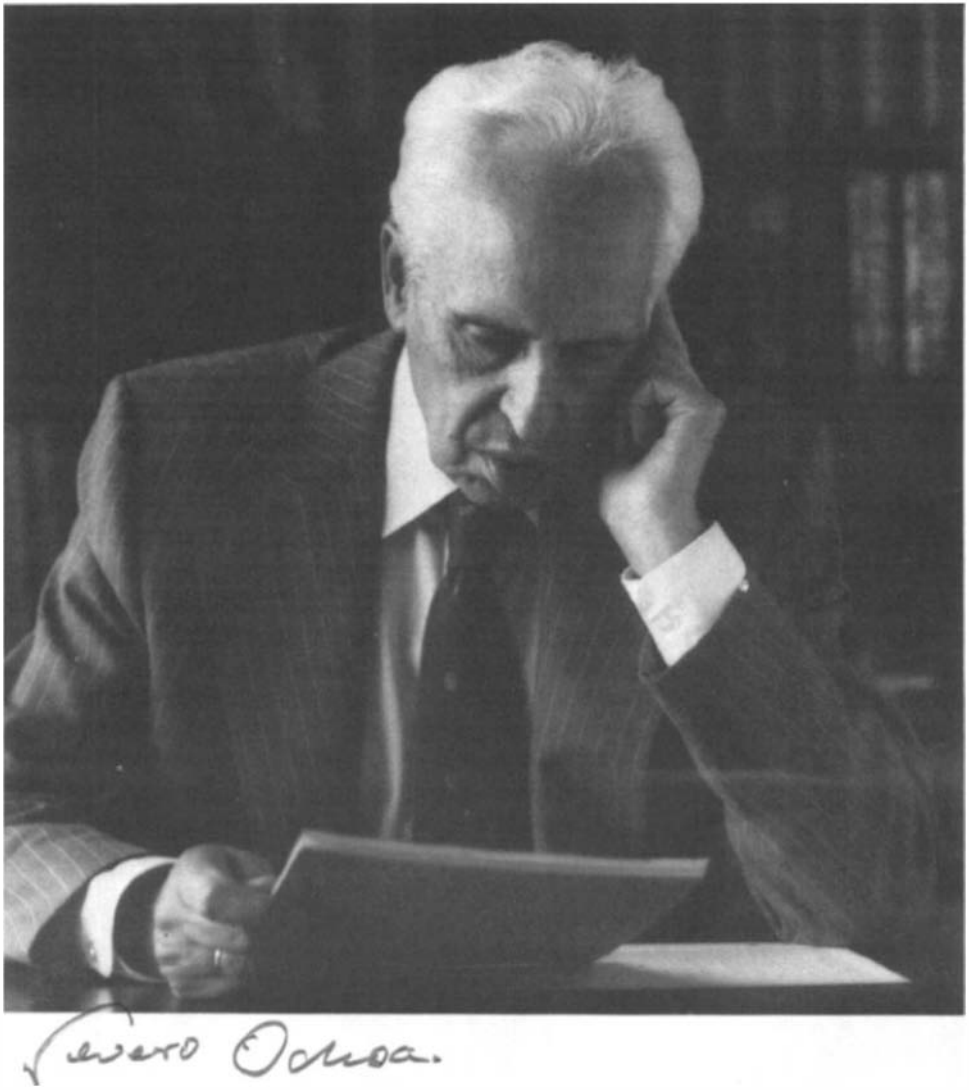

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THE PURSUIT OF A HOBBY

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One evening in the late nineteen forties my wife and I were at a party given in honor of Otto Loewi and Sir Henry Dale, corecipients of the 1936 Nobel Prize in Medicine for their discovery of the chemical transmission of the nerve impulse. We were all asked to sign the guest book and state our hobby and I did this as Sir Henry looked over my shoulder. As I put down my hobby as Biochemistry he roared with laughter. At that time I was Professor of Pharmacology and Chairman of the Department at the New York University School of Medicine and Sir Henry said, "now that he is a pharmacologist, he has biochemistry as a hobby." I tell this story to justify the title of this essay, because in my life biochemistry has been my only and real hobby.

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A GLIMPSE AT MY LIFE

I was born in 1905 in a small town in the north of Spain, on the Atlantic Coast; a rainy, beautifully green country where the mountains slope down to the ocean. In search of a milder climate, my family started going to Málaga on the Mediterranean coast, from mid-September to mid-June when I was seven years old. After going to a private school for several years I entered high school from which I earned a BA degree in 1921. It was in the last few years of high school that I began to be enormously attracted by the natural sciences. Much of this was due, I am sure, to the stimulating teaching of a young, bright professor of chemistry, Eduardo García Rodeja. For a while I contemplated becoming an engineer but, on the one hand, I had little talent for mathematics and, on the other I eventually realized that what really interested me was biology. It was for this reason that I entered Medical School at the University of Madrid in 1923. It had never entered my mind to practice medicine but at that time, in Spain at any rate, medicine provided the best access to the study of biology. My imagination had been fired by the accomplishments of the great Spanish neurohistologist Santiago Ramón y Cajal and I was dreaming of studying histology under him when I entered medical school after one premedical year of study of physics, chemistry, biology, and geology. I cannot describe how disappointed and unhappy I felt when I realized that the 70-year old Cajal had retired from his Chair although he continued his research at the laboratory that the Spanish Government had built for him in Madrid. Fortunately, in my second year at Medical School I came under the influence of a young, bright, and inspiring teacher, Juan Negrín, who had been trained in Germany. He received an MD degree from the University of Leipzig where he became a physiologist under Ewald Hering. He had returned to Spain to chair the Physiology Department at the University of Madrid Medical School after working for several years at the Physiological Institute in Leipzig. Negrín opened wide, fascinating vistas to my imagination, not only through his lectures and laboratory teaching, but through his advice, encouragement, and stimulation to read scientific monographs and textbooks in languages other than Spanish. He also advised us to read Cajal. At that time the only foreign language I could read was French but that came in handy for it allowed me, during a summer vacation, to read an excellent textbook of biochemistry by H. Lambling, a professor of biochemistry at Lille in northern France. It was fascinating reading. At that time I also read and reread a book by Maurice Arthus, Professor of Physiology at the University of Lausanne, on the experimental method in physiology. My greatest fascination, however, was reading Cajal. I never got tired of reading his autobiography and his book *Advice on Scientific Research.* My devotion

and admiration for the man who created modern neurology was boundless. However, I never met him for I did not think that a young student should dare disturb the great man. When I was beginning my third year at Medical School, where I had the good fortune to be taught pathology by Cajal's student, Francisco Tello, my decision to devote my life to biological research was irrevocable.

An Apprentice Scientist

The study of medicine in Spain required six years to graduate with a degree equivalent to a Bachelor of Medicine degree. One further year of study and the presentation of a thesis were requirements for the MD degree. When I completed my second year, Professor Negrín offered me and a classmate and close friend of mine, José G. Valdecasas, the possibility of doing some research in our spare time. There was no research laboratory at the Medical School (the available laboratory space was used only for teaching) but Negrín directed a small research laboratory run by the Council for Scientific Research (CSR). This was located at some distance from the Medical School in one of the buildings of an institution which I can best describe as being the equivalent of an Oxford or Cambridge College. About 100 students from the various faculties of the University and the schools of engineering and architecture lived there. This institution, run by the CSR, provided tutoring in various fields of knowledge including foreign languages, the tutors being in general some of the more advanced senior students. Under the leadership of Alberto Jimenez Fraud, a gentleman and scholar, this Student Residence, La Residencia, as it was familiarly called, engaged in a number of cultural activities, including lectures on art, literature, history, philosophy, science (Albert Einstein once lectured there), and music recitals. Three people who lived at the Residencia made it famous in later years: the poet and playwright Federico García Lorca, the painter Salvador Dalí, and the film director Luis Buñuel. I mention this to underline the fact that the Residencia provided those people who lived there and/or worked in its research laboratories a strong stimulus toward creative accomplishment. Scientific stimulation and encouragement were also provided by a research laboratory that was next door to Negrín's, the laboratory of the great neurohistologist, Pío del Río Hortega, the discoverer of new types of glial cells, where the famous McGill brain surgeon Wilder Penfield once worked.

Valdecasas and I wanted to work together and asked Professor Negrín for a problem. Negrín wisely thought that we should start by getting some laboratory experience and suggested that we isolate creatinine from the urine. We had some problems before we got crystals of creatinine hydrochloride. During this time we had been reading and became more and more

interested in the function and metabolism of creatine and creatinine in the body. It seemed to us that whereas there were a number of methods for determination of these compounds, all based on the Jaffe color reaction with picric acid, it would be desirable to have a good micromethod for the determination of creatine in muscle. In a few months we came up with a rather simple micromethod for this determination. I had become interested in the then prevailing theory that creatine was synthesized from guanidine, believed to be a normal metabolite that was thereby detoxicated, and I started applying our micromethod to the determination of creatine in the muscles of rabbits before and after guanidine injections. This was in the winter and spring of 1927. In the summer of that year I decided to go to the laboratory of Professor D. Noel Paton at Glasgow who was the proponent of the theory. I was then in my fourth year at medical school. I wrote to Noel Paton and was delighted to hear that he would be glad to have me spend a couple of months in his laboratory that summer. At Glasgow I injected frogs with guanidines and, naturally, did not find any increase in muscle creatine; but I found that the guanidines had a pronounced contracting effect on the melanophores of the frog skin. This work gave rise to my first paper which Professor Paton communicated to the Proceedings of the Royal Society of London. Back in Madrid, with a fairly good knowledge of English, I proposed to Valdecasas that we submit our creatine method for publication to the Journal of Biological Chemistry (JBC). We did so and, in a few weeks, the paper came back with some minor criticisms and suggestions of the Editors for revision. I was delighted that my English was left practically untouched. We did the suggested control experiments, revised the paper, and resubmitted it. It was published in 1929 (1). Not even in my wildest dreams could I have dreamt that, years later, I would become a member of the editorial board of the JBC and serve as President of the American Society of Biological Chemists.

In his own work on the mechanisms regulating the secretion of epinephrine, Negrín had used perfusion of the hind limbs of the frog with Ringer solution as a bioassay for epinephrine. This was known as the Loewen-Trendelenburg preparation. The saline entered through a canula placed in the abdominal aorta and exited through a canula in the inferior vena cava. It occurred to Valdecasas to use this preparation to see if there was any flow of creatine or creatinine out of the muscles either at rest or during contraction elicited by stimulation of the sciatic nerves. The answer was unequivocal, there was no creatine or creatinine in the perfusion fluid at rest but considerable amounts of creatine appeared upon stimulation. A search of the literature revealed that an Australian physiologist had made a similar observation using the sartorius muscle of the frog immersed in saline; upon stimulation it released creatine to the medium. We thought that Valdecasas'

observation meant that stimulation increased the permeability of muscle to creatine. We failed to connect this observation with the earlier discovery by Phillip and Grace Eggleton in Cambridge, of phosphagen, a compound that releases inorganic phosphate upon stimulation of muscle. In 1929 Fiske and Subbarow at Harvard and Lohman in Berlin showed that phosphagen was in fact phosphocreatine. Had Valdecasas determined inorganic phosphate as well as creatine in his perfusion fluid he might well have discovered phosphocreatine before the American and German workers.

During some of my time at Medical School I helped myself financially by tutoring medical students in physiology and biochemistry. At that time there was no separate biochemistry in Spanish medical schools; it was taught by the physiology department in the sophomore year. Negrín was an exacting teacher and failed a high proportion of students, so that tutoring in physiology was very much sought after. One of my students, later my close friend Francisco Vega Diaz, became one of Spain's foremost cardiologists.

Postdoctoral Years

Early in the summer of 1928 I had finished the requirements for my BM degree and I began to think about carrying out my most cherished wish, to go abroad for further training. I had become greatly interested in the work of Otto Meyerhof on the chemistry of muscular contraction. Meyerhof was then head of a division of the Kaiser Wilhelm Institute for Biology in Berlin-Dahlem. Otto Warburg headed another division at the same Institute and Carl Neuberg was Director of the Kaiser Wilhelm Institute for Biochemistry. The scientific atmosphere at Dahlem has been beautifully described by David Nachmansohn in his Prefatory Chapter in the *Annual Review of Biochemistry* a few years ago (2). I was very fortunate in being accepted by Meyerhof and in the fall of 1929 I set out for Berlin. I had a nominal fellowship (with no stipend) from the CSR. I had been granted a regular fellowship but, having some means of support of my own, I relinquished it in favor of one of my other fellow students who did not. I was then fairly fluent in French and English but my German was still spotty. Fortunately, Meyerhof spoke English and this helped me along until, after a couple of months, I got sufficient mastery of the German language. At that time Meyerhof had only one scientific assistant, Karl Lohman, and a superb technical assistant, Walter Schultz. Other postdoctoral fellows were David Nachmansohn, Fritz Lipmann, Frank Schmitt from St. Louis, and Ken Iwasaki from Japan.

An American physiologist had reported that the muscles of frogs subjected to repeated insulin convulsions became virtually free of glycogen but could nevertheless contract when stimulated, presumably utilizing a source

of energy other than carbohydrate. Meyerhof wanted me to check on this observation. My results were by and large confirmatory. By the time my work was finished, Einar Lunsgaard had already reported that the muscles of frogs poisoned with iodoacetate, although unable to produce lactic acid, were able to contract, presumably at the expense of phosphocreatine breakdown.

In late 1929 Meyerhof moved from Berlin to Heidelberg. The Kaiser Wilhelm Gesellschaft had built a beautiful new building in Heidelberg that housed, in four separate wings, research institutes for physics, chemistry, physiology, and experimental medicine. Meyerhof had been appointed director of the Physiology Institute. In the meantime I went back to Madrid to take examinations in various subjects, including biochemistry, in partial fulfillment of the requirements for the MD degree; then I went back to Meyerhof in Heidelberg. The story of the energetics of muscular contraction is an interesting one. It was believed from the work of Meyerhof that the conversion of glycogen to lactic acid supplied directly the energy utilized in contraction. This despite indications from Embden's laboratory that lactic acid production was not directly related to contraction for, in fact, the bulk of the lactic acid was produced during relaxation when contraction was over. Meyerhof would not accept this view and stubbornly maintained that Embden's results were the result of overstimulation. He received Lunsgaard's results with skepticism but Lunsgaard was quick to realize that to have his results accepted he would have to repeat them in Meyerhof's own laboratory. He came to Heidelberg in the summer of 1930 and convinced Meyerhof that he was right. Iodoacetate-poisoned muscles contracted without producing lactic acid until their store of phosphocreatine was exhausted. Evidence was obtained that lactic acid production was coupled with the resynthesis of phosphocreatine from its breakdown products. It remained an open question whether or not phosphocreatine breakdown was the chemical process most closely connected with contraction. ATP had already been discovered and it was known to be fully hydrolyzed at the time iodoacetate-poisoned muscles stopped contracting and went into rigor. It was not until several years later that biochemistry in Lohman's hands provided the answer: Lohman found, with muscle extracts, that phosphocreatine could not break down unless ADP, the hydrolysis product of ATP, was present. Phosphocreatine regenerated ATP by transferring phosphate to ADP, the so-called Lohman reaction.

Back in Madrid in late 1930 I collaborated with Francisco Grande Covian, an Asturian like myself, in a study of the role of the adrenal glands in muscular contraction, a study which eventually became my MD thesis. In 1931 I married Carmen García Cobian and a year later we went abroad for a further period of postdoctoral study. I wanted to be exposed to British

science and, on Negrín's suggestion, I went to the National Institute for Medical Research in London which was directed by Sir Henry Dale. There I joined Harold W. Dudley to work on my first enzyme problem, glyoxalase. This enzyme has been discovered independently by Neuberg in Germany and by Dakin and Dudley in the United States. Its mode of action was elucidated years later by my good friend Efraim Racker. During my post-doctoral period in London I was supported by a fellowship from the University of Madrid. We stayed in London for about two years and during my second year I moved to Sir Henry Dale's own laboratory to collaborate with some of his students in a study of the influence of the adrenal glands on the chemistry of muscular contraction. One of these students was Oliver Cope, the well known Massachusetts General Hospital surgeon. Sir Henry, together with Feldberg and Gaddum, was following up Loewi's discovery suggesting that the transmission of the nerve impulse to effector cells is chemical in nature. Loewi had found that acetylcholine is released in the heart of the frog by stimulation of the vagus nerve. I had the good fortune of watching the demonstration by Dale and co-workers of the release of acetylcholine in the perfused superior cervical ganglion of the dog upon stimulation of the cervical sympathetic nerve fibers, and I shared in the general excitement.

On returning to Madrid I rejoined Negrín's laboratory as an assistant Professor of Physiology and combined teaching with research. My work on glyoxalase had aroused in me an interest in enzymes and, since methylglyoxal, the compound that glyoxalase converts to lactic acid, was considered by some to be an intermediate in glycolysis, I had become familiar with the literature on glycolysis and fermentation. I decided to study glycolysis in heart muscle. There were some claims that whereas muscle glycolysis involved phosphorylated intermediates this was not so in heart.

At this time there was a development that could have important consequences for my scientific career. Professor Carlos Jimenez Díaz, head of one of the three Departments of Medicine at the University of Madrid Medical School, had conceived the idea of creating an Institute for Medical Research and he counted on considerable private support for this project. Jimenez Díaz had a deep appreciation of the crucial role of basic science in medicine; he was not only a great teacher and a great physician but was one of the most famous medical practitioners in Spain. Thus he was able to persuade some of his wealthy friends to provide a large sum of money to purchase scientific equipment for the Institute and to underwrite a generous annual budget for salaries, supplies, and other expenses. The University provided the space (several floors in one of the wings of the new Medical School buildings at the University City) and the maintenance. The Institute consisted of four sections: biochemistry, physiology, microbiology, and experi-

mental medicine. I was offered the direction of the Physiology Section which I accepted eagerly, and began to work with two young collaborators and one technician in the fall of 1935. Unfortunately, work came to a standstill a few months later because of the start of the Spanish Civil War. Clearly, to continue doing research without a long interruption that might forever destroy my chances of becoming a scientist, I would have to go elsewhere and, after much thought, my wife and I decided to leave Spain.

Wander Years

We left Spain in September 1936. My plan was to go back to Meyerhof, for some time at any rate, if he could accept me. I contacted him from Paris and he very kindly consented to receive me in his laboratory. We arrived in Heidelberg in October. We left one troubled country for another. Germany was at the height of the Nazi furor and was no longer the country I had loved so much. Meyerhof was in a precarious position. Some of his family had already left the country but his laboratory was still highly productive. How we were left relatively unmolested by the German authorities, having come from Spain on a Spanish republican passport to work in the laboratory of a Jewish scientist and live with a Jewish family, appeared rather miraculous. However, our passports were taken away by the police soon after our arrival. About a month later I received a letter from the Franco Embassy in Berlin expressing surprise at my being in Germany and not in Spain serving my country. I replied that as a scientist I thought I served my country much better by studying in Germany, a country that owed its greatness in a large measure to the highly advanced state of its science and technology. I must have convinced them because I never heard from the Embassy again; instead our passports were returned shortly thereafter.

Scientifically there was a profound change in Meyerhof's laboratory. When I left it in 1930 it was basically a physiology laboratory; one could see muscles twitching everywhere. In 1936 it was a biochemistry laboratory. Glycolysis and fermentation in muscle or yeast extracts or partial reactions of these processes catalyzed by purified enzymes, were the main subjects of study. My old friends Nachmansohn and Lipmann were no longer there. Karl Lohman was still in Heidelberg where he had just demonstrated that cocarboxylase was thiamine pyrophosphate. There were two new assistants, Walter Kiessling and Paul Ohlmeyer. Walter Schulz was also there, and there was also a superb technical assistant, Walter Möhle, a mechanic (Fein Mechaniker) by training. Ohlmeyer and I did some work together and became very close friends. I finished work on glycolysis in heart, isolated pure NAD from skeletal muscle, and carried out some transphosphorylation studies in muscle extracts with Ohlmeyer. This period in Heidelberg did not last very long, for Meyerhof was about to leave the country. He left

for Paris in August 1937. We did not meet again until several years later in the United States. Before leaving, Meyerhof saw to it that I had some place to go and, through his friend A. V. Hill, arranged a fellowship for me to work for six months at the Marine Biological Laboratory (MBL) in Plymouth, England. The time in Plymouth, very happy otherwise, was one of much worrying for us. We had relatives on both sides of the fence in Spain and were fearful of the hardships and risks to which they might be exposed. Although my wife had no prior laboratory training, she came to work with me at the MBL and very ably helped me in studies on transphosphorylation reactions and NAD distribution in invertebrate muscle; so much so that we published a joint paper in *Nature*. We shall never forget the kindness of our Plymouth friends, in particular that of Dr. W. R. G. Atkins who did not rest until he found me a job so that I would not be left in the air when my Plymouth fellowship expired. He found me an excellent job indeed, with Professor R. A. Peters at Oxford. I could have wished for nothing better.

I was familiar with the work of Peters on the role of thiamine in pyruvate oxidation in brain and fascinated by it. Peters was looking for an organic chemist but, nevertheless, he invited me to Oxford for an interview and when he heard that I had been in Meyerhof's laboratory and was acquainted with Lohman's work on cocarboxylase he became interested and got me a fellowship from the Nuffield Foundation. The Oxford period was a very happy and productive one. A relatively small university town with a deep cultural tradition is ideal for intellectual accomplishment. Moreover, the British have a knack for accomplishing very much without apparently working too hard, a knack that I fortunately found to be contagious. Among the good friends we made at Oxford was Ernst Chain. I can still see him getting so excited that he literally jumped on his chair when he described to us the wonderful properties of penicillin which he was then purifying. Unfortunately, the Oxford period was cut short by the Second World War. The whole Biochemistry Department became involved in war work and I, an alien, had to be left out of it. I began to feel lonely and isolated and started thinking of going to America. In fact, I very much wanted to work in Cori's laboratory. Again my wife and I debated the question thoroughly and came to the conclusion that for the sake of my scientific career we should endeavor to go to America and Peters also advised us to go. I wrote to Cori and was elated to hear that he was willing to accept me in his laboratory and provide some support. So, one day in August 1940 Carmen and I sailed for the New World, not without sadness, but full of hope and expectations.

The New World

The laboratory of Carl and Gerty Cori at the Washington University School of Medicine in St. Louis was an exciting place. There, enzymes were the

really important thing and work on phosphorylase was in full swing. There were also exciting people around: Herman Kalckar, Earl Sutherland, and Sidney Colowick among others. I was very happy in St. Louis even though my work turned out to be somewhat frustrating. Cori proposed that I study the enzymatic mechanism of conversion of fructose to glucose in liver extracts which ought to have been a cinch. Fructose would be phosphorylated to fructose-6-phosphate by a hypothetical fructokinase, fructose-6-phosphate would be isomerized to glucose-6-phosphate by phosphohexoisomerase, and glucose-6-phosphate dephosphorylated by glucose-6-phosphatase to yield glucose. When fructose was incubated with liver homogenates in the presence of fluoride, a phosphate ester accumulated all right, but it proved to be fructose-1-phosphate rather than fructose-6-phosphate. The occurrence of fructose-1-phosphate in liver had previously been reported by Kosterlitz. All my efforts to show that fructose-1-phosphate would be converted to fructose-6-phosphate or isomerized to glucose-1-phosphate, either of which would then form glucose-6-phosphate, were in vain. To make matters worse, there was a rather large accumulation of inorganic pyrophosphate along with fructose-1-phosphate. I left the Cori laboratory a bit frustrated having explained nothing. The rather involved mechanism of conversion of fructose to glucose was explained some time later by Leuthardt and Hers and that of pyrophosphate formation by Arthur Kornberg. The Leuthardt-Hers mechanism involved cleavage of fructose-1-phosphate by liver aldolase to glyceraldehyde and dihydroxyacetone phosphate, the latter then isomerizing to an equilibrium mixture of dihydroxyacetone phosphate and glyceraldehyde phosphate. Aldolase then formed fructose-1,6-diphosphate and this was dephosphorylated to fructose-6-phosphate by fructose-bis-phosphatase. It is often hard to guess the ways of nature. Fructose-6-phosphate is formed in liver by a roundabout route and then in only half the yield that could be obtained by phosphorylation of fructose in the 6 position. Inorganic pyrophosphate was generated by reversal of the Kornberg NAD pyrophosphorolysis reaction, i.e. ATP + nicotinamide ribotide $\leftrightarrows$ NAD + PP_i.

In Oxford I had met Bob Goodhart, a nutritionist from New York University (NYU), who was a Rockefeller Foundation Fellow in Peters' laboratory, and we became very good friends. He stubbornly labored to bring me to NYU and succeeded in early 1942. I became a research associate in medicine. This was the beginning of a fruitful and happy association with NYU that lasted for many years. At NYU I had my first graduate student, Alan Mehler, and my first postdoctoral students, Santiago Grisolía and Arthur Kornberg. My association with the Department of Medicine lasted but a couple of years. Eventually the space I occupied at Bellevue Hospital was needed for other people. At that time Dr. Isidor Greenwald kindly let me have space in his own laboratory across the street in the

Department of Biochemistry of the NYU Medical School. A part of the school that included the biochemistry department was housed in the old building of Cornell Medical College where Graham Lusk and Stanley Benedict had once worked. Keith Cannan, an English biophysicist, was Chairman of the Department. I was appointed Assistant Professor of Biochemistry, the first staff position I had outside of Spain. Two years later the Chair of Pharmacology became vacant through the retirement of George Wallace, and Cannan had the idea that I, having had a medical training, might be fit for the job of Chairman of that department. I was not very eager to assume this responsibility. However, two considerations made me decide to accept the position were it offered to me. On the one hand I thought I would be better able to expand and deepen my research effort. On the other, the Department of Pharmacology, although housed in the old 26th Street building, had some beautiful new laboratories. They had been built by Jim Shannon who had been Wallace's successor for a short time. I moved to Pharmacology in the fall of 1946. I was the second biochemist to become Professor of Pharmacology in an American Medical School, or anywhere for that matter. Since Cori was the first, I was in good company. I spent nine exciting and productive years (1946–1954) in the Department of Pharmacology. They were also years of hard work. In 1953 the new NYU Medical School Building was about to be completed. At that time Keith Cannan resigned as Chairman of the Department of Biochemistry to become head of the Medical Division of the National Research Council in Washington and I was offered the Chairmanship. I stayed in Pharmacology until, in the summer of 1954, we moved into the new building. Although, contrary to my expectations, the teaching of pharmacology had not been a chore but very often a pleasure, I was far more at home in biochemistry and its teaching was more congenial and attractive. My philosophy was, and still is, that a Medical School as part of a University, a center of higher learning, must impart to its students a culture, in this case a culture in biology, besides training them to be good physicians. Accordingly, we taught biochemistry at a fairly high level, almost as a graduate course, and tried to have the students do in the laboratory, not research, but meaningful, significant experiments in newly developing areas of biochemistry, e.g. oxidative phosphorylation, polynucleotide synthesis, chromatography of proteins and amino acids. Each of five or six staff members lectured on a given topic for a few years and we all attended each other's lectures. This kept us in touch with basic biochemistry year after year and kept us on our toes as each of us was being judged by his or her peers. Biochemistry was a popular subject and our lectures were attended by many a staff member from other departments, a further reason to be on our toes. As I had done in Pharmacology, I kept Biochemistry as a rather small, closely knit department where we all knew each other well and appreciated one another. We

got together for coffee every afternoon and discussed our problems. This was immensely helpful and kept everyone, including graduate and postdoctoral students, well informed on and interested in the activities of the whole department. The scientific productivity and the spirit of the Biochemistry Department in a human as well as an academic sense were excellent. I firmly believe that a leading medical school, devoted to the ideals of basic medical research and training, is second to no other branch of the University or other research institution in carrying out these functions. This requires the understanding and support of an enlightened administration and faculty, in particular of the clinical departments. The NYU School of Medicine always had men among its faculty who, like Homer Smith, William Tillet, Colin McLeod, Donal Sheehan and more recently Saul Farber, to name but a few, devoted their lives to the furthering of these ideals.

In the summer of 1974, twenty years after I had taken charge of the Department of Biochemistry, I retired as Chairman. I was 69 years old. I disliked the idea of becoming Emeritus and I requested the University Administration to keep me simply as Professor of Biochemistry to which they graciously consented. It was my good fortune at this time to be able to join the Roche Institute of Molecular Biology. Sidney Udenfriend, the Director of the Roche Institute, was a graduate student in Biochemistry at NYU when I was an Assistant Professor in the Department. He likes to tell the story that he was considering becoming my graduate student but did not do so because of my moving to Pharmacology. At the Roche Institute I once more found friendship, warmth, and understanding and I am grateful to Sid Udenfriend and Herb Weissbach for having given me the chance to continue my work in a superb, stimulating scientific environment with more facilities than I ever had, and with as much if not more understanding. In coming here I joined a group of good old friends, including my former colleague at NYU, Bernie Horecker, and have made new and stimulating ones. Commuting between Manhattan and Nutley, New Jersey, is a small price to pay for all I have gained.

RESEARCH

Vitamin B_1 and Cocarboxylase

My work at Oxford, much to my liking, had to do with vitamin B_1 (thiamine) and cocarboxylase. Peters had found that the brains of thiamine-deficient pigeons had a decreased capacity to oxidize pyruvate in vitro which could be restored to normal levels by thiamine but not by cocarboxylase, its pyrophosphate ester. This suggested that although cocarboxylase was the coenzyme of pyruvate decarboxylation in yeast, thiamine, the non-phosphorylated form, rather than cocarboxylase was the cofactor of pyruvate oxidation in animal tissues. We decided to look at the problem in a

different way, namely to assay pigeon brain for thiamine and cocarboxylase and see which form of the vitamin was present. We assayed cocarboxylase through its stimulation of the decarboxylation of pyruvate by alkaline-washed yeast and very much increased the sensitivity of the method through the finding that added thiamine markedly augmented the effect of cocarboxylase. We also made use of this observation to determine free thiamine in tissue extracts. Once we had the method in shape the answer was clear-cut; pigeon brain contained cocarboxylase but no thiamine (3). We also found that the level of cocarboxylase, about two μg per gram of tissue, decreased to a fraction of a μg in the brain of pigeons in acute thiamine deficiency. This level returned to normal in a matter of minutes upon injecting the pigeons with thiamine coincident with the disappearance of all symptoms of thiamine deficiency. Consistent with these findings, liver and brain were found to convert thiamine to cocarboxylase in vitro; in fact, the effect of thiamine on pyruvate oxidation in the brain of thiamine-deficient pigeons was due to its conversion to cocarboxylase. The inactivity of cocarboxylase was due to the fact that, whereas the brain brei preparations used by Peters were permeable to thiamine, they were impermeable to cocarboxylase. With brain homogenates we showed that cocarboxylase was more active than thiamine. Dialysis of normal brain homogenates markedly decreased their capacity to oxidize pyruvate; this capacity was restored by the addition of small amounts of a dicarboxylic acid, such as succinic or fumaric acid, and adenine nucleotide, e.g. AMP or ATP (4). The effect of dicarboxylic acids on the respiration of muscle homogenates had been discovered by Szent Gyorgyi who thought that they acted as hydrogen carriers; but, as later found by Krebs, they act catalytically as intermediates of the citric acid cycle. The requirement of adenine nucleotides for pyruvate oxidation, on the other hand, was shown by us for the first time and suggested a tight coupling of oxidation with phosphorylation. Several years later Lehninger obtained direct evidence for such a coupling using mitochondrial preparations.

Oxidative Phosphorylation

The above experiments directed my attention to oxidative phosphorylation, a problem that I began to study in Oxford in the summer of 1938 and continued later in New York. In Oxford I found that the oxidation of pyruvate in brain homogenates, in the presence of catalytic amounts of AMP or ATP, was coupled with the phosphorylation of hexosemonophosphate or glucose. I showed that oxidation is actually coupled to phosphorylation of AMP to ATP followed by a transfer of phosphate from ATP to the sugar. The atomic ratio of phosphorus esterified to oxygen consumed (P/O ratio) in the first few minutes was about two. I concluded that it would be hard to understand how the primary dehydrogenation of the substrate,

i.e. the transfer of two hydrogens to the first hydrogen acceptor, probably NAD or NADP, could give rise to an uptake of more than one atom of phosphorus unless the further transfer of hydrogens to oxygen were also linked with phosphorylation. This had already been made likely by the work of Kalckar in Denmark and of Belitzer in the USSR. The detailed account of my work on phosphorylation in brain homogenates was written up after we left England and was published in the JBC (5). This, my second JBC paper, appeared in 1941, twelve years after the first.

When I came to NYU I took up the problem of oxidative phosphorylation again. It looked as if previous determinations of the P/O ratio had given values lower than the real ones because of leakage caused by the hydrolysis of ATP by ATPase. Using heart homogenates I compared the P/O ratio of triose phosphate oxidation by NAD, known from the work of Warburg to be equal to 1, with that of pyruvate oxidation by O_2. The quotient, observed P/O ratio of pyruvate oxidation to observed P/O ratio of triose phosphate oxidation, should give the true value of the former. I found it to be about 3 (6). Lehninger later confirmed this value by direct determination of the P/O ratio using mitochondria. At this time it was already clear that NAD was the primary hydrogen acceptor of the respiratory chain. It was also clear that a phosphate uptake higher than 1 must be caused by coupling of phosphorylation with the further transfer of hydrogens from NAD to oxygen. Using my heart homogenates I did the obvious experiment with chemically reduced NAD; it was rapidly oxidized but I got no traces of phosphorylation. However, Lehninger later obtained a positive result with mitochondria.

CO_2 Fixation and Citric Acid Cycle Enzymes

It seemed to me then that the mechanism of oxidative phosphorylation was not likely to be understood without much more knowledge of the enzymatic reactions that were coupled to phosphorylation. Krebs had shown that the citric acid cycle was the main pathway for the oxidation of foodstuffs in the cell. Through the work of Keilin, Warburg, and others, it was also known that the transfer of hydrogens and electrons from oxidizable substrates to molecular oxygen, involved pyridine nucleotides, flavoproteins, and cytochromes. However, we did not know much about the nature and mode of action of the enzymes concerned with the primary oxidation of citric acid cycle substrates. This led to my decision to study some of the enzymes of the citric acid cycle. I began with isocitric dehydrogenase. This enzyme was known to catalyze the NADP-dependent oxidative decarboxylation of isocitrate to α-ketoglutarate and CO_2. Isocitrate was formed from citrate, as Martius had shown, via *cis*-aconitate in a reaction catalyzed by aconitase. Martius thought that the isocitric dehydrogenase reaction probably proceeded in two steps, oxidation of isocitrate by NADP to oxalosuccinate, and

decarboxylation of oxalosuccinate to α-ketoglutarate and CO_2. To check on this idea I decided to prepare oxalosuccinic acid to study its interaction with preparations of isocitric dehydrogenase. Oxalosuccinate acid is very unstable and is readily decarboxylated. My first attempt to hydrolyze commercially available oxalosuccinic ethyl ester with aqueous hydrochloric acid was successful. Oxalosuccinic acid was reduced to isocitric acid by NADPH, in the presence of crude isocitric dehydrogenase, and the same enzyme preparations greatly accelerated the release of CO_2 from oxalosuccinate in the presence of manganese ions. Imagine my desperation when, having exhausted my first supply of oxalosuccinic acid, I tried to prepare some more and failed. I don't remember how many times I tried. I used every time less and less dilute hydrochloric acid to no avail until I finally used concentrated HCl. This worked like a charm much to my surprise. Not wishing to take any more chances I proceeded to prepare a large amount of oxalosuccinic acid which, as the barium salt, was quite stable. I made enough to do all the experiments I wanted to do and to give to other people; I suspect there is still some left in some refrigerator at NYU.

The demonstration by Wood and Werkman that CO_2 fixation is not the exclusive prerogative of green plants and other autotrophic organisms but can also occur in heterotrophic bacteria produced considerable excitement in the biochemical world. With use of isotopic carbon Wood and Werkman showed that propionic acid bacteria incorporated CO_2 into the carboxyl groups of dicarboxylic acids, the so-called Wood and Werkman reaction. The mechanism of the Wood and Werkman reaction (called jokingly by Racker the wouldn't work reaction) was unknown. It occurred to me that if the isocitric dehydrogenase reaction were reversible it would provide a mechanism for CO_2 fixation in animal tissues. Earl Evans, Birgit Vennesland, and their collaborators had in fact shown incorporation of isotopic CO_2 into dicarboxylic acids in pigeon liver. At that time only a few laboratories were equipped to use isotopes but I thought that maybe I could observe the reversibility of the oxidative decarboxylation of isocitric acid in the spectrophotometer; it should lead to oxidation of NADPH in the presence of the enzyme, α-ketoglutarate, and CO_2. For some reason I felt this was too good to be true and I procrastinated doing the experiment until, having told my good friend Ef Racker (Racker and I spent hours at that time talking biochemistry) that I was thinking of doing a crazy experiment, he encouraged me to do it; he did not think it was so crazy. I was seldom so thrilled in my life as I was when I saw the spectrophotometer needle move in the proper direction (indicating oxidation of NADPH) when I added a drop of CO_2-containing bicarbonate solution to a mixture that contained isocitric dehydrogenase, α-ketoglutarate, NADPH, and manganese ions (7). I got so excited that I ran out of the room yelling "come and watch

this" but nobody came; in my excitement I had forgotten that it was past 9 P.M.

I had purchased the Beckman spectrophotometer, used in these experiments, through a grant from the American Philosophical Society. The grant was for one year and the instrument had to be returned to the Philosophical Society at the end of the grant period. At the end of the year, in the midst of my CO_2 fixation experiments, I sent a progress report to the Philosophical Society along with a strong plea for permission to keep the instrument for one more year. I based this request on the importance of the work and the indispensability of the Beckman to pursue this approach, and they kindly consented. At the end of the second year, their response to a similar plea was to grant NYU ownership of the instrument upon payment of a nominal fee. This, my first and only Beckman spectrophotometer for a long time, was christened by Alan Grafflin "The Philosophical Beckman"; it died of old age.

I thought that what we called isocitric dehydrogenase was in reality a mixture of two enzymes, isocitric dehydrogenase and oxalosuccinic carboxylase, but I could never get any evidence for this idea with my system. At this time, having moved to the Pharmacology Department and having a graduate student (Alan Mehler) I decided to combine two known enzymes, malic dehydrogenase and oxaloacetic carboxylase, to see whether we could decarboxylate malate to pyruvate and CO_2 in the presence of NAD, and/or convert pyruvate and CO_2 to malate in the presence of NADH. This was a complete failure. However, one day Alan observed a rapid oxidation of malate on adding NADP, but not NAD to a pigeon liver extract. This observation marked the discovery of the malic enzyme. It catalyzed the reversible reaction, malate + $NADP^+$ $\leftrightarrows$ pyruvate + CO_2 + NADPH + H^+. The enzyme was studied in detail by Mehler, Kornberg, and others in our laboratory (8). Since the malic enzyme could catalyze the decarboxylation of oxaloacetate to pyruvate + CO_2, as well as its reduction to malate by NADPH, just as isocitric dehydrogenase could catalyze the decarboxylation of oxalosuccinate to α-ketoglutarate + CO_2 and its reduction to isocitrate, we concluded that a single enzyme with two active centers was probably involved in both cases, something we loosely referred to as a double-headed enzyme. The malic enzyme was later used by Wolf Vishniac to obtain a light-dependent reductive carboxylation of pyruvate to malate in the presence of spinach grana and NADP (9). This was the first demonstration of the photochemical reduction of pyridine nucleotides by chloroplast preparations.

In 1948 Joe Stern, who had been a graduate student of Hans Krebs in Sheffield, came to our laboratory as a postdoctoral student. I thought the time was ripe to tackle the most elusive enzyme of the citric acid cycle, the enzyme that condensed oxaloacetate with "active acetate" to form citrate,

the condensing enzyme as we called it. I had become extremely interested in the mechanism of this reaction and I couldn't help thinking about it at all times. However, I never came up with a bright idea. I believed that the condensing enzyme itself was soluble but the enzyme(s) that made "active acetate" from pyruvate, acetate, or acetyl phosphate, were insoluble, at least in animal tissues. However, they might be soluble in bacteria. There were indications that Lipmann's coenzyme A might be involved in the condensing reaction. By combining an *Escherichia coli* extract with an extract of pig heart we got good citrate synthesis from acetyl phosphate and oxaloacetate; it required catalytic amounts of CoA. Using this reaction as an assay, Joe Stern, Mort Schneider, and I crystallized the condensing enzyme, the first crystalline enzyme of the citric acid cycle, from pig heart (10). We soon realized that the *E. coli* extract provided transacetylase, an enzyme discovered by Earl Stadtman, that catalyzed the transfer of phosphate from acetyl phosphate to CoA to form acetyl CoA and phosphate. Thus, as Feodor Lynen had predicted, acetyl CoA was the active form of acetate. In joint experiments with Lynen we showed that the condensing enzyme catalyzed the reversible conversion of acetyl CoA and oxalacetate to CoA and citrate (11). We were naturally very interested in the early steps of pyruvate oxidation and the mechanism by which pyruvate and oxaloacetate react to form citrate during the operation of the citric acid cycle. At this time I. C. Gunsalus spent a few months in our laboratory as a Guggenheim Fellow and he and Seymour Korkes took a closer look at the early steps of pyruvate oxidation in *E. coli* extracts. They found that NAD was reduced in the presence of pyruvate and CoA, which indicated the occurrence of the reaction, pyruvate + CoA + NAD^+ = acetylCoA + CO_2 + NADH + H^+ (12). Fractionation of this system indicated that at least two enzyme fractions were required.

An interesting enzyme of the citric acid cycle, discovered by Seymour Kaufman, catalyzed the synthesis of ATP from ADP, P_i, and succinyl CoA. Succinyl CoA was deacylated to succinate and CoA (13). We referred to this enzyme as the phosphorylating enzyme or P enzyme for short; it was later named succinic thiokinase. Work of Joe Stern and Minor Coon led to the discovery of a new enzyme of fatty acid metabolism, CoA transferase (14). The P enzyme accounted for one substrate level phosphorylation coupled with the oxidative decarboxylation of α-ketoglutarate in the citric acid cycle.

1. α-Ketoglutarate + CoA + NAD^+ → Succinyl-CoA + CO_2 + NADH + H^+
2. Succinyl-CoA + ADP + P_i ⇆ Succinate + CoA + ATP

   Σ α-Ketoglutarate + NAD^+ + ADP + P_i → Succinate + CO_2 + NADH + H^+ + ATP

CoA transferase catalyzed the transfer of CoA from succinyl CoA to acetoacetate. This enzyme is present in heart and muscle and accounts for the activation of acetoacetate which is formed in the liver and largely oxidized in the peripheral tissues.

One line of work that went on for several years was concerned with the metabolism of propionate. My interest in this subject was aroused by reports that propionate oxidation involved CO_2 fixation and led to the formation of succinate. The work was begun by Martin Flavin. He used pig heart extracts and found that they converted propionate to a dicarboxylic acid. Martin was very exacting and meticulous and was not convinced that the product was succinate because it differed slightly from succinate in chromatographic behavior. He eventually identified the product as methylmalonate thereby triggering a series of interesting discoveries (reviewed in 15). The work of Martin Flavin and later Yoshito Kaziro, Enzo Leone, Peter Lengyel, Raj Mazumder, Joaquin Cannata, and others eventually showed that propionate is first converted to propionyl CoA and carboxylated to methylmalonyl CoA, in the presence of ATP, by the biotin-containing enzyme propionyl CoA carboxylase. Methylmalonyl CoA is then isomerized from the A to the B form and the latter undergoes mutation, catalyzed by methylmalonyl CoA mutase, to form succinyl CoA. Methylmalonyl-CoA mutase was shown to be a vitamin B_{12} enzyme. Propionyl CoA carboxylase was crystallized by Kaziro who demonstrated the stoichiometric participation of the enzyme in the reaction. Propionyl CoA carboxylase is itself carboxylated and then transfers the carboxyl group to propionyl CoA. It was Lynen who showed that carboxybiotinenzyme was formed thus elucidating the chemical mechanism of the reaction.

Polynucleotide Phosphorylase and the Genetic Code

In 1954 it seemed to me that the time might be ripe to come back to oxidative phosphorylation. I thought that one should look for a source of enzymes capable of converting ADP to ATP and that one might follow such a reaction by measuring the incorporation of radiophosphate into ATP. I also thought that one would have a chance of finding such enzymes in extracts of *Azobacter vinelandii,* which very actively oxidize carbohydrates and other compounds. Two postdoctoral students had arrived in the laboratory, Ernie Rose from Chicago and Marianne Grunberg-Manago from Paris. Another problem that interested me was the mechanism of phosphorylation of acetate to acetylphosphate in bacteria. I explained the two projects to the students and asked them to tell me their preference. Ernie chose acetylphosphate, Marianne selected oxidative phosphorylation.

The *Azobacter* extracts appeared to be very active in incorporating phosphate labeled with ^{32}P into ATP, and this activity was partially purified. In

the meantime crystalline ATP became commercially available. This meant that one could use a purer ATP than the amorphous kind that we were currently using. However, with crystalline ATP there was no reaction. This made us happy for we thought we were on the verge of discovering a new coenzyme. Chromatographic examination of the amorphous ATP showed, however, that it was contaminated with ADP and that ^{32}P was being incorporated into ADP, not ATP. We soon discovered that the *Azobacter* enzyme promoted the incorporation of ^{32}P not only into ADP, but also into other nucleoside diphosphates like IDP, UDP, CDP, and GDP. We also found that the enzyme catalyzed the release of phosphate from nucleoside diphosphates. At this point we seemed to have a rather uninteresting reaction, namely a hydrolysis of ADP and other diphosphates to AMP and other monophosphates: $ADP + H_2O \rightarrow AMP + P_i$. It was curious, however, that this reaction appeared to be reversible, for, after all, ^{32}P was incorporated into nucleoside diphosphates. However, reversibility of a hydrolytic reaction seemed very unlikely. Several weeks were spent trying unsuccessfully to characterize the AMP that would be formed from ADP. The eventual answer was that what was being formed from ADP was not AMP but a large polymer composed of AMP residues; an RNA-like polyadenylic acid or poly(A). Other nucleoside diphosphates gave rise to poly(I), poly(U), poly(C), or poly(G). The reaction catalyzed by the new enzyme was formulated thus:

$$n\ XDP \leftrightarrows (XMP)n + n\ P_i$$

where X is a nucleotide base (adenine, uracil, etc). The enzyme was named polynucleotide phosphorylase because in one direction (right to left) it cleaved polynucleotides by phosphorolysis rather than hydrolysis.

The first report on polynucleotide phosphorylase was published in the summer of 1955 as a Letter to the Editors of the Journal of the American Chemical Society (16) despite very adverse criticism by a referee. The work was presented at the spring 1955 meeting of the Federation of American Societies for Experimental Biology in San Francisco and at the International Congress of Biochemistry held in Brussels that summer. It aroused considerable interest. This was understandable for it was the first time that high molecular weight RNA-like polynucleotides had been synthesized outside the cell.

Bob Warner in our laboratory found that mixing solutions of poly(A) and poly(U) yielded a viscous solution of double-stranded poly(A)-poly(U). Its double-stranded helical structure was established by Alex Rich by X-ray diffraction analysis; the structure of this polymer was similar to that of DNA. Paul Doty later made extensive use of these polynucleotides in his fundamental work on annealing of complementary nucleic acid strands, the basis of the modern hybridization techniques.

With the collaboration of Bob Warner at NYU and Leon Heppel, Russell Hilmoe, and Maxine Singer at NIH it was established that, with a mixture of nucleoside diphosphates, e.g. ADP, UDP, GDP, and CDP, polynucleotide phosphorylase made RNA-like polymers but with a randomly-ordered base sequence (17). With Matthys Strahelin and Dewey Brummond we found that, whereas polynucleotide phosphorylase was present in many species of bacteria, it was by and large absent from mammalian cells. It was thus clear that, despite early hopes to the contrary, polynucleotide phosphorylase was not involved in RNA synthesis and must function somehow in RNA degradation. However, to this day, the function of the enzyme is not really known. The importance of polynucleotide phosphorylase lies in the many studies on fundamental properties of the nucleic acids made possible by the ready availability of the synthetic polynucleotides used as models and, above all, in the use of the enzyme by Nirenberg and co-workers and by ourselves in the early deciphering of the genetic code. Polynucleotide phosphorylase may be considered to have been the Rosetta Stone of the genetic code.

By 1960 the concept of messenger RNA had been formulated. There was evidence that after infection of *E. coli* with T_2 phage the newly formed mRNA was utilized by the bacterial ribosomes to make phage proteins. These findings indicated that mRNA is the template in protein synthesis. This suggested the use of synthetic polyribonucleotides as messengers in cell-free systems of protein synthesis to study the genetic code. Peter Lengyel and Joe Speyer firmly believed that this approach would open the way for deciphering the code. In early 1961 they started to work with cell-free protein synthesis systems. The expectation was that systems depending on the addition of mRNA for incorporation of amino acids into protein might respond to the addition of synthetic polynucleotides and would incorporate different amino acids depending upon their base composition. When we were beginning this work Nirenberg reported that a system from *E. coli* translated poly(U) into polyphenylalanine. We confirmed Nirenberg's observations and found, in addition, that whereas poly(U) promoted the incorporation of only phenylalanine into trichloroacetic acid–insoluble material, copolynucleotides like poly(UC) promoted the incorporation of phenylalanine, serine, and leucine, and poly(UA) that of phenylalanine and tyrosine. Lengyel, Speyer, and I were watching the counter and were thrilled. This result, obtained for the first time anywhere, showed that incubation of *E. coli* extracts with copolynucleotides containing C or A besides U residues promoted the synthesis of polypeptides containing serine, leucine, and tyrosine, along with phenylalanine. I remember this as one of the most exciting moments of my life. At a meeting of the Microbiology section of the New York Academy of Medicine, in September 1961, Nirenberg presented his

results on the poly(U)-directed synthesis of polyphenylalanine. For some reason copolynucleotides supplied to him by Leon Heppel and Maxine Singer had not been active. At that meeting I announced our positive results with copolynucleotides, an announcement that aroused considerable excitement. The first paper of a series on "Synthetic Polynucleotides and the Amino Acid Code" appeared in the December 1961 issue of PNAS (18). In our conclusions we stated "these and other results reported in this paper would appear to open up an experimental approach to the study of the coding problem in protein biosynthesis."

In the following months, we ran a close race with Nirenberg's group on the effect of an increasing variety of synthetic polynucleotides on amino acid incorporation. The results were published by both laboratories in PNAS. They agreed beautifully. At first, poly(A) had been inactive in our hands. A bright medical student, Bob Gardner, working in our laboratory in the summer of 1962, found that poly(A) directed the synthesis of polylysine; the product, which was rather soluble in TCA, could be precipitated by the combined addition of TCA and phosphotungstic acid. This gave us the possibility of determining the coding properties of a variety of copolynucleotides that were rich in A. It did not take very long for the workers at NIH and NYU to have the base composition of the triplets coding for all 20 amino acids and to establish that the genetic code is degenerate or redundant, i.e. that in many cases several triplets code for the same amino acid. The use of synthetic polynucleotides in deciphering the genetic code was the subject of Peter Lengyel's PhD dissertation (19).

The last chapter of the genetic code story was written by Marshall Nirenberg and Gobind Khorana. Nirenberg discovered that trinucleotides of specified base sequence promoted the ribosomal binding of specific aminoacyl-tRNAs. For example, UUU promoted the binding of phenylalanyltRNA. GUU promoted the binding of valyl tRNA, and UGU that of cysteinyl tRNA. Using this method Nirenberg and his collaborators determined the base sequence of most codons. This was confirmed by Khorana. In addition, Khorana synthesized polyribonucleotides of specified base sequence that, when used as messengers in cell-free translation systems, directed the synthesis of polypeptides with a unique amino acid sequence. The results agreed with the binding results. Khorana's procedure was based on the chemical synthesis of oligodeoxyribonucleotides which, after amplification and transcription with DNA polymerase and RNA polymerase, provided appropriate messengers. UAA, UAG, and UGA coded for no amino acids. Ingenious genetic experiments of Sydney Brenner at Cambridge and Al Garen at Yale suggested that these triplets are chain termination signals. K. A. Marcker at Cambridge discovered that AUG is a chain initiation codon.

Protein Synthesis

Polynucleotide phosphorylase could be used in various ways for the preparation of short polynucleotide messengers having specified codons at the 3'- or the 5'-end of the chain. Wendell Stanley, Jr., prepared a number of them. For example, poly AAA ... AAAAAC, with a 3'-terminal (asparagine) codon, was prepared by digesting random poly(AC) (A to C ratio, 25 to 1) with pancreatic ribonuclease. A_nC polymers of various chain lengths were isolated by chromatography on DEAE-cellulose in 8 M urea. These polymers directed, in *E. coli* cell-free translation systems, the synthesis of lysine oligopeptides with one C-terminal asparagine. Other synthetic messengers, e.g. AAAAACAAA .... AAA and AAAACAAAA.... AAA, were synthesized with polynucleotide phosphorylase that had been sufficiently purified to require oligonucleotide primers (e.g. AAAAAC and AAAACA) for activity. These messengers directed the synthesis of lysine oligopeptides (e.g. Asn-Lys.... Lys, Thr-Lys.... Lys) having N-terminal asparagine or threonine (20). These results indicated that mRNA is read in the direction from the 5'- to the 3'-end. i.e. from left to right. Polynucleotides having the initiation codon AUG at the 5'-terminus, followed by A's or U's, directed the synthesis of lysine or phenylalanine peptides with N-terminal methionine (e.g. Met-Lys-Lys.... Lys or Met-Phe-Phe.... Phe). Codons other than AUG were not translated at the 5'-terminus, e.g. AACAAA.... AAA yielded polylysine and ACAUUU.... UUU polyphenylalanine. Interestingly, such polynucleotides required no initiation factors for translation whereas polynucleotides beginning with 5'-AUG did (see below). Other polynucleotides were used to show that UAA is a chain termination codon. Thus, whereas AUGUUUAAAAAA.... AAA was translated to yield Met-Phe-Lys-Lys....Lys oligopeptides, AUGUUUUAAAAA...AAA yielded, upon translation, the dipeptide Met-Phe (21).

In 1964 Margarita Salas and Wendy Stanley found that natural messengers, such as MS2 or Qβ phage RNA, were translated by crude ribosomes but not by ribosomes that had been chromatographed on DEAE-cellulose or washed with 0.5 M salt. However, the purified ribosomes readily translated poly(A) or poly(U). We suspected that purification of the ribosomes removed factors required for polypeptide chain initiation. However, we were not sure of this until we found that synthetic polynucleotides beginning with AUG at the 5'-terminus behaved like natural mRNA. Only then did we report the discovery of the chain initiation factors (22). At this time their existence had also been made likely by other workers. We isolated two proteins, F1 and F2, from salt washes of *E. coli* ribosomes that restored translation of natural mRNAs and 5'-AUG-containing polynucleotides by purified ribosomes (22). They were found to be required for the AUG-

dependent ribosomal binding of fMettRNA$_f$ but not for the binding of aminoacyl-tRNAs directed by codons other than AUG (23). Later we found that one other initiation factor, F3, was required for mRNA translation by ribosomes that had been washed with 1.0 M salt (24). Formation of an initiation complex appeared to involve two steps, the F3-dependent binding of mRNA to the 30S ribosome, and the F2-dependent binding of fMet-tRNA$_f$ to the mRNA-30S complex. With homogeneous F3 labeled with ^{35}S, Steve Sabol, an MD-PhD student, showed that F3 binds to the 30S ribosome and is released when the 50S ribosome joins the 30S complex to form a 70S initiation complex. Sylvia Lee-Huang isolated two species of F3 exhibiting specificity for different groups of mRNAs.

The occurrence of initiation factors in eukaryotes was first reported by Richard Sweet in 1968. Their systematic study was undertaken by French Anderson and his collaborators at NIH. M1, a protein isolated from salt washes of rabbit reticulocyte ribosomes, promoted, like bacterial F2, the AUG-dependent binding of initiator Met-tRNA to the small (40S) ribosomal subunit. In the summer of 1970 during a visit to Stockholm on IUB business, Torsten Hultin introduced me to *Artemia salina,* the brine shrimp. I returned home with the feeling that *A. salina* embryos would be an excellent material for the isolation and study of eukaryotic initiation factors. Thus, when Mike Zasloff came to work with me as an MD-PhD student, I proposed to him that we study initiation in *Artemia.* A factor isolated from the postribosomal supernatant appeared to be the counterpart of prokaryotic F2. It promoted the binding of Met-tRNA to eukaryotic 40S ribosomal subunits, whether from *Artemia* or rat liver. However, the reaction differed from that catalyzed by F2 in its lack of a GTP requirement (25). Andersons M1 had the same properties. This investigation, though not without interest, was disappointing. M1 proved to have no function in chain initiation. This was shown by Cesar Nombela with use of antibody against the Zasloff factor. In the meantime, the real counterpart of F2 was isolated in several laboratories (Gupta, Levin, Staehelin, Stanley). Different names were given to it and this created some confusion. This factor is now named eIF-2 (for eukaryotic initiation factor 2). eIF-2 consists of three polypeptide chains, with a combined molecular weight of about 150,000, and requires GTP for activity. It functions by forming a ternary complex with GTP and eukaryotic (but not prokaryotic) Met-tRNA initiator that, in the presence of a 40S ribosomal subunit, gives rise to the 40S initiation complex. The function of M1 (if any), like that of polynucleotide phosphorylase, remains a matter for speculation. I seem to have a knack for isolating proteins of unknown function.

Whereas eIF-2 has a multi subunit structure, F2 (its prokaryotic counterpart) consists of a single polypeptide chain of M_r around 80,000. The two

factors, however, do the same thing; they recognize and promote the ribosomal binding of initiator Met-tRNA. Similarly, whereas the prokaryotic initiation factor F3 consists of a single polypeptide chain of M_r about 23,000, eukaryotic eIF-3 consists of ten or more subunits with a combined M_r of over half a million. The reason for the greater structural complexity of the eukaryotic initiation factors is unknown. It has been speculated that it may be related to the need for regulation of translation in eukaryotes. It has been known for some time that globin synthesis in reticulocytes is controlled by the level of heme, the prosthetic group of hemoglobin. Protein synthesis in reticulocyte lysates is but briefly maintained in the absence of added hemin. Hemin is believed to prevent the activation of an inhibitor of polypeptide chain initiation (hemin-controlled inhibitor or HCI). Several laboratories (Hunt, London, Hardesty) have recently identified HCI as a cAMP-independent protein kinase that phosphorylates the small (38 K) subunit of eIF-2, which results in inhibition of initiation. The mechanism of this inhibition was not clear, for the capacity of purified eIF-2 to form initiation complexes was not impaired by phosphorylation of its 38K subunit. However, we observed that phosphorylation of partially purified eIF-2 led to inhibition of ternary complex formation. Looking for a factor required to inactivate phosphorylated eIF-2 Cesar de Haro came up with a protein that greatly enhanced the activity of intact eIF-2 but had virtually no effect on the activity of the phosphorylated factor. We called the new protein ESP for eIF-2 stimulating protein. Now eIF-2 is present in reticulocyte lysates at very low concentrations (about 15 nM) and, at these low levels it is essentially inactive in the absence of ESP. Hence our results are consistent with the notion that HCI inhibits initiation by blocking the interaction of ESP with eIF-2 (26).

The mechanism of activation of HCI and the mode of action of hemin are still poorly understood. Our results indicate that HCI is activated by phosphorylation catalyzed by a cAMP-dependent protein kinase, which suggests that cAMP may be involved in control of protein synthesis. We further found that hemin blocks cAMP activation of protein kinase by binding to the regulatory subunit of the enzyme. This blocks cAMP binding in an allosteric fashion (27).

SOME RECOLLECTIONS FROM NYU

In 1946 I invited Sarah Ratner to join the Pharmacology staff at NYU and she greatly enriched the department in both the scientific and human aspects. There she carried out much of her beautiful work on the enzymatic steps of the Krebs urea cycle. Later, when I moved to Biochemistry,

she joined Racker's department at the Public Health Research Institute of the City of New York. She has recently given an account of her work (28).

Otto Loewi had long been a research professor in the Department of Pharmacology when I moved in. We all profited from his culture, his experience, his wisdom, and greatly enjoyed his extraordinary charm. Everyone knew the details of how, having had the idea in the middle of the night, he discovered that stimulation of the vagus nerve to a perfused frog's heart released a chemical substance, "der Vagusstoff," which slowed down the heart of another frog. He stressed the importance of intelligence, ingenuity, and curiosity over physical facilities in the laboratory and often quoted the German proverb "es kommt nicht an Kaefig an ob der Vogel singen kann."

An important discovery, that of "active" methionine, was made by Giulio Cantoni during the tenure of an American Cancer Society fellowship in the Department of Pharmacology. Cantoni showed that in enzyme preparations from rat and pig liver ATP converted methionine to an active form which reacted with nicotineamide to form N-methylnicotineamide. Some years later he crowned this discovery by characterizing "active" methionine as S-adenosylmethionine, a beautiful piece of work.

The elucidation of the biosynthetic pathway of diaminopimelic acid in *E. coli* by Charles Gilvarg was another important contribution. Gilvarg had been a postdoctoral student in the Department of Pharmacology and had moved with me to Biochemistry as an independent staff member. It always amused me that Gilvarg, a man of unusual intelligence and superb training, was scared to work on competitive problems. He looked very carefully for a problem in which he would have no competition. His success in doing so amazed me. With an elegant combination of genetic and biochemical techniques he succeeded in identifying the enzymatic steps of diaminopimelic acid synthesis before he ran into any serious competition.

I think that if I were to start life all over again I would still become a biochemist, but I would begin with chemistry rather than medicine. I have always been handicapped by the lack of a formal training in chemistry. I owe much to two of my colleagues in the Department of Biochemistry, Bob Warner and Bob Chambers, for helping me fill this gap. Bill Robinson's good memory and factual knowledge of biochemistry were also often helpful.

Warner did most of the physical studies on proteins and nucleic acids. Through the use of physical methods he made the important discovery that mixing solutions of poly(A) and poly(U) yielded an aggregate of higher molecular weight. It migrated electrophoretically as a single component with a mobility intermediate between those of its constituent polynucleo-

tides. This, together with the observed hypochromic effect, suggested formation of a double-stranded poly(A)·poly(U) complex (29). Among other important contributions Warner observed the presence of concatenated DNA rings in bacteria that he thought might be intermediates in genetic recombination. Bob Warner enriched the Department in many ways. He trained, over the years, a number of outstanding graduate students.

Bob Chambers had been trained in organic chemistry. He was a postdoctoral student with Khorana and acquired an excellent background in nucleic acid chemistry. He made important contributions to tRNA biochemistry by trying to define the structural features that are recognized by aminoacyl tRNA ligases.

Some very nice work on the mechanism of replication of phage RNA was done by Charles Weissmann. He has given elsewhere an account of his life at NYU (30). He came as a postdoctoral fellow from Switzerland and remained on the staff of the Biochemistry Department for several years. Not having Gilvarg's reluctance to face competition he came into conflict with Sol Spiegelman and their relations were strained for a while. They are now the best of friends.

Two important additions to the Department of Biochemistry were those of Albrecht Kleinschmidt and Dan Lane. Kleinschmidt, a superb biophysicist and electron microscopist, had been a pioneer of the visualization of DNA in the electron microscope through his development with Rudolph Zahn and others of the protein monolayer mounting technique. Their picture of the DNA released by osmotic shock from the head of a T2 phage particle is one of the most dramatic in molecular biology. It probably shares with Lowry's protein assay method (the most frequently quoted biochemical paper) the honor of being the most frequently reproduced biological illustration. Weissman used to call Kleinschmidt, half seriously, half jokingly, der behrümte (the famous) Kleinschmidt. As for Dan Lane, I was impressed by his work from the time our two laboratories were studying propionyl-CoA carboxylation. I was fortunate in persuading him to join our department. He made a beautiful study of acetyl-CoA carboxylase, the rate-limiting enzyme in fatty acid synthesis, showing that its activation by citrate involves a polymerization of the enzyme with formation of very large, fibrillar aggregates.

Wlodzimierz Szer, a biophysicist from Poland, was the last addition to the Biochemistry staff before my retirement. A former student of David Shugar's, he was interested in the physical properties of macromolecules underlying their interactions. He became interested in protein synthesis and made important observations on the nature and function of some ribosomal proteins. Among other things he found that S1, the largest protein of the *E. coli* 30S ribosome, plays an essential part in the binding of mRNA to the ribosome.

ET ALIA

I have lived in New York City half of my life and, despite the difficulties inherent to life in a big metropolis, I don't regret it. It would now be hard for my wife and me to adapt to life elsewhere. No other city can offer so much in every facet of cultural and intellectual life. In earlier years, when we had more energy, we rarely missed an important art exhibit. We often visited not only the museums, but many of the art galleries in the city. Moreover, we seldom missed an exciting chamber music recital, theater show, or choral or symphonic concert. More recently, opera and ballet provide us with much enjoyment and relaxation.

As for science, New York offers an impressive variety of seminars and lectures, such as the Harvey lectures. In these times, when the scientific literature has grown to such an extent that it is impossible to keep abreast of progress even in one's own field, seminars, lectures, and other meetings are essential to keep informed.

The Enzyme Club

There is an institution in New York City that has no counterpart anywhere, the Enzyme Club. Its origins date back to 1940 or 1941 when a few people at the Rockefeller Institute got together at regular intervals to read and comment on David Green's book on enzymes. In a few years the Enzyme Club grew to a membership of 20 or 30. The group meets monthly during the academic year to hear a talk by an invited speaker on recent, exciting topics in enzymology or other areas in biology. For several years meetings were held at the Columbia Faculty Club, at 114th Street and Morningside Drive. Later they moved to the Rockefeller Institute. Meetings start with cocktails and dinner. The speaker then presents his topic using a blackboard (no slides allowed) in a very informal, relaxed manner. There is no limitation of time and the speaker may be interrupted many times in the course of his presentation. The Enzyme Club is managed by a secretary who serves for several years and he or she, alone or with the help of other members, selects the speakers for the year. The members can bring invited guests. While the secretaries serve for periods rarely exceeding three years, the financial aspects of the operation of the club have been under the competent management of Phil Siekevitz for the last fifteen years or more. The audience has changed and increased over the years. Among the very few oldtimers that attend the meetings regularly nowadays are Fritz Lipmann, Sarah Ratner, and myself. I have given a number of talks at the Enzyme Club; the list of topics corresponds more or less to the highlights of research by myself and my collaborators. They are as follows: April 1943, Phosphate Bond Energy in Oxydation of Pyruvic Acid; October 1945, Isocitric Dehy-

drogenase and CO_2 Fixation; April 1951, Enzymatic Synthesis of Citric Acid and Pyruvate Oxidation; December 1961, Synthetic Polynucleotides and the Amino Acid Code; January 1967, Translation of the Genetic Message; May 1971, Initiation of Protein Synthesis; October 1973, Conformational Control of Interaction of Eukaryotic Elongation Factors and Ribosomes; April 1978, Regulation of Protein Synthesis. A talk on polynucleotide phosphorylase given by Marianne Grunberg-Manago in 1956, should be added to this list.

The International Union of Biochemistry

At the first International Congress of Biochemistry, held in Cambridge in 1949, the participants present at the closing session voted for the creation of an International Union of Biochemistry (IUB) and, for this purpose, elected an International Committee under the chairmanship of Sir Charles Harington. This committee organized the second International Congress of Biochemistry which was held in Paris in 1952. Two international unions were then in existence or about to be established: Pure and Applied Chemistry (IUPAC) and Physiological Sciences (IUPC). Both of these unions wanted the biochemists to join them. However, the biochemists were not inclined to lose their identity. At the Paris Congress the International Committee met with delegates of biochemical societies from various countries, and on March 1, 1953, the International Union of Biochemistry, was formally established with an "Interim Council" chaired by Marcel Florkin of Belgium. In January 1955 delegates from fifteen countries met at the Ciba Foundation House in London. At this meeting the statutes of the new union were approved and the first Council and General Assembly were elected (31). This meeting was attended by Sir Rudolph Peters and Robert Thompson (UK), Jean Roche and Jean Courtois (France), Marcel Florkin (Belgium), Kurt Felix and Karl Lohmann (Germany), Vladimir Engelhardt and Alexander Oparin (USSR), Fujio Egami (Japan), A. H. Ennor (Australia), Otto Hoffmann-Ostenhof (Austria), Kaj Linderstrom-Lang (Denmark), R. Nicolaysen (Norway), E. Hammersten (Sweden), H. G. K. Westenbrink (The Netherlands), A. Virtanen (Finland), Alessandro Rossi-Fanelli (Italy), and Elmer Stotz and myself (USA). IUB's first Executive Committee consisted of Marcel Florkin (President), Robert Thompson (Secretary General), and Elmer Stotz (Treasurer).

The adhering bodies to IUB are biochemical societies, national academies, or national committees for biochemistry, elected, through the corresponding biochemical societies, by the biochemists of a given country or geographic region, with or without participation of academies, national research councils, or similar organizations. Like other international unions

IUB is governed by an Executive Committee, a Council, and a General Assembly. Whereas the Executive Committee (composed of the President, the President-Elect, the Immediate Past President, the Secretary General, and the Treasurer) meets more frequently, the Council and the General Assembly meet at international congresses of biochemistry every three years.

Originally the President served for six years. Florkin served from 1955 to 1961. He was to be succeeded by Kaj Linderstrøm-Lang of Denmark. Unfortunately Linderstrøm-Lang died before taking office and I was elected to be the second President of IUB at the International Congress of Biochemistry in Moscow in 1961. I served until 1967, through the New York (1964) and Tokyo (1967) congresses, when I was succeeded by Hugo Theorell of Sweden.

When I took office the membership of IUB included all of the Western European countries, the USSR, USA, Canada, Japan, Australia, and the People's Republic of China. At my suggestion Latin America was invited to join as one member; this was done with the Latin American Association of Physiological Sciences as the adhering body. In later years IUB membership became open to individual Latin American countries.

At each international congress IUB considers invitations from various countries to organize and host the next congress and selects one by majority vote of the General Assembly. One other function of IUB is to organize and sponsor international symposia guided by the recommendations of a Symposium Committee. The IUB Commission on Nomenclature and the Commission of Editors of Biochemical Journals meet regularly to make recommendations on nomenclature of biochemical compounds or set up guidelines and editorial policies. The work of the Nomenclature Commission is well known to biochemists the world over. Who has not struggled with the recommended names for Warburg's pyridine nucleotides? For a long time I had a mental block for NAD and NADP. If one considers the fast growth of biochemistry, and the confusion often produced by the existence of different names for the same thing, the arduous task of the Enzyme Commission is a meritorious one.

During my term as President I strove hard to keep politics out of IUB and to maintain high scientific ideals and standards. I always disliked the intromission of politics in science. I recall in this connection that, when IUB received an application for membership from the biochemists of the Republic of China (Taiwan), the People's Republic of China threatened to resign if Taiwan was admitted. There were insinuations from the State Department that Taiwan be admitted that were, to say the last, not very proper. How different things look today! In any case Taiwan was admitted by a majority vote of the General Assembly and Red China walked out. Shortly

thereafter I received a strongly worded letter from the delegate of the People's Republic to the IUB Council accusing me of political maneuvering. Events have now taken a turn for the better. The People's Republic of China has a new biochemical society and, in negotiations with the IUB Executive Committee, representatives of the Chinese Biochemical Society showed interest in reapplying for membership in IUB. An agreement was reached calling for the coexistence in IUB of two adhering bodies from China: the Chinese Biochemical Society and the Biochemical Society of Taipei, the latter to replace (in name) the present adhering body, the Academia Sinica (Taiwan). During the last International Congress of Biochemistry in Toronto (July 1979) an account of these developments was given by a local newspaper under the headline "China to join scientists' group which includes Taiwan." This points to the role that science can play in the realm of human relations.

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