage for *K. aerogenes* had been discovered that enabled me to return to the study of the intriguing problem of the ability of this organism to produce the enzymes needed for the degradation of histidine in the presence of glucose when ammonia was omitted from the growth medium.

1971–Present: Nitrogen Regulation in Bacteria and Yeast

Some of the studies I have described in the preceding section continued into this period. Our major research efforts, however, were directed toward elucidating the mechanism responsible for the regulation of the expression of the genes for enzymes capable of supplying the cell with glutamine and glutamate, the precursors of all nitrogen-containing cellular constituents, in response to the availability of a source of nitrogen.

We discovered the existence of such a mechanism when we found that mutants of *K. aerogenes* lacking the ability to produce CAP or cyclic AMP had lost the ability to activate the expression of the *hut* genes in response to the absence of glucose, but could activate the expression of these genes in response to the absence of ammonia (47). Similarly, these mutants had lost the ability to use proline as source of carbon, but could use it as source of nitrogen with glucose as the carbon source. Further studies revealed a close relationship between the regulation of the synthesis of glutamine synthetase and of histidase in response to the availability of ammonia (46). In these experiments, we used a constitutive (*hutC*) mutant and found that lack of ammonia was a sufficient condition for an increase in the intracellular concentration of both histidase and glutamine synthetase. However, while mutations in *hut* had no effect on the expression of glutamine synthetase, all mutations affecting glutamine synthetase altered the regulation of histidase synthesis.

We eventually showed that glutamine-requiring mutants either lacked the ability to increase the level of histidase in response to ammonia deprivation or produced histidase at a high level even in the presence of ammonia. Some of these mutations turned out to be in *glnA,* the structural gene for glutamine synthetase. A mutation in a site closely linked to these mutations resulted in high levels of both enzymes in cells grown with ammonia. In addition, mutations unlinked to *glnA* could result in failure to increase the levels of both enzymes in response to ammonia deprivation. One was in a gene we called *glnB,* which was subsequently found to code for P_{II}, a component of the adenylylation-deadenylylation system that regulates the activity of glutamine synthetase (9). The *glnB* mutation resulted in an altered P_{II} capable of facilitating the inactivating adenylylation, but not the deadenylylation of glutamine

synthetase. Consequently, the enzyme was present in these mutant cells in low concentration in its fully adenylylated inactive form.

Because of these observations, as well as with some prejudice stemming from the discovery of autogenous regulation in the *hut* system, I proposed that glutamine synthetase in its nonadenylylated active form served as activator of the expression of *glnA, hut,* and other nitrogen-regulated genes (32). During this period, a young investigator, Sydney Kustu, began to study the regulation of *glnA* expression in *S. typhimurium.* I must have resented this competition by a young upstart who on occasion scooped us and on other occasions raised doubts concerning the validity of our interpretations. I suspect that Sydney had similarly unkind feelings about me. However, the competition sharpened the analysis of our results and speeded up the progress of our investigations. Over the years, Sydney and I have become good friends, though we have continued to compete. In general, only competitors have the particular interest and knowledge for a meaningful discussion of their common area of research.

Kustu et al (15) and Pahel & Tyler (45) finally showed that the true activator of *glnA* expression was the product of *glnG* (called *ntrC* by Kustu), a gene closely linked to *glnA*. I do not regret having proposed an incorrect hypothesis, because by having a definite concept in mind, one can plan the experiments that eventually lead to the correct interpretation. I analyzed the experiments that suggested the incorrect and eventually the correct interpretation in a section of a 1982 review subtitled "Glutamine synthetase as regulator of gene expression: the rise and fall of a hypothesis" (22). A similar analysis has recently appeared, without reference to my earlier review (34).

We could not have achieved our current understanding of this mechanism without cloning the segment of DNA carrying *glnA* and the closely linked *glnL* and *glnG* genes whose products are the regulators of the genes subject to nitrogen regulation (2). As a small remnant of our old antagonism, Sydney refers to these genes as *ntrB* and *ntrC* and to their products as NtrB and NtrC, while I call these products nitrogen regulator II (NR_{II}) and NR_I, respectively; she and I studiously avoid any discussion of our conflicting nomenclature. We found that the three genes are organized as a complex operon (44), with two promoters, *glnAp1* and *glnAp2* preceding the *glnA* gene and a third promoter, *glnLp,* located between *glnA* and *glnL* (48). In cells grown with an excess of nitrogen, transcription is initiated by the common σ^{70} RNA polymerase at *glnAp1* and *glnLp*, and is repressed by NR_I, which binds to sites overlapping *glnAp1* and *glnLp*. The autogenous repression of NR_I at *glnLp* results in the maintenance of this regulator at a level of about five molecules per cell. This concentration allows sufficient transcription of *glnA* to be initiated at *glnAp1* to produce an amount of glutamine synthetase adequate for providing the cell with glutamine for incorporation into protein and to synthesize purine nucleotides and some of the amino acids specifically requiring glutamine. When the

cells are subjected to ammonia limitation, glutamine synthetase assumes the responsibility for the synthesis of glutamate. Under these conditions, NR_I activates the initiation of transcription at *glnAp2,* which results in a great increase in the levels of both glutamine synthetase and NR_I.

Both Kustu and her collaborators and we were able to show that an RNA polymerase associated with the newly discovered σ^{54}, the product of the *rpoN* gene was responsible for the initiation of transcription at *glnAp2* (11, 12). This transcription requires NR_I and NR_{II} as activators, and the role of NR_{II} is to bring about the phosphorylation of NR_I (43). In the presence of ammonia, NR_{II} combines with P_{II}, the product of the *glnB* gene, and halts further activation of transcription initiation at *glnAp2* by bringing about the dephosphorylation of NR_I-phosphate. As shown previously by Stadtman and his coworkers in their studies on the regulation of glutamine synthetase activity, ammonia deprivation causes UTase, the product of *glnD,* to uridylylate P_{II} (9). The resulting P_{II}-UMP promotes the deadenylylation and consequent activation of glutamine synthetase, but does not affect transcription of *glnA;* an excess of ammonia causes UTase to convert P_{II}-UMP to P_{II}, which in turn stimulates the adenylylation of glutamine synthetase and, by acting on NR_I-phosphate in concert with NR_{II}, halts the initiation of transcription a *glnAp2.*

NR_I and NR_{II} have been identified on the basis of the sequence of their structural genes as members of the large class of so-called two-component regulatory systems. Subsequent to our discovery of the phosphorylation of NR_I by NR_{II}, in every case studied so far the homologue of NR_{II} activates the corresponding homologue of NR_I by phosphorylation. We showed that the initial step is the transfer of the γ-phosphate of ATP to a histidine residue to NR_{II}, and that this phosphate is then transferred to an aspartate residue of NR_I (59).

NR_I-phosphate exerts its effect by binding to two sites overlapping the *glnAp1* promoter located more than 80 bp upstream from the promoter. Moreover, these binding sites can be moved more than 1000 bp in either direction without interfering with the ability of the NR_I-phosphate bound to them to activate the initiation of transcription at *glnAp2* (49). In other words, these binding sites correspond to the enhancers previously discovered in eukaryotic cells. The flexibility of the DNA allows the interaction of the activator bound to its sites with the σ^{54} RNA polymerase bound to the promoter in a closed complex, which results in the isomerization of the closed to open σ^{54}-RNA polymerase promoter complex.

The fact that in both eukaryotic and prokaryotic cells DNA flexibility allows regulation of gene expression from distant sites raises the interesting question of why this is the common mechanism in eukaryotic cells, but is restricted in prokaryotic cells to the rare genes with σ^{54}-dependent promoters, while the common σ^{70}-dependent promoters are all regulated from adjacent

sites. I have suggested that regulation from distant sites requires separation of the promoters by long stretches of DNA to prevent inappropriate activation and that prokaryotic cells have evolved to favor regulation from closely linked sites, eliminating excess DNA in order to shorten the time and energy cost of replication (23).

A recent study has revealed why phosphorylation is required to enable NR_I to activate the initiation of transcription (58). We found that phosphorylation favors a cooperative interaction of the NR_I dimers to form the tetramer responsible for this effect. This hypothesis is supported by the observation that all promoters activated by NR_I-phosphate have two binding sites upstream of the promoter located on the same face of the DNA helix. The presence of these sites greatly facilitates the cooperative interaction of the NR_I-phosphate molecules. The binding sites differ in their affinity for NR_I but generally have less affinity than those at *glnAp2*. This fact explains why NR_I, in the low concentration present in cells grown with an excess of ammonia, can upon phosphorylation fully activate transcription at *glnAp2* but not at the other promoters. For this reason, the first effect of ammonia deprivation is an increase in the intracellular concentration of glutamine synthetase, which enables the cell to utilize the traces of ammonia for the synthesis of glutamine. The other nitrogen-regulated genes have products whose activities are responsible for the better uptake of ammonia and of glutamine and for the generation of ammonia from other nitrogen compounds; their expression is activated subsequently owing to the increase in the level of NR_I, which results from the transcription initiated at *glnAp2,* rather than at *glnLp*.

The problem of why *S. typhimurium* fails to activate the expression of *hut* and similar genes in response to ammonia deprivation was finally solved by a former student of mine who had isolated a mutant of *K. aerogenes* that had normal regulation of the *glnALG* operon but that could not activate the expression of *hut* and other nitrogen-regulated genes in response to ammonia deprivation. The affected gene, *nac,* has a σ^{54}-dependent promoter (4). The activator for the initiation of transcription at this promoter is NR_I-phosphate, and the product of the *nac* gene in turn can activate the σ^{70}-dependent promoters of *hut* and other operons. *S. typhimurium* lacks the *nac* gene but can acquire the ability to use histidine as source of nitrogen in the presence of glucose upon transformation with a plasmid carrying the *nac* gene of *K. aerogenes.* This discovery finally fully explains, almost 40 years after it was discovered, the ability of *K. aerogenes* to use histidine as source of nitrogen in the presence of glucose.

Another problem explored in our laboratory during this period was nitrogen regulation in a eukaryotic microorganism, *Saccharomyces cerevisiae* (24). We knew the inducible enzymes enabling this organism to use arginine and proline as sources of nitrogen were not formed when the cells were grown in an

ammonia-containing medium. We found that ammonia and also glutamine, another excellent nitrogen source for this organism, inactivated the appropriate permeases and in this manner prevented the induction of the degradative enzymes (8). On the other hand, we were able to show that glutamine was a repressor of glutamine synthetase, of the NAD-linked glutamate dehydrogenase and of the general amino acid permease.

The expression of the structural genes for these proteins, *GLN1, GDH2,* and *GAP1,* respectively, was activated by the product of the *GLN3* gene (38), and the product of another regulatory gene, *URE2,* was responsible for disabling the *GLN3* product in response to the presence of glutamine (7). The *GLN3* product binds to sites upstream of these genes that have reiterated sequences of 5′-GATAAG-3′ or 5′-GATTAG-3′, a motif also found as an enhancer in higher eukaryotes (35–37). We recently discovered the existence of another activator, the product of the *NIL1* gene, which is capable of activating the expression of *GAP1,* perhaps in response to a deficiency of glutamate, rather than of glutamine. The products of *GLN3* and of *NIL1* are similar zinc finger proteins and appear to recognize similar, or perhaps the same sites.

Obviously, our understanding of nitrogen regulation in *Saccharomyces cerevisiae* is far from complete. Much work remains to be done.

CONCLUSION

As the title of this biographical essay indicates, I have been very lucky. I left Vienna in time to escape the Holocaust and came to New York where I could attend a first-rate college charging no tuition. I did my duty in the US army without being exposed to much danger. I was promptly admitted to graduate school at Columbia University and at appropriate times was offered faculty positions at Harvard and at MIT. I carried out my research during a period of good government support and was permitted, and even paid, to do exactly what I wanted to do. I was married to the same woman for 41 years, and we always shared our interests. I enjoyed the association with my predoctoral and postdoctoral students; many of them have remained my friends and, as far as I know, only one hates me. As far as honors are concerned, I was elected to the National Academy of Sciences in 1969, was given its Waksman award for excellence in microbiology in 1993, and finally, was invited to write this autobiographical essay.

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