TO BE THERE WHEN THE PICTURE IS PAINTED

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Roots

I have lived my whole professional life in Sweden, most of it at the Karolinska Institute, the medical school in Stockholm. However, my roots go back to another European country.

In 1925, I was born in Wiener Neustadt, a small town in Austria, about 40 km South of Vienna. In school, we learned about our town’s heroic past as a bastion against invasions from the east by Magyars and Turks. By the time I arrived, that was very past history, and people made their living from small industry and the agriculture of the surrounding area, the Steinfeld (stone field). Most people were poor; during the depression of the 1930s they were very poor. Many of my classmates’ fathers were out of work year after year. Their
hopeless situation makes it easy to understand their enthusiasm for the Nazi takeover in 1938; within a few weeks, everybody had a job.

My parents had a very different background. Both were born during the last years of the Habsburg Monarchy, but in different parts of the country. My mother’s family was in Wiener Neustadt. The maiden name of her mother was Haiden (heathen). It was said to indicate that her ancestry went back to some of the infidels that stayed behind when the Turks were driven back from Austria in 1683. Be that as it may, the town and surrounding settlements were full of mother’s relatives. Grandmother had married a manual worker, a simple man without major intellectual or political aspirations. She organized the women working in an ammunition factory during World War I, and later, after the war, became a member of the city council and then a representative for the Social Democrats in the Austrian parliament—one of its first female members. She must have been a remarkable woman since she accomplished all of this with only six years of grammar school. She died when I was one year old, and I am sorry that I never knew her.

My father was the youngest of 14 children of a Jewish family living in the ghetto of Kremsier, a small town situated in today’s Czech Republic. His father was the cantor of the congregation and made part of his living by teaching the violin. Both grandparents started life somewhere in Hungary. According to family legends, my grandfather spent his early years wandering with gypsies and, while with them, learned to play the violin. Later, both grandparents came to the Austrian town of Graz, where grandfather sang in the Opera. Grandmother was a strong-willed woman who did not like the attention grandfather gave to the female singers, so the family moved to Czechoslovakia.

My parents met when my father started his career as a lawyer after World War I. Like so many bright young Jews who grew up in the outskirts of the monarchy, he had gone to Vienna to study law. He became an ardent organized socialist and was on friendly terms with many of the future leaders of the Social Democratic Party. I do not know what attracted him to the small provincial Austrian town, but once there, he could hardly avoid meeting my mother, who was secretary to the mayor. Father built a highly successful general practice, which included everything from traffic accidents to murders as well as civil and business law.

Thinking of my early childhood brings back many disparate images. I lived in a large house surrounded by a beautiful garden on the outskirts of the town. In grammar school, our teacher ruled 50 to 60 boys by the stick. Our summer vacations were in Italy, where I swam for the first time in the Adriatic and learned some Italian. This must have been the time when I fell in love with Italy and the sea. Now 60 years later, our summer place on a Swedish island is surrounded by the Baltic, and I work part time with my Italian wife at the University of Padova.
In February 1934, the economic misery in Austria exploded in a short civil war between the Social Democrats and the Catholic government that was easily won by the government. Some of the socialist leaders escaped to Czechoslovakia, but a few were executed. Father was briefly imprisoned in a small concentration camp in the vicinity of our town but was soon released.

In the meantime, I left grammar school and entered the gymnasium of the town. I received a classic education with daily Latin and Greek lessons. Chemistry and physics practically did not exist. If I ever thought of it, I presumed that I would enter law school and eventually join my father in his practice.

With the annexation of Austria by Germany in 1938, life changed completely. School became a nightmare. At home, the police searched the house in the middle of the night; I do not know for what. My parents began to talk of emigration. During the organized pogroms of the Kristallnacht in November, father was taken to the concentration camp of Dachau for one month. Fortunately, the former Secretary General of the Austrian Social Democratic Party, who was a close friend, had emigrated to Sweden and persuaded the Swedish party to provide an immigration visa for my father as a political refugee. Thus my Swedish life began at the age of 14 in the summer of 1939, a few months before the war. This is also one reason why, later in life, I found it difficult not to vote for the Social Democrats in Swedish elections.

Early Years in Sweden

I obtained a fellowship in a private boarding school in a small village near Stockholm. The school had a strong Quaker background and its friendly and quiet atmosphere was a tremendous relief. The school did not give grades, which was unheard of at that time. For me, it was an excellent system that provided time to adapt to new challenges and develop skills at my own pace. From Latin and Greek, I switched to modern languages and science. I was fortunate to be taught physics by a wonderful man who encouraged my growing interest in science. He recognized my limited mathematical ability and wisely directed my interest towards Chemistry. The school had a well-equipped laboratory suitable for easy analytical experiments, and I obtained considerable satisfaction from analyzing the composition of unknown mixtures of inorganic salts prepared by my teacher.

At the end of three years, I passed the examinations required to start a university education and began to think about my future life. My first choice was to become a chemical engineer. To enter the school of engineering in Stockholm, I needed some practical experience. I therefore worked for one year in a factory in the far north of Sweden. I enjoyed neither the long cold winter nor the dull work, which did not live up to my expectations. I decided no more chemistry for me.
Back in Stockholm, I was rather undecided on what to do. I had earlier considered entering medical school, but such a choice involved two problems. Medical school meant seven-and-a-half years of study followed by several years of little or no income. More important, as an alien citizen, I was not allowed to study medicine, even though I spoke Swedish fluently and had passed my examination with excellent grades. But times and rules changed, and my lack of citizenship suddenly no longer mattered. Equally important, I obtained a small monthly stipend that enabled me to contribute to the family budget. In January 1944, I was accepted as a medical student by the Karolinska Institute.

We were a small group of 40 and started with parallel classes of anatomy and general chemistry. Each subject had only one professor and one associate professor. Two senior medical students served as salaried teaching assistants (amanuenss) and organized dissections or laboratory courses, helped by an unspecified number of younger unpaid medical students (extra amanuenses). These unpaid students usually also carried out a small research project while attending medical school. Graduate courses and examinations in relation to the research work did not exist. The combination of research, teaching younger students, and attending medical school meant hard work for the extra amanuenses, and most gave up after a year or two. A few remained and competed for the two salaried teaching assistantships. Successful research could finally, after considerable time, lead to a doctoral thesis.

Pehr Edman gave our first lectures in General Chemistry. This was a few years before he invented the famous Edman method for the sequential degradation of proteins. The lectures in Biochemistry were delivered by the professor, Einar Hammarsten, who clearly did not enjoy lecturing. Our ignorance during that time is difficult to comprehend today. Our German textbook, the Lehnartz, was descriptive, presenting chemical formulas of biological materials. It contained a few pages on nucleic acids and represented them as tetranucleotides, with stoichiometric amounts of the four bases. The general impression that we students received was that catabolic reactions could be described in chemical terms, whereas anabolism depended on cellular structure and therefore was not available for chemical experimentation. I remember the excitement with which I read Dynamic Aspects of Biochemistry by E. Baldwin at the end of the biochemistry course, and somewhat later, Schoenheimer’s The Dynamic State of Body Constituents. Both books gave me a first glimpse of biochemical dynamism that was very different from the static view of Lehnartz.

Much to my delight, the introductory laboratory course involved qualitative analyses of mixtures of inorganic salts that did not cause me any problem. It was at this stage that I began to realize how much I enjoyed shaking test tubes and the outcome of simple experiments. I have done this throughout my life and still maintain contact with bench work as much as possible.
The Beginning of a Scientist

I passed the examination in Medical Chemistry with good grades, thought that maybe chemistry was not so bad after all, and began to consider a career in biological research. I was not particularly attracted to Einar Hammarsten as a teacher and decided to approach Torbjörn Caspersson, one of his former students. On my way, I met Pehr Edman by chance and told him about my intentions. He strongly discouraged me. I had looked at Caspersson as a genius who had discovered the importance of nucleic acids for protein synthesis. However, his largely technical research poorly suited my talents. In my youthful ignorance, I had not recognized this incompatibility, but followed Edman’s advice and never approached Caspersson. A second incident finally brought me to Hammarsten. He invited one of my fellow students, Håkan Arvidsson, who was a distant relative of his, to start as an extra amanuensis in the department. Håkan knew about my interests in chemistry and suggested that we both start. Thus, I was enrolled as an extra amanuensis in Hammarsten’s research.

Hammarsten was professor of Chemistry and Pharmacy, a title that started with Jons Jacob Berzelius in 1810 when the Karolinska Institute was founded. The establishment of a new medical school had met violent opposition from the old medical faculty of the University of Uppsala. A major argument was that, with people like Berzelius as teachers, the students would not obtain the appropriate philosophical and humanistic education but be turned into a bunch of scientists. The opposition remained for a long time, and even though graduates from the Institute were allowed to practice medicine, it was not until 1908 that the Karolinska Institute could bestow upon its students the research degree of “Doctor of Medicine.” No doubt, the professors of the Institute were consoled by the fact that since 1901 they selected the winners of the Nobel prize in Medicine or Physiology.

Many famous Swedish professors—among them Caspersson, Theorell, and Jorpes—started their scientific career in Einar’s laboratory. He was a man who lived for his science and had the ability to instill his enthusiasm for research into his collaborators. His uncle, Olof Hammarsten, had been a well-known scientist who wrote a much-used textbook and discovered pentose in nucleic acid. Later in life, he became president of the University of Uppsala. Einar considered the decision to take this position to be an example of his uncle’s poor judgment.

In his thesis, Einar Hammarsten had demonstrated that DNA from thymus was a high-molecular-weight substance when prepared without the then-usual alkaline treatment. Somewhat later, Erik Jorpes demonstrated that RNA from the pancreas did not contain equimolar amounts of the four bases and thus did not conform to the generally accepted tetranucleotide model. Their work did not receive the attention it deserved, largely due to the authority of P. A. Levene...
at the Rockefeller Institute, who was an advocate of the tetranucleotide concept.

At the time of my arrival, Einar was building a mass spectrometer in order to study nucleic acid metabolism with $^{15}$N-labeled compounds. He was much taken by Schoenheimer's work that used $^{15}$N-amino acids to measure protein turnover and planned to do similar work with nucleic acids. $^{14}$C-technology was still in its infancy; moreover, the American Atomic Energy Commission had prohibited export of this radioactive isotope to Sweden, fearing that it might find its way to the Soviet Union. Thus, isotope work had to be carried out with stable isotopes or with $^{32}$P.

My first assignment was to assist in the construction of the mass spectrometer. The physicist in charge soon realized my limitations. When, after one week, my screwdriver slipped and broke a major glass part of the machine, he discharged me from my duties. My friend Arvidsson had a much better hand with screwdrivers and stayed with the machine for the next two years. Not surprising, he became frustrated, left, and with time started a career in radiology.

I did not realize how lucky I was to be released from the project but thought that this was the end of my scientific career. After a week or two, I went back to the department prepared to resign. To my pleasant surprise, Einar just looked at me, with a twinkling smile in his eyes, and asked if I would prefer to crystallize deoxyribonuclease. I had never heard of the enzyme but was of course delighted. As a starting point for my work, he gave me a reprint. It was a paper by McCarty (1) describing the partial purification of deoxyribonuclease. Avery and he had used this enzyme to inactivate the pneumococcal transforming principle. I had never heard of the transforming principle and only much later did I realize the reason for Einar's interest.

Unfortunately, nobody in the laboratory, including Einar, knew how to purify an enzyme. Theorell was working next door in the building, and he would probably have been pleased to advise me. However, I did not know that he was working with enzymes, and nobody enlightened me on that point. It is difficult today to imagine the circumstances under which we worked at that time. Seminars or group meetings did not exist; everybody had to struggle by himself. The isolation and lack of guidance provided a strong selective pressure for independent students with a considerable degree of optimism. Our equipment was quite good then even though it was pitiful by present standards. I particularly remember the Köhler spectrophotometer that was used to measure the ultraviolet absorption of solutions at the wavelengths provided by a mercury lamp. The machine was placed in a small dark room and was preferentially used between midnight and four am when the streetcars outside the department were not running.

The arrival of several American visitors to the laboratory boosted my bio-
chemical education. First came Richard Abrams. His PhD work had been in enzymology, and from him, I learned the importance of enzyme units, specific activity, and yields during purification. He was knowledgeable in many different fields, participated in the final stages of the construction of the mass spectrometer, and played a major role in getting the machine into workable shape. I am grateful for all he taught me and was happy when, 25 years later, he returned to Sweden on his sabbatical to work with me in the Nobel Institute. A little later, when I no longer worked with deoxyribonuclease, David Rittenberg and David Shemin arrived. Both were former collaborators of Rudolf Schoenheimer, my scientific hero. Shemin stayed in Stockholm for six months and determined the life span of chicken erythrocytes. He strongly influenced my development into a functioning scientist. I became his part-time assistant, took blood samples at odd hours from the wings of the chicken, and prepared heme for the $^{15}\text{N}$ analyses. More importantly, Shemin spent a good deal of time talking to me about how to do $^{15}\text{N}$ experiments, pointing to the lack of appropriate methods for the isolation of pure $^{15}\text{N}$-labeled purines and pyrimidines from small amounts of nucleic acids.

At that time, chromatography in its different forms was introduced into biochemistry. Chargaff used paper chromatography to analyze purines and pyrimidines of nucleic acids. It occurred to me that partition chromatography might provide the solution to our problem. Chromatography on starch columns indeed proved a satisfactory tool, and my first publication in *Nature* described the separation of the four common ribonucleosides (2). Pehr Edman, who had conveniently just returned from a postdoctoral stint at the Rockefeller Institute, gave some advice and, more importantly, contributed to my efforts with his fraction collector. A large, old-fashioned alarm clock drove an eccentric cog-wheel that made a round turntable move one step every hour and with it a series of beakers that were placed on its top. Each run was exciting because the beakers were placed loosely on the rubbermat of the turntable and sometimes moved to the wrong place before they were in their position under the column.

Now my work progressed rapidly. Starting from nucleic acids prepared by Hammarsten’s method (3), I obtained ribonucleotides by alkaline hydrolysis, dephosphorylated them with prostatic phosphatase, and separated the nucleosides on my starch column. DNA was hydrolyzed with formic acid, and the free bases were again separated by using starch chromatography. This methodology was then used to analyze the $^{15}\text{N}$ content of separated nucleosides and bases from experiments in which labeled precursors had been administered to animals. The results of these analyses are today of little interest, except for one case involving $^{15}\text{N}$-orotic acid (4). At the suggestion of Sune Bergström, we tested labeled orotic acid as the precursor of nucleic acid pyrimidines. The results were a resounding success and demonstrated a very efficient incorporation into the pyrimidines of both RNA and DNA.
These events took place within two years, and in the fall of 1949, I was ready to defend my thesis for a doctorate in medicine. A dissertation was a serious affair during which the defendant and two opponents, all dressed up in white tie, discussed the merits of the work. A faculty committee graded both the scientific content and the defense of the thesis, and on the basis of these grades, the faculty then decided if the defendant could become a docent, which was a prerequisite for an academic career. Olov Lindberg, professor in Cell Biology at the University of Stockholm, was one of the opponents appointed by the faculty. His kindness then and all his later encouragement were invaluable. I obtained the highest grade for both content and defense. This grade did not reflect the quality of my work alone. Hammarsten was of the opinion that all dissertations in Chemistry were much better than those in any other subject and therefore automatically deserved the highest grade.

Much had happened in Swedish academia between 1945 and 1949. I was fortunate to start my work at a time when politicians began to understand the importance of fundamental medical research. With government support the number of academic positions increased considerably. The Swedish Medical Research Council started its activity. The move of the Karolinska Institute from its downtown localities to the new campus at Solna had started. Everything pointed to a bright future for a person starting his academic career in biochemistry. Nevertheless, I hesitated. Parallel to my scientific work I had continued my medical education. I was tempted to leave halfway through medical school and do research fulltime. However, I needed only two more years to qualify as a doctor and a well-paid job in academic medicine. In addition, I married in 1949, and my wife Dagmar had postponed her budding career in law because we expected our first child. Fortunately, the Swedish system at that time permitted me to finish medical school and to continue research simultaneously, thus postponing a final decision. I am afraid that my knowledge of medical matters was rather limited when I took my final exams in 1951. Some of the research done during the previous two years had had a decisive influence on my scientific future and had eclipsed my medical studies.

The Beginnings of Ribonucleotide Reduction

The methodology I had worked out in my thesis was designed to analyze labeled pyrimidines obtained by degradation of nucleic acids. It struck me that the same methods could be used to prepare $^{15}$N-nucleosides from biosynthetically labeled nucleic acids. The nucleosides could then be injected into rats as precursors of nucleic acids, similar to our previous experiments with orotic acid.

The striking outcome of experiments with labeled pyrimidine ribonucleosides (5) and deoxyribonucleosides (6) led to some bold conclusions. Ribonucleosides were incorporated into both RNA and DNA, whereas deoxyri-
bonucleosides were used exclusively for DNA synthesis. From these results, we proposed the existence of an enzyme that transforms ribose to deoxyribose when the sugar is attached to a pyrimidine. Ribonucleotide reduction was born. Because labeled deoxynucleosides were exclusively incorporated into DNA, we hypothesized that radioactive thymidine might prove a useful tool for the visualization of DNA by radioautography. I remember with sorrow that I could not persuade any of my Swedish friends working with this technique that such experiments could be of interest.

During this time I also made the first transition from in vivo to in vitro experiments. Using slices from regenerating rat liver, I demonstrated that orotic acid was a normal intermediate in pyrimidine synthesis. Looking at these experiments today, I am struck by the scale: slices of 5 regenerating livers were incubated in a volume of 50 ml for up to 8 h. Today, we work with 50 µl samples and incubate for 20 min. Nevertheless, with this clumsy system, Ulf Lagerkvist and I later demonstrated that aspartic acid is a precursor of pyrimidines (7). Ulf had joined the laboratory a few years after myself and in his thesis devised a method for the chemical degradation of the pyrimidine ring (8). We now synthesized aspartate labeled in different positions with $^{13}$C, $^{14}$C, and $^{15}$N and demonstrated incorporation of the whole molecule into orotic acid. By a similar technique, we also showed that carbamyl aspartate was an intermediate. Our findings were never properly recognized. We published them in *Acta Chemica Scandinavica*, aware that this was not a major biochemical journal. I suppose that we acted in the mistaken belief that our publications would transform it into one.

**First Visit to the United States**

After finishing medical school, I was ready for a postdoctoral period in the United States. I could count on receiving a fellowship from the Rockefeller Foundation. Support from the foundation was of enormous importance for biological research in Sweden, and Hammarsten's, as well as Caspersson's and Theorell's research at the Karolinska Institute, would not have been possible without it. I had decided to work one year at Stanford with Hubert Loring, whose work was related to my interests. One of Loring's former students, Elizabeth Anderson, was a postdoctoral student in Sweden and described the beauty of Stanford to Dagmar and myself in glowing terms. So Stanford it had to be. Gerald Pomerat, the emissary from the Rockefeller Foundation, did not think that a visit to Loring's laboratory was a good idea and tried to persuade me to go somewhere else. I was stubborn already then and he gave in. His only condition was that we would stay at Stanford for 11 months only and would visit various American laboratories of my choice during the last month. This was not hard to accept, since travel expenses were to be paid by the Foundation.
In November 1951, Dagmar, our one-year-old son Per, and myself left Oslo by boat for New York on our first trip to the United States. My first encounter with American biochemistry made a profound impression. As Pomerat had predicted, my work in Loring's laboratory was not exciting. However, my visits to other laboratories during the final month introduced me into modern biochemical research. The period at Stanford was also the beginning of a long love story with that place. As a result, I returned many times for shorter or longer periods and worked there a total of almost four years, mostly in Arthur Kornberg's laboratory.

Traveling up and down the American continent in a ten-year-old car from Palo Alto to New York, with a two-year-old child in the backseat, was quite exciting. We must have visited almost ten towns that housed scientists active in the nucleotide and nucleic acid field and saw the best sides of my fellow scientists. I knew them through their work but had not met a single one of them. Everybody received us with open arms, me as a fellow scientist, Dagmar and Per as dear visitors to be taken care of by the family.

The discovery of my own scientific ignorance came as a shock to me and probably also to my hosts. For the first time in my life I gave seminars. The scientific content was reasonable, but I shudder when I think of the quality of my presentation. Two places stand out in my memory, Cleveland and Bethesda. In Cleveland, Harland Wood had brought together a wonderful group of scientists. We were house guests of Bob Greenberg who had begun to unravel the enzymology of purine synthesis. Before then, I did not really see a possible connection between the isotope studies that I had carried out and the enzyme work concerning nucleosides and nucleotides done by others. In Bethesda, I met Arthur Kornberg for the first time; he was to play an important role later in my life.

Our trip stopped short in New York. Our car had behaved erratically on several occasions but on the whole had served us faithfully. Now it suddenly became impossible to shift into reverse gear, which caused problems, not the least during parking. I was happy to sell it to a used-car dealer on Broadway, and after a few days extra vacation, we sailed for Gothenburg.

The Beginning of an Enzymologist

After my return to the Karolinska Institute, I continued to work on pyrimidine biosynthesis. The encounter with American science changed my approach, and I now started my second career as an enzymologist. Important for my development was a summer stay in Copenhagen during 1954, where I learned basic techniques in enzymology from Hans Klenow during efforts to prepare ribose 1,5-diphosphate. Bob Hurlbert, a postdoctoral student in Stockholm, and I had found that tissue extracts converted orotic acid to uridine nucleotides, and we suspected the involvement of ribose 1,5-diphosphate. In the meantime, Arthur
Kornberg demonstrated that 5-phosphoribosyl pyrophosphate was the true intermediate (9).

For quite some time, I studied the enzymatic formation of carbamyl aspartate from aspartate and demonstrated that two steps were required (10). The first was identical to the previously known first step in citrulline synthesis and led to the formation of an “active” carbamyl compound (Compound X) that in the second step was transcarbamylated to aspartate. Compound X had been discovered by Grisolia and required acetylglutamate for its formation. This was my first encounter with an allosteric effect, but I was not aware of it. Instead, I believed erroneously that acetylglutamate was part of the active carbamyl compound structure until it was shown, by chemical synthesis in Lipmann’s laboratory, that the active compound is carbamyl phosphate (11). Purification of aspartate transcarbamylase then became simple (12). The experience was somewhat painful but emphasized the importance of outguessing an intermediate in a reaction sequence.

Aspartate transcarbamylase is famous as the first allosteric enzyme. This story began in 1956 with a report by Yates & Pardee (13) showing that the enzyme activity in extracts of E. coli was inhibited by cytidine and CMP. Much to my surprise neither cytidine nor CMP inhibited my pure transcarbamylase. Later experiments showed that CTP is the allosteric inhibitor. In the crude extract, CTP was formed by phosphorylation of cytidine and CMP. For once, the pure enzyme was not superior.

I now supervised my first graduate students, Nils Ringertz and Ola Sköld. Nils was officially in Caspersson’s department working with sulfated nucleotides and mucopolysaccharides, somewhat outside Caspersson’s own interest. I had some knowledge of nucleotide chemistry and was pleased to learn about polysaccharides in order to advise him in his work. Ola’s work was on uridine kinase and phosphorylase. His experiments aroused medical interest when, together with George Klein, we showed that the development of resistance against fluorouracil could result from the loss of one or both enzymes. This was one of the first demonstrations that resistance to base analogues in mammalian cells is caused by loss of the activating enzymes.

My studies concerning pyrimidine synthesis were reasonably successful and provided me with funding from the Medical Research Council. My attempts to advance the ribonucleotide reduction story were for many years less successful. Incubation of labeled ribonucleotides with extracts from various cells never provided convincing evidence for the formation of deoxyribonucleotides. Discussions with people more knowledgeable in organic chemistry than myself were discouraging. There was no chemical precedence for this kind of reaction, and therefore the reaction did not exist. However, isotope experiments with intact cells continued to provide positive evidence. I was also greatly encouraged by Arthur Kornberg during his visit to Stockholm. When I complained...
about the small traces of radioactivity in dCMP after incubation of an *E. coli* extract with labeled CMP, he told me that these amounts of radioactivity were greater than what he had recovered in DNA during early efforts to demonstrate DNA replication in vitro. And then suddenly success arrived (14). I vividly recall when the sample recorded hundreds of counts instead of the usual five to ten. On that occasion, I had added just the right amount of ATP and Mg\(^{2+}\) to the bacterial extract. The function of ATP is twofold: it is an allosteric effector for the reductase, and it transforms CMP to CDP, the substrate in the reaction. With too much ATP, all CDP is transformed to inactive CTP, but I realized this much later. The first inkling of an allosteric regulation came in 1960 when I collaborated with Van and Zoe Canellakis in New Haven, Connecticut, and we observed complicated patterns of inhibition and stimulation of ribonucleotide reduction by deoxyribonucleotides with extracts from chick embryos (15). However, allostery was not yet invented, and we did not understand these phenomena for several years.

By 1960, I had become a reasonably well established scientist. At the end of my stay in New Haven, Dagmar and I again crossed America by car, this time going from New Haven to Berkeley. Many more scientists were working in my field of interest, and this time I knew them in advance. The friendly reception was the same. My seminars were better than those given in 1952 and were well received, although I was somewhat subdued by one of Arthur Kornberg’s commentaries after my talk at Stanford. I recounted some complicated experiments with a crude *E. coli* extract that indirectly showed that CDP, and not CMP or CTP, is the substrate reduced by the enzyme. Arthur said that these were nice experiments but that he would have purified the enzyme first and then it would have become apparent that CDP was its substrate. Nevertheless, he was favorably impressed, and our lifelong friendship began on this occasion.

The decade from 1950 to 1960 was a period of growth. Our three children were born. We moved from a small apartment in central Stockholm to a house in the suburbs and, in 1960, bought our summer place in the Stockholm archipelago. I widened my professional competence and became a reasonably good enzymologist. Arne Tiselius, who was professor of Biochemistry with the Science faculty in Uppsala, once expressed his surprise about the number of Swedish medical doctors who were excellent biochemists. After all, none of us had any formal chemical education, and we all had to pick up knowledge while we were moving along in our science.

In 1952, after my return from the United States, I had been appointed assistant professor of Medical Chemistry. My teaching obligations were considerable. I lectured not only to medical students but also to students of dentistry and to laboratory technicians. I rather enjoyed teaching, and I believe that with time I became a good teacher. I obtained my first grants
from the Medical Research Council, and later from the Swedish Cancer Society. My grants paid for two laboratory technicians. Such people, who are trained in special professional schools, constitute an important part of the Swedish research establishment. My present associate, Rolf Eliasson, comes from such a school. We started our collaboration more than 30 years ago. At the end of the decade, I obtained my first American grant from the Damon Runyon Foundation and later from the Public Health Service. These grants were tremendously important. For example, I was one of the first scientists at the Institute who could afford a scintillation counter. I could buy commercially available $^{13}$H-CDP to use as a substrate for the reductase instead of $^{32}$P-CDP, which we had to synthesize ourselves once a month starting from 30 mC $^{32}$P.

This period also had its darker aspects. My position as assistant professor was not renewable. I applied for several professorships, one of them as associate professor at the Institute. I optimistically thought that I would get the job but was mistaken, nor did I obtain any of the other professorships. This event occurred at a time of transition in the department. Einar Hammarsten retired and Sune Bergström came from Lund to replace him. He brought a very active, large group of young people with him. Ulf Lagerkvist and myself, the leftovers from the previous era, felt rather isolated. For some time, I was torn by doubts about my future. I had an attractive offer from Philip Handler to come to Duke University, but I did not wish to leave Sweden for family reasons. There was the alternative of leaving biochemistry altogether and making use of my medical education. However, my wife Dagmar strongly advised against it. She was a pillar during these difficult times and her belief in my scientific ability provided me with the necessary staying power, and everything worked out. At the end of my seven years as assistant professor, the Medical Research Council offered me a permanent research position. Two years later, I applied for my fourth professorship and this time I succeeded. As of July 1, 1961, I was Professor of Medical Chemistry at the University of Uppsala.

**Professor in Uppsala**

I arrived in Uppsala at a good moment and full of energy. Compared to Stockholm, both the medical faculty and the Department of Medical Chemistry were small. My predecessor, Gunnar Blix, had discovered sialic acid but by the time I arrived had more or less retired from research. The second professor and chairman of the department, Gunnar Ågren, was also a student of Einar Hammarsten and received me with open arms. I brought all my equipment from Stockholm to Uppsala and the university provided the required funds for the remaining purchases. Within a few weeks, the three graduate students and two technicians that accompanied me started new experiments which were soon aided by new students eager to join our team.
I was also pleased that Torvard Laurent, an old friend from Stockholm, returned from several years of research in the United States and chose to come to Uppsala. He carried out pioneering work in the polysaccharide field and his expertise in physical chemistry was helpful in our work. He became my successor when I left Uppsala in 1964 and since then has built up one of the strongest Swedish departments of medical chemistry. At that time, a university professor was an important person. We were fewer than we are now. In Uppsala, every professor was formally inaugurated. On this day the church bells rang in the morning. Later, I marched in the academic procession between the president and the vice president of the university together with most of my colleagues of the faculty to the large lecture hall to give a lecture on Biochemistry and Heredity to the general public. In the evening, we had invited the whole faculty and some honoratiore from the university to a formal dinner, together with family and friends. This was the first and only time that my father, who had come from Austria was dressed in white tie. Our 11-year-old, Per, served as toastmaster. It was a memorable occasion. A few years later, this century-old tradition was abandoned: there were too many new professors each year.

I now had the means and power to organize my own department in what I considered to be the proper way. I myself had received no formal training and little instruction as a graduate student and wished my students a better fate. Soon we began to have small meetings to discuss ongoing research. In Stockholm, I had started a journal club together with Joe Bertani and his group. We now had alternating meetings in Stockholm and Uppsala once a week and were soon joined by Hans Boman from Arne Tiselius’ department. Joe and Betty Bertani had arrived in Stockholm in 1958 to teach us microbial genetics, at that time an unknown subject in Sweden. During their 20 years at the Karolinska Institute, they educated the first crop of Swedish microbiological geneticists. Betty worked with me during one year to learn some biochemistry and participated in the discovery of dUTPase (16). Joe eventually became disgusted with the educational and political system of Sweden and he and Betty returned to the United States. Hans Boman was something of an anomaly in Tiselius’ place. There almost everybody discovered new methodology, but Hans discovered RNA methylation (17). He later moved to the University of Umeå and founded an outstanding Department of Microbiology. Then he moved to Stockholm and became a pioneer in insect immunology. Few people have his scientific imagination. Our journal club was of high quality even though my students sometimes complained that they could not follow all the genetic lingo. I admitted I had the same problem but assured them that they would understand if they heard it often enough.

In Tiselius’ Biochemistry Department were also Jerker Porath and Bo Malmström. The proximity to Jerker’s group gave us an edge in our work since we
could use their ingenious methods for protein fractionation before they were published. Bo Malmström became a good friend. Later, when he was professor of Biochemistry in Gothenburg and had established an outstanding group working on metalloenzymes, I often obtained their advice concerning the peculiarities of the iron center of ribonucleotide reductase. In Uppsala, I also met Lennart Philipson for the first time, then a hungry, budding molecular biologist. Lennart had a certain ability to make enemies because of his extreme outspokenness. He also had, and still has, an even greater ability to infect others with his enthusiasm and to organize research. We became good friends and together fought the battles with taxonomists and journalists to make Sweden accept molecular biology.

The first American postdoctoral students arrived. In Stockholm, American visitors had been my first teachers. Later they came to Einar Hammarsten but usually ended up working with me. A third and most distinguished group came to Hugo Theorell. Their presence contributed considerably to my growth as a scientist. Two of them, Jack Buchanan and Joe Neilands, became good friends. Jack corrected the broken English in my first paper in Nature (2); Joe later returned to work with me during a sabbatical. I always greatly enjoy visiting him and Juanita at their home in Berkeley, which he had built with his own hands.

And now the young Americans came to Uppsala to join our research enterprise. We began to harvest the fruits of enzyme purification. Foremost was the identification of the hydrogen donor system for the reduction. Already, after a moderate purification, we found that ribonucleotide reduction depended on reduced lipoate, but for several reasons we did not believe that lipoate was the physiological hydrogen donor. Instead, we discovered that a 12-kb dithiol-protein, named thioredoxin, could fulfill this function (18). A second protein, thioredoxin reductase, maintained the reduced status of thiols of thioredoxin with the aid of NADPH (19). Two of the new Uppsala students purified and characterized the two proteins during their thesis work. Arne Holmgren continues his elegant work on thioredoxin and in the meantime has discovered many new functions for this molecule (20). He also identified glutaredoxin as a second hydrogen donor in ribonucleotide reduction. Arne is now my successor at the Karolinska Institute. Lars Thelander developed considerable skill in physical chemistry and enzymology during his studies of the flavoprotein thioredoxin reductase (21). He later applied these skills to studies of the structure and mechanism of the E. coli ribonucleotide reductase and then to the mammalian enzyme. He is now professor of medical chemistry in Umeå where he can also satisfy his yearning for outdoor life and hunting. Arne, Lars, and a third student, Uno Lindberg, who was one of the few who did not work with ribonucleotide reduction, were wonderful examples of what Uppsala could offer in the way of enthusiastic future scientists.
Back at the Karolinska Institute

Even though everything was wonderful I left Uppsala after only two and a half years and returned to the Karolinska Institute to succeed Erik Jorpes as professor of Medical Chemistry. I had many friends in Stockholm with whom I hoped to be able to collaborate, foremost among them Joe Bertani and George Klein at the Karolinska Institute, and Olov Lindberg and Lars Ernster at the University of Stockholm. Dagmar and I had started our friendship with Lars Ernster, then a docent at the University, and his wife Edit, a concertmaster at the Opera. Lars was beginning to be an authority in bioenergetics, a subject he had started as a student of Lindberg and continued with such great success during his time as a professor of biochemistry at the university and into his retirement. The Ernsters remained our best friends over the years.

Also important for my decision to move was that Uppsala University started to build a new large center for biomedical research. I did not look forward to becoming heavily involved in the planning and had my doubts about how such a huge organization would function without involving a large bureaucracy. In actuality, the center functions extremely well, largely because the various groups are wisely allowed to maintain their organizational independence. My short period in Uppsala was happy and productive. It has helped me to remember that first-class biomedical research in Sweden can also be done outside the Karolinska Institute.

In Stockholm, our growing group continued the line of research started in Uppsala. All students, old and new, and several of the technicians came with me to Stockholm. The number of guest scientists increased. I found that a good way to maintain the necessary interaction within the department was to eat lunch together. One of the dishwashers who proved to be a good cook was released from her duties, and a hot lunch was served in the lunchroom every day at noon sharp. The quality of the food as well as its price sufficed to persuade almost everybody to participate. Visitors were always invited and the lunchroom also provided excellent dinners for seminar speakers. Many visitors later recalled the food better than the science. The lunches were started without authorization, but none of the top administrators of the institute protested when invited. The lunches became a resounding success that I would keep up for 25 years. Then one of the technicians in the department denounced the lunches to the central authority that oversaw all government expenditure and that was the end of our noon meals.

Ribonucleotide Reductase from E. coli

I was now back in my original department, Medical Chemistry. Enzyme purification became a major activity and eventually resulted in a pure E. coli ribonucleotide reductase. During purification, the reduction of CDP provided
WHEN THE PICTURE IS PAINTED

the assay. Agne Larsson, one of my early graduate students, had already found in Uppsala that the activity vis á vis ADP and GDP disappeared early during purification and had assumed that purine and pyrimidine ribonucleotides were reduced by different enzymes (22). But Agne never could purify a purine-specific enzyme. I then remembered the experiments in New Haven with the reductase from chick embryos, in which we had found that GDP reduction was stimulated by dTTP (15). To our great satisfaction, Agne found that the purified reductase preparation rapidly reduced purine ribonucleotides when supplied with dTTP. This story ended four years later with the understanding of the remarkable allosteric properties of ribonucleotide reductase (23). The enzyme consists of two proteins, at the time called B1 and B2, later renamed R1 and R2. R1 contains two sets of allosteric sites that bind nucleoside triphosphates. This chapter is not the place to describe in detail the complicated and beautiful allosteric regulation of ribonucleotide reduction that makes one enzyme produce equimolar amounts of the four building blocks for DNA synthesis. Suffice it to say that we had always used ATP in our assays, which made the protein a pyrimidine specific enzyme. With dTTP, the enzyme could reduce purine ribonucleotides.

Agne Larsson also began to study the chemical mechanism of the reaction by showing that the reduction of carbon-2' of the ribose occurred with retention of configuration (24). Other investigators found the same stereochemistry for the reaction catalyzed by the adenosyl cobalamin—requiring reductase, discovered by Blakley and Barker (25), even though the protein structures of the two enzymes differ greatly from each other. More recently, Joanne Stubbe's elegant work has clarified the mechanism of both types of reductases in great detail and extended their mechanistic similarity (26).

It was hard work to obtain large enough amounts of the E. coli reductase. DNA-technology for overexpression of enzymes was not yet invented, and the methods for enzyme purification were less sophisticated than today. After a joint effort of many technicians and postdoctoral students, foremost among them Lars Thelander and Neal Brown, we arrived at a reproducible method that after two weeks of hard work gave us several milligrams of R1 and R2 (27). Structural work became possible. R2 gave a characteristic spectrum, which suggested that the presence of iron in the enzyme was linked to its activity (28). My next-door neighbor was professor Anders Ehrenberg, a former student of Hugo Theorell, and a pioneer in the application of EPR spectroscopy to biological problems. When he analyzed the R2 protein by EPR spectroscopy we were in for a major surprise. No iron signal was found, but a sharp signal with all the hallmarks of an organic free radical (29). At first, we did not believe that the protein could contain a free radical, because it had spent two weeks or more in water solution during its preparation. This was the beginning of a long and fruitful collaboration with Anders Ehrenberg, and
later with Astrid Gräsland, his student and now his successor, that led to the identification of the radical and to an understanding of the interplay between the iron center and the radical. Without their expertise and enthusiasm, this work would not have been possible. Britt-Marie Sjöberg played a decisive role in this collaboration, first as a postdoctoral student and now as Professor of Molecular Biology. She localized the organic radical to tyrosine-122 of the polypeptide chain of R2 (30). The iron center consists of a pair of Fe(III) ions linked by oxygen (31). Each R2 monomer contains one iron center. This feature became evident from the three-dimensional structure of the protein (32) even though our analyses erroneously first indicated only one center per dimer. The number of tyrosyl radicals is still not settled; radicals cannot be localized by X-ray crystallography. Tyrosine-122 is deeply embedded in the protein structure in close vicinity to the iron center.

The presence of an organic free radical in R2 suggested that ribonucleotide reduction proceeds by radical chemistry and that R2 provides the required radical (33). What is the function of R1? This protein contains not only the earlier-mentioned allosteric sites but also the catalytic sites for substrates (34). In 1974, Lars Thelander did an important experiment that demonstrated the stoichiometric oxidation of thiols of R1 to disulfides during the reduction of CDP (35). The dithiol functionality of the enzyme is restored by reduced thioredoxin or glutaredoxin. Later experiments done independently by Britt-Marie Sjöberg (36) and Joanne Stubbe (37) carried this work further and identified two separate groups of dithiols. One group participated directly in the reduction of the ribose, and the other interacted with thioredoxin. On the basis of extensive, elegant experiments, Stubbe proposed a model that describes the interaction of the tyrosyl radical with the thiols at the active site during the reduction of the ribose moiety (38). Recent crystallographic results by Ulla Uhlin and Hans Eklund concerning the structure of R1 (39) strongly support this model. I look forward to an extension of the crystallography to complexes between R1 and allosteric effectors.

This brings the account of research on ribonucleotide reductase from E. coli up to 1994. I may have spent too many words on this subject, but the enzyme has been the most important part of my scientific life. A brash conclusion in 1950 by a 25-year-old beginner in science, in part founded on my ignorance of organic chemistry, had a decisive influence on the rest of my life in science.

The Nobel Institute and the Nobel Prize in Medicine

In 1971, I succeeded Hugo Theorell as Professor of Biochemistry. He had been the head of the Medical Nobel Institute for Biochemistry at the Karolinska Institute since 1937. The institute was originally founded with Nobel funds accumulated mainly from prize money that was not used during the war years.
In 1959, the Institute became a Department of Biochemistry within the Karolinska Institute.

According to the statutes of the Nobel foundation, a Nobel institute should assist in the work of the prize-awarding institution. The original idea may have been to check on the results of prize candidates. In medicine, the prize-awarding institution (the Nobel Faculty) originally consisted of all the professors at the Karolinska Institute. In 1982, the Swedish law concerning matters of secrecy in government affairs was liberalized drastically, and to maintain secrecy of the deliberations concerning the prize, a private Nobel assembly was put in place of the previous federal Nobel Faculty. The assembly is a self-perpetuating institution of 50 members who must be professors at the Karolinska Institute.

A first attempt to start a medical Nobel institute was made in 1918 when influential members of the Nobel Faculty suggested starting an Institute of Racial Biology. However, this was not done since it was not apparent how such an institute could help in the deliberations for the Nobel prize. A federal Institute for Racial Biology was instead started a few years later at Uppsala University. The name is disgusting, but I remember the director of the institute during the war as an outspoken enemy of the Nazi ideology.

The second attempt to start a medical Nobel institute came in 1935, when Einar Hammarsten started a campaign for an Institute in Biochemistry with Theorell as its director. This time the argument could be made that the creation of the institute would allow the Karolinska Institute to retain Theorell and that his collaboration with the Nobel Committee was of importance for the selection of worthy prize winners. The proposal stressed that the director of the institute must continue his research activities in order to maintain his usefulness for the prize work. In 1937, the faculty of the Karolinska Institute was small, and a person of Theorell’s caliber must have been a great asset for the Nobel work. He could now write expert opinions on prize candidates and closely collaborate with the Nobel Committee in other ways. He won the prize himself in medicine in 1955 and was therefore excluded from prize discussions during the early 1950s.

More than just the prestige of being director of the Nobel Institute induced my move from Medical Chemistry to Biochemistry. The number of medical chemistry students increased each year and so did the size of the department and its teaching load. I was attracted by the possibility of moving to a smaller department exclusively dedicated to research and of still maintaining my professorship at the Karolinska Institute.

On my return from Uppsala in 1964, I automatically became a member of the Nobel Faculty. I was immediately elected an adjunct member of the Nobel Committee and participated heavily in its work for the next 20 years. The directorship of the Medical Nobel Institute for Biochemistry could hardly
increase my obligations in this respect. My interests in Nobel matters dated back to my early days in Hammarsten's laboratory. For many years he had considerable influence on the choice of prize winners and sometimes commented rather freely on what went on, in particular when he returned from a committee meeting that did not go his way. His interest in Avery's work was why he suggested I attempt the crystallization of deoxyribonuclease. Avery's discovery in 1944 that DNA is the carrier of genetic information is certainly one of the greatest biochemical discoveries of this century and should have been rewarded with a Nobel Prize, either in medicine or chemistry. At the time of Avery's discovery, the idea that DNA and not protein is the genetic material was foreign even to scientists like Hammarsten and Caspersson who realized that nucleic acids are complicated molecules and not tetranucleotides. Avery's evidence was far from complete, and among the outspoken nonbelievers was Alfred Mirsky, a close colleague of Avery at the Rockefeller Institute. I can therefore understand why Hammarsten was hesitant in the beginning. A mistake had happened once before in 1926 when a Nobel prize in medicine was awarded for a discovery that later turned out to be incorrect. However, Avery lived until 1955. By that time the evidence for DNA was overwhelming and included Watson & Crick's discovery of the double helix. In the early 1950s, many important discoveries were backlogged because no prizes had been given during the war but it remains something of a mystery how the Medical Nobel Faculty could avoid giving a prize to Avery.

I have sometimes been asked why a certain person is not a Nobel Prize winner. No omission is as blatant as Avery's, but I can name a few other scientists who, in my opinion, would have deserved a Nobel Prize. One must remember that a Nobel Prize is given for "the most important discovery within the domain of physiology or medicine" and not for the accumulated work of a whole life. Also, Nobel's will stipulates that the prizes should be given "to those who, during the preceding year, shall have conferred the greatest benefit on mankind." For obvious reasons it is hardly ever possible to give the Prize for a discovery that was made the preceding year. The sentence is interpreted by the prize-awarding institutions to mean that the importance of the discovery should have become apparent during the previous years. In medicine, the prizes awarded to Peyton Rous and Barbara McClintock represent two extreme cases where the importance of the discoveries was appreciated only much later. However, as a rule old discoveries may find it difficult to compete with more recent ones. An incubation period of ten years is certainly not unusual.

I found my work in the Nobel Committee highly stimulating and rewarding, certainly much more so than work in any of the other committees that I attended during my professorial years. I became familiar, at least to some extent, with many areas of biomedicine outside of my own specialty. As a biochemist, I was pleased to see that an increasing number of prizes went to scientists whom
I would classify as biochemists even though their research was classified as immunology, virology, pharmacology, or something else. My colleagues usually protest when I point out that the aim of today's biomedical research is to understand life in chemical terms.

Some say that the Nobel Prizes are a disservice to science and that Prize winners stop doing important research. However, scientists usually make their most important discovery before the age of 50, and most winners are older than that when they receive the prize. There are also many examples of the Prize winner continuing his or her research successfully, two of them being Arthur Kornberg and Gobind Khorana. Once a year, the award of the Nobel Prizes focuses the interest of society on intellectual achievement. The fact that so many other Prizes, instituted more recently, often wish to relate to the Nobel Prize is also telling. There can be no doubt about the usefulness of the Nobel Prize for Swedish science. It forces the Swedish scientists participating in the prize work to maintain contact with the cutting edge of science, and it brings the most outstanding international scientists to Sweden before and after they receive their Nobel Prize.

Sabbatical Leaves

In 1966, I took my first sabbatical leave at Stanford. The medical school had been started, and Arthur Kornberg had a laboratory there in which I worked for six months. Stanford was no longer "The Farm" it had been in 1952, and on trips to Santa Cruz we no longer went through blooming orchards. But such nostalgic feelings rapidly disappeared when I viewed the scientific atmosphere. Arthur is not only an outstanding scientist but he had also created a new department with other outstanding young people who could work together. Over the years, my many visits to Stanford, first to Arthur and more recently to Roger Kornberg, gave me the opportunity of uninterrupted bench work in a congenial atmosphere. Every visit, the latest in 1994, provides stimulation and pleasure.

My work in 1966 set the pattern for later visits: I participated in ongoing work on DNA replication in Arthur's group and initiated collaborative experiments linked to my work in Stockholm. I studied the replication of phiX DNA with Arthur, measured the fluorescence of thioredoxin with Lubert Stryer, and did NMR experiments aimed at the stereochemistry of ribonucleotide reduction with Lois Durham in the chemistry department.

After my return to Stockholm, I began to think about experiments that would connect ribonucleotide reduction with DNA replication. Influenced by Arthur's concept of viral windows on DNA replication, I spent my next sabbatical in 1970 at the Salk Institute in La Jolla and learned from Walter Eckhart how to handle polyoma virus. This experience led to a decade of experiments in Stockholm in which we used isolated nuclei from polyoma virus infected cells.
to study the replication of the viral DNA. The ultimate goal was to be able to extract the enzymes from the nuclei and to achieve a complete replication of the DNA. In addition, I hoped to be able to couple DNA replication with the enzymes isolated by Lars Thelander that were involved in mammalian ribonucleotide reduction. This was utopia. We were not able to initiate new rounds of replication, mainly because the large amounts of genetically engineered T antigen required for a successful experiment were not available. Our major contribution was the discovery and description of a particular kind of RNA that initiated the synthesis of Okazaki fragments (40). This RNA was characteristically 10 nucleotides long, started with A or G, but lacked sequence specificity. Many postdoctoral and graduate students in Stockholm worked diligently on this project, foremost among them Ernst Winnacker and Göran Magnusson. Both soon became independent professors—Ernst in Munich and Göran in Uppsala—and continued to work with animal viruses.

For many years, I returned to Stanford for one month each November to do some experimental work and to interact scientifically and socially with good friends. Stanford became my second intellectual home. On returning to Sweden, I always felt that I traveled back with a portion of Stanford in my luggage. Life became poorer when, in the late 1980s, I no longer could return because of the illness of my wife Dagmar.

**A Return to Intact Cells**

There came the time when I wished to find out if the complicated regulation of the pure ribonucleotide reductase in the test tube was relevant for the function of the enzyme in intact mammalian cells. In his thesis, Lambert Skoog devised an enzymatic method for the measurement of the small intracellular dNTP pools (41). In S-phase cells, the pools suffice only for a few minutes of DNA replication. The sensitivity of Skoog's method is such that it could be used to measure the dATP pool in resting lymphocytes, which amounts to 1 pmol/10^6 cells. We could now study pool fluctuations caused by manipulations of the reductase. One example was the paradoxical inhibition of DNA replication by thymidine. Gunnar Bjursell found that addition of thymidine to cells not only increased the dTTP pool as expected but also depleted the dCTP pool and increased the dGTP pool, as predicted from the known allosteric regulation of the reductase (42). Also, other examples showed that intracellular events mirrored the results found with the isolated reductase.

Measurements of pool sizes represent only snapshots and tell us little about the dynamics of dNTPs in the cell. The size of a pool depends both on the rate of its synthesis and the rate of its disappearance via DNA replication and catabolism. Questions concerning the dynamics of dNTP metabolism can be answered from experiments using isotope flow kinetics. We began such experiments in the early 1980s by feeding cells in culture with labeled nucleosides
and measuring the flow of isotope into deoxyribonucleosides, dNTPs, and DNA. In these experiments, measuring the specific radioactivity of dNTP pools is crucial. Unfortunately, it is all too common that investigators carrying out experiments of this type are satisfied to determine the total amount of radioactivity in the pool, assuming that the size of the pool does not change with the manipulations involved in the experiment.

We used both normal cultured cells and cells carrying specific mutations in enzymes involved in deoxyribonucleotide metabolism. We could conclude that ribonucleotide reductase is the primary target for the regulation of dNTP synthesis but that an additional type of control, involving nucleoside kinases and nucleotidases, is also important. The kinases are often called salvage enzymes, which implies that their only function is to reutilize nucleosides arising from the catabolism of nucleic acids. However, our experiments indicate that kinases also form part of a regulatory circuit, which explains why they are strictly allosterically regulated. Intracellular deoxynucleotides are continuously broken down by nucleotidases and resynthesized by kinases. This process results in substrate (or futile) cycles that help maintain the proper size of dNTP pools (43). This regulatory circuit comes into play, for example, when cells have lost the enzyme dCMP deaminase (44) or when DNA synthesis is blocked during S-phase by inhibitors (45). In both instances, dNTPs are continuously overproduced by ribonucleotide reductase catabolized via substrate cycles and excreted as deoxynucleosides. Substrate cycles are also of considerable medical interest since they are involved in the activation of nucleoside analogues used in the treatment of viral and malignant diseases.

Experiments on substrate cycles were initiated ten years ago in Björn Nicander’s thesis and are continued today by Vera Bianchi, who joined our group during a sabbatical in 1984. She has later returned many times. Vera is also Professor of Cell Biology at the University of Padova and, most importantly, has been my dear wife since 1991.

In the course of these experiments, our laboratory became involved in a controversy concerning multienzyme complexes that are supposed to channel dNTPs from ribonucleotide reductase to the site of DNA replication. Channeling of intermediates, i.e. the movement of small molecules from one enzyme to the next without mixing with a general cytoplasmic pool, is certainly an attractive idea but must be supported by solid evidence. A multiprotein complex containing enzymes of dNTP synthesis and DNA replication, called replitase, was postulated to be assembled in mammalian-cell nuclei during S-phase (46). The evidence for this concept was shaky from the beginning (47), and much later work has contradicted it, not the least the finding that mammalian ribonucleotide reductase is a cytoplasmic enzyme (48). Nevertheless, it has been difficult to bury the replitase, probably because the concept
is diffuse enough to provide apparent explanations for half-baked data from experiments with intact cells.

More Ribonucleotide Reductases

Blakley & Barker had discovered that ribonucleotide reduction by extracts of *L. leichmannii* requires addition of a vitamin B₁₂ derivative(25). The enzyme present in extracts from *E. coli* did not require addition of cobalamin, and when we purified the reductase from bacteria grown in the presence of ⁶⁰Co-labeled vitamin B₁₂, the radioactivity disappeared during purification. Clearly, *E. coli* and *L. leichmannii* contain different ribonucleotide reductases. The amino acid sequences of the two enzymes were later found to be very different. The two enzymes can be considered prototypes of two classes of ribonucleotide reductases. Class I enzymes have an R₁:R₂ structure and contain a stable tyrosyl radical; class II enzymes consist of a single polypeptide chain and require adenosyl cobalamin.

What about ribonucleotide reduction in higher organisms? Mammalian cell extracts did not require addition of cobalamin for the reduction of ribonucleotides, and when purified enzymes became available, we learned that reductases from higher organisms in almost all respects behave as the *E. coli* reductase. Final proof came from Lars Thelander's work that produced the first pure mammalian enzyme (49). Today one can generalize that the reductases from all higher organisms are class I enzymes.

DNA technology greatly contributed to this generalization as it has to many other insights concerning ribonucleotide reduction. Britt-Marie Sjöberg constructed the first plasmids that were used to produce large amounts of *E. coli* reductase (50) and now applies recombinant DNA technology to identify functionally important amino acids of the enzyme. Rolf Eliasson and I familiarized ourselves in the late 1980s with the new technique. Now we happily clone new genes related to new reductases and obtain the required overproducers, but we prefer to do this in collaboration with experts in the field.

I realized early on that I would not be able to learn all the technology required to study the reductase. During my first professorial years, I encouraged my students to develop the required special skills. Lars Thelander showed a good ability to master physical-chemical techniques; Arne Holmgren specialized in amino acid sequences; and Olle Karlström was our microbial geneticist. However, this approach was limited. The students became independent scientists and started to dig in their own gardens. I began to collaborate with other groups and soon relied on a network of experts who were a prerequisite for our success. I have already mentioned my indebtedness to the biophysical expertise of Anders Ehrenberg and Astrid Graslund. It would not have been possible to apply recombinant DNA technology so successfully without Britt-Marie Sjöberg's and Elisabeth Haggård-Ljungquist's help. Outside Sweden, Fritz
Eckstein from the Max Planck Institute for Medicine in Göttingen has been a long-standing friend and collaborator whose deep knowledge of synthetic nucleotide chemistry has left many imprints on our work.

In recent years, we have had very close relations with Marc Fontecave's laboratory in Grenoble. Marc came to Stockholm as a postdoctoral student in 1985. During his graduate work on cytochrome P-450, he obtained a solid knowledge of metal biochemistry. At the time, we had just started to fractionate the components of an enzyme system from *E. coli* that generates the radical of tyrosine-122 in an inactive form of protein R2. With Marc's help, we separated the system into four proteins and identified a hitherto unknown flavin reductase as a key component that reduces the Fe(III) center of the reductase to Fe(II) (51). Molecular oxygen reoxidizes the iron center and at the same time generates the radical in Tyr122.

How does *E. coli* synthesize dNTPs in the absence of oxygen? Of the several possible answers, Nature has chosen the most interesting one which opens up new vistas into biochemistry and evolution and has provided Marc and me with a fascinating problem to solve. Not all the answers are in yet, but the collaborative efforts of our two laboratories have begun to give us a good grasp of the general picture. Collaboration with Marc has been a joy. In 1990, at the young age of 34, Marc became Professor of Inorganic Biochemistry at the University of Grenoble. There he has already built up a strong laboratory with many enthusiastic co-workers. During recent years they have spent time in Stockholm, and I visited Grenoble on several occasions.

Anaerobic *E. coli* produces a new ribonucleotide reductase with an iron-sulfur center and, in its active form, a glycyl radical (52). The amino acid sequence differs from that of a class I or II enzyme and opens a new class III of ribonucleotide reductases that can be expected to contain other anaerobic enzymes.

The glycyl radical of the new enzyme is extremely oxygen sensitive. The enzyme is isolated inside an anaerobic box, but by the time we have obtained a pure protein, most of the radical and the activity are lost. However, both can be restored when the inactive reductase is incubated with S-adenosyl methionine, NADPH, and an enzyme system of *E. coli* that consists of three defined, and by now, cloned proteins. We were greatly helped in our work by earlier elegant experiments by Joachim Knappé and his group in Heidelberg. They had shown that a similar system generates a glycyl radical in pyruvate formate lyase from *E. coli*. This enzyme catalyzes the dissimilation of pyruvate to acetyl-CoA and formate, a central route in anaerobic energy metabolism (53).

One fascinating aspect of our present work deals with the evolution of DNA (54). It is now generally believed that RNA preceded DNA during the early evolution of life and, in the beginning, provided the means for both catalysis and self-replication. This discovery raised the question, at what stage did
proteins enter? DNA synthesis requires deoxyribonucleotides. It is not unreasonable to assume that these were produced from ribonucleotides. Thus, either the first ribonucleotide reductase was a protein or RNA itself could catalyze the reduction of ribonucleotides (55). Ribonucleotide reduction requires radical chemistry, and free radicals are a highly reactive species useful only if they can be contained. I find it difficult to believe that RNA can do this without being damaged itself, whereas proteins can obviously be designed for this purpose; I therefore favor the idea that the first ribonucleotide reductase was a protein. This means that proteins preceded DNA during evolution. One problem is the current existence of three apparently different reductases, but we do not know the extent of those differences. A more definite answer must await knowledge of the 3D structure of the enzymes. Currently, Joan Stubbe’s data on the active cysteines of the \textit{L. leichmanni} enzyme suggest that class I and II enzymes are structurally related. Also, the class III \textit{E. coli} reductase shares certain features with enzymes from the other two classes, particularly with respect to allosteric regulation. In my scenario, the first protein-reductase emerged during evolution before photosynthesis and was a prerequisite for DNA synthesis. The radical chemistry required for ribotide reduction was provided by the oxygen-sensitive glycyl radical that the reductase could have usurped from the older pyruvate formate lyase. Today’s class III enzymes are the closest relative of this ancient enzyme. When oxygen appeared on Earth, it required a new radical generating mechanism involving major restructuring of the protein, and this mechanism resulted in the appearance of class I and II enzymes.

\textbf{Active Retirement}

In the Swedish university system, a professor retires at the age of 66 to make room for a successor. For me, the date was July 1, 1991. I had prepared for retirement by not accepting new graduate students or postdoctoral fellows, but I did not cut down my research. I was in the middle of the exciting discoveries concerning the new anaerobic ribonucleotide reductase described above. The policy of the Karolinska Institute is to provide laboratory space to active professors after retirement if they can finance their research with outside funds. So far I have been able to do so and have continued working together with two long-time associates, Rolf Eliasson and Elisabet Pontis. I am also fortunate to continue collaborating with other groups, the foremost being Marc Fontecave’s in Grenoble. Recently, our circle was widened by Albert Jordan from Jordi Barbé’s laboratory in Barcelona. Their work has resulted in the discovery of an additional ribonucleotide reductase in \textit{E. coli} (56). This bacterium has the potential to produce three different ribonucleotide reductases.

The most important change in my life occurred immediately after my retirement in 1991, when Vera and I married. We had collaborated in research
since 1984, and after the death of my wife Dagmar, decided to extend our collaboration to the private sector. Vera teaches Cell Biology in Padova and has a research group there. As a consequence, we divide our time between Sweden and Italy and get the best of each country.

I have borrowed the title for this chapter from Francis Crick's autobiography, "The Important Thing Is to Be There when the Picture Is Painted" (57). I was there, and I hope to be there for some time to come. In looking back, I find that the citation describes a good part of my life. So much happened outside my control; so many events of my life came to me without my own doing. My own contribution was to grab the opportunities.

Most important was that I was taken to Sweden and a school that made the transition easy. My new country provided me with the opportunity to start a career in science and later gave me all the support I needed for success. Arthur Kornberg recently asked me if it would not have been better to have gone to the United States and practice biochemistry there. My answer was no. My preference for Sweden in part comes from my preference for Swedish society. But it also has to do with the satisfaction I obtained from doing what I did. I would not have been a better scientist in the United States, and given that, I believe that my achievements as a scientist and teacher left a larger imprint in Sweden than they would have in the United States.

The next most important event came in the person of Einar Hammarsten. The example of his dedication to science made a lasting impression on me, and his way of running, or rather not running, a department was what made me develop rapidly into an independent biochemist.

The third event occurred when ribonucleotide reduction came to me. Little did I know what would happen when I injected labeled ribonucleosides into rats in 1950. From there, one thing led to another. I did plan to find the enzyme for ribonucleotide reduction but did not know that when I added ATP to the extract I would get deeply involved in allosteric effects of a unique kind. Again, I planned to obtain a pure enzyme but had no plan to make the unprecedented discovery of the first stable protein radical. It may be lack of imagination that has kept my mainstream in research to a single subject for so many years, but there were so many fascinating side streams from other fields to be explored. There was of course DNA replication but also disulfide metabolism, cobalamin, allosteric effects, organic free radicals, metallo-enzymes, anaerobic metabolism, and now evolution. It was a question of keeping the eyes and mind open. To be there when the picture was painted.
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