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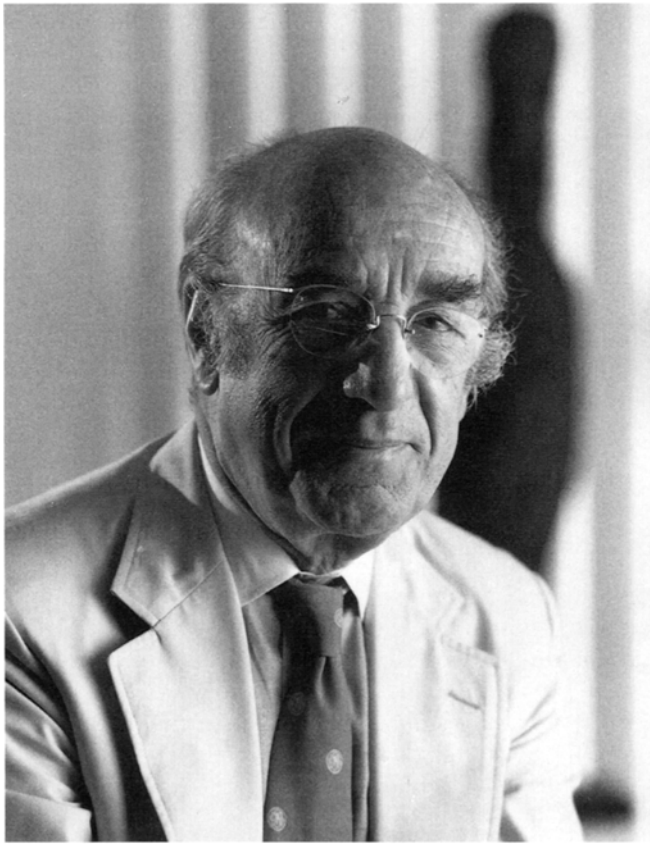

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January 11, 1924

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Lycée Carnot, Dijon, B.A. (1941)
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National Academy of Sciences, U.S.A. (1974)
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Roger Guillemin and his collaborators clarified the neuroendocrine control of the anterior pituitary gland by identifying a series of novel peptide molecules of hypothalamic origin. The same molecules were later found to have other functions in many parts of the central and peripheral nervous system, in the gastrointestinal tract, and more recently in the immune system.

The History of Neuroscience in Autobiography, Volume 2

Roger Guillemin

Childhood and Youth

I was born in Dijon, France, on January 11, 1924. My brother was born on May 4, 1927. That is the earliest of my long-term memories, as I remember vividly the beautiful bushes of Persian lilac in full bloom in the garden that surrounded the house where we were both born, that morning, when riding back home on my small red tricycle from the nearby house of my paternal grandparents, I was told that I had acquired a brother. My parents, our parents, were simple people with only a modicum of education. My mother had a junior high school education. Her parents were small farmers in the northern part of Burgundy, and my grandfather on that side had been, at times, a wine merchant. My father had completed a trade school education as a metal tool maker. He had read the French classics on his own and owned a small library, which became my first contact with books. He worked as a lathe operator in the nearby industrial plant that my paternal grandfather owned and operated and in which they were then manufacturing several types of riveting machines for which my grandfather had obtained some early patents. Later, my father changed jobs but always remained in this field of fine toolmaking, a trade that my brother later acquired formally. My father had a good voice as a lower baritone and sang in a men's choir that became famous as part of a traditional guild society created in the 1930s during the recession years and which aimed at promoting the sale of the wines of Burgundy throughout the world. The "Chevaliers du Tastevin" is still a flourishing association. He also wrote poetry, some dealing with the wine songs of the guild. As an expression of a different part of his personality he was a referee of boxing matches; as a younger man he had been involved, like his father, in French boxing, also known as *savate,* or foot boxing. I never was interested in that sort of thing. Dijon had been the capital of the Duchy of Burgundy when the Dukes of Burgundy were more powerful than the fledgling King of France, owned all of the Flanders, and maintained a rich court life. The superb collections of Flemish and early Burgundy masters at the Musée des Beaux Arts go back to those days. Those paintings and the sculptures of Claus Sluter marked my younger years.

As a child, I always was interested in things dealing with science; for instance, botany—I knew hundreds of wild plants, which I collected in a

large herbarium, quite a few wild mushrooms, which I collected and ate, of course; biology—the usual dissection of frogs *et alia*; electronics—radio crystal sets, transmitters (early one vacuum tube), ham radio, blowing of fuses not only in the house but even at the nearby electric pole, the resetting of which by the power company took care of all my piggy bank, etc. I was educated in the public schools in Dijon all the way through the two baccalaureates: in letters, the solid classic curriculum, including French literature, Latin for 5 years, Greek for 4 years, German for 5 years (a foreign language I learned to speak fluently), and in elementary mathematics (algebra, analytical geometry, elementary calculus). By that time, I was 18, and it was 1942. France had incredibly lost the war to Hitler's panzer divisions in May 1940, and Dijon was in that part of France which the German army occupied since that date. But I had heard the call (*l'appel*) that General de Gaulle had broadcast from London on June 18, 1940. It was by listening to the programs in French from the British Broadcasting Corporation, though strictly forbidden by the Germans, that we knew how the war progressed. Those years of youth were awfully bleak and sad. Being fluent in German helped me out of a couple of brushes with the occupation army having to do with involvement in the Résistance, the underground movement, and when I received my marching orders from the local Kommandantur to proceed to some munitions plant in Bavaria as part of the forced civilian labor, I tore them apart and disappeared into the underground. I somehow managed to reach the Jura mountains near the town of Besançon, where, with some local friends, until the liberation in the fall of 1944 by the 5th American Army coming from the south of France, I ran a passage point to nearby Switzerland for all sorts of people fleeing the German army, the Gestapo, and the French milice of Vichy. Our cover was a camp of about 100 children moved by the Red Cross from the industrial suburbs of Paris, which were often bombed by the Allies. There were some tense encounters with exploring German patrols. The day the American tanks came up, there was a short skirmish with the rear guard of the German garrison; I was wounded by a piece of shell that scraped my skull, but far worse, one of the children of the camp was killed. I was back on my feet within a few weeks, though for years, small boils would erupt from the scar left by the shrapnel on my skull and scalp and small pieces of steel would come out. I still have the scar.

Medical School and Early Training

After the baccalaureates, I had hesitated between a career as an engineer, as I loved practical things, doing things with my hands, and a career in medicine. I entered medical school in Dijon in 1943. Late in 1944, I returned from the Jura mountains to Dijon, to continue my second year at the local medical school. I received the M.D. degree from the Faculté de Médecine of

Lyon in 1949. The two schools were then administratively connected, with the larger school of Lyon granting the degrees. All my medical studies and training were totally clinically oriented, with 3 years of what we could call rotating internship. There was no laboratory facility of any sort in Dijon, except for gross anatomy—in which I was *prosecteur,* a teaching assistant, for 2 years. During these 5 years of medical studies, I had become interested in endocrinology, probably because two of my best teachers of clinical medicine, P. Etienne-Martin and Jacques Charpy, were themselves interested in what were in those days the early concepts of endocrinology and the beginning logical therapy it appeared to offer. I always hoped that somehow I could one day work in a laboratory. In France, one terminated medical studies after 5 years of curriculum; one could then practice medicine—which I did for some time. To obtain the degree of Doctor in Medicine, one had to write and defend a dissertation, a thesis, which was usually *pro forma.* I decided, however, to write a dissertation for the M.D. degree that I would enjoy and, I hoped, on some work I could perform in a laboratory.

While I had always been interested in endocrinology as a medical student, my interest in neuroendocrinology undoubtedly started later with my collegial and friendly contacts with Claude Fortier when in 1948 I joined the group of young people attracted to the just-created Institute of Experimental Medicine and Surgery at the Université de Montréal.[1] Its young director, Hans Selye, then 43, was at the peak of his attractive powers over young(er) minds intrigued by his extraordinary experimental abilities, the novelty of his observations, and the far-reaching implications he derived from them. At the end of World War II, medical literature from the United States started to trickle into Europe. One day, in the small village where I was practicing medicine, I heard that Selye would be lecturing in Paris on "stress" and the "diseases of adaptation." I decided to go to hear him. Selye lectured, in French, at la Pitié (a charity and teaching hospital built in the 17th century and where the cardiologist Paul Lian had for a number of years organized an annual teaching event with distinguished invited lecturers). The magnetism of the man was extraordinary. For me, just out of 5 years of medical school, 2 years of which had been in Nazi-occupied France, with teaching entirely directed toward medical care and no laboratory opportunities whatsoever, the lectures of Selye were from a different world. I went to talk to him after one of his lectures. After some sort of an interview in the office of Robert Courrier in the old building of Collége de France, Selye assured me of a modest fellowship of $120 per month from his own research funds to come to Montreal for one year, where I would complete a dissertation for the M.D.

[1] The following 30-odd pages, with minor changes, are from a chapter entitled *Pioneering in Neuroendocrinology 1952–1969,* published in *Pioneers in Neuroendocrinology,* Vol. 2, Plenum Press, 1978; with permission from the publisher.

A couple of months at that institute, in the midst of other young people from Canada, England, Holland, the United States, and Brazil, led me to conclude that I had grossly miscalculated my abilities. There was no way I could ever reach their ease at handling the knowledge, techniques, and concepts which I had never heard of in those dark years of my "medical studies" in Dijon. I was thus inclined to regard the year as a rather unique escapade and then go back to the practice of medicine in Burgundy, probably in that medieval little town where I had already established a modest reputation as a young and alert practitioner in part-time assignments during my last year of medical schooling.

Selye had asked me to set up a technique to keep rats alive long enough after bilateral nephrectomy to see whether large doses of desoxycorticosterone acetate would still produce the vascular lesions he had shown earlier to be regularly produced by the mineralocorticoid in unilaterally nephrectomized rats.

While he was a remarkably lucid and elevating lecturer, Selye was not a teacher of graduate students or postgraduate fellows in the sense that he would make specific efforts to spend any of his own time to teach one anything. The tools, the environment were there and available, but it was left to everyone to make the best of it. Personal contacts, discussions, collaboration among the younger people were of major importance. It was through these that I learned of Fortier's interest in elucidating the mechanisms involved in the physiological control of ACTH secretion, one of the primordial events, as had been shown by Selye, in the response to stress. Besides my work with the bilaterally nephrectomized rats, I read the available literature and observed what Fortier was doing, at that time transplanting the anterior lobe of the pituitary in the anterior chamber of the eye. Geoffrey Harris, then at the Maudsley Hospital in London, came to Selye's institute as a Claude Bernard Lecturer in 1949 and spent a week with us. Very different from Selye as a scientist, he convinced some of us that there were major problems in classical physiology to be answered in elucidating the physiological mechanisms involved in the hypothalamic control of adenohypophysial secretions. In his laboratory at the Maudsley, he had recently shown, with several younger collaborators such as Curt von Euler, Seymour Reichlin, Bernard Donovan, K. Brown-Grant, and Jack deGroot, that it was possible specifically to stimulate, acutely or chronically, the secretion of one anterior pituitary hormone or another [adrenocorticotropin-(ACTH), thyrotropin-(TSH), gonadotropin(s)-(FSH/LH)] by electrical stimulation of different loci in the ventral hypothalamus in studies with rabbits; conversely, high frequency or electrocoagulation of the same hypothalamic nuclei would inhibit or prevent secretion of the same adenohypophysial hormones. Similar conclusions had been reached quite independently by David Hume, Don Fredrickson, and W. F. (Fran) Ganong at the NIH in Bethesda and Samuel (Don) McCann and John Brobeck in Philadelphia.

These studies had followed early reports by Charles (Tom) Sawyer, J. E. Markee, and W. H. Hollinshead in 1946 and by Jack Everett and C. H. Sawyer in later years.

At that time the prevalent concept was that the hypothalamus was somehow involved in controlling the secretions of the anterior lobe of the pituitary, most likely through some neurohumoral transmitter, reaching the adenohypophysial parenchyma through the exquisite network of capillary vessels first described by Popa and Fielding (*J. Anat.* Vol. 675, 88, 1930). The vascular pathway was the sole possible link, because it was by that time clearly established that no nerve fibers could be shown to exist between hypothalamus and adenohypophysis, in contradistinction to the well-recognized tract of nerve fibers joining the supraoptic and the paraventricular nuclei of the hypothalamus to the posterior lobe of the pituitary. The concept of neurosecretion had originated with morphologists such as Berta Scharrer, first in Germany and later in the United States, observing in invertebrates, neurons which contained granules stained by various dyes and which thus resembled cells of exocrine or endocrine glands—the thyroid, the pancreas, the pituitary, etc. Extended to vertebrates by Ernst Scharrer, and the school around Bargman in Germany, the concept was essentially based on morphological observations, again dealing with the hypothalamo–hypophysial system of fibers I mentioned above and relating to the neurohypophysis. As early as 1924, the chemist J. J. Abel, then at Johns Hopkins, had mentioned that aqueous extracts of the ventral hypothalamus of several mammalians had the same bioactivities as similar extracts of the posterior pituitary. Geoffrey Harris and Dora Jacobson had shown that secretion of adenohypophysial hormones (ACTH, TSH, FSH/LH) would cease following complete section of the pituitary stalk, only to resume if and when the capillary vessels between hypothalamus and adenohypophysis would rejoin and reirrigate the pituitary tissue. And the flow of blood had been shown by Harris in the rabbit, by Bernardo Houssay in Buenos Aires in the frog, by Jacques Benoit and Ivan Assenmacher in the duck to be from brain to pituitary.

What was the chemical nature of this single? multiple? message from hypothalamus to pituitary? Nobody knew. And Selye, in the daily rounds in the laboratory or at the weekly seminar, kept insisting that it was all important to know the chemical nature of that "first mediator" that would acutely activate the pituitary to secrete ACTH upon exposure to any kind of stress, thus leading to his "alarm reaction." Somewhat after the visit of Harris in Montreal, the idea occurred to me to use what Selye had called "adaptation to nonspecific stress" in an effort to dissociate the nonspecific release of ACTH by drugs exerting other, very specific pharmacological actions, drugs such as antihistaminics. Why antihistaminics? Probably because we had had another set of Claude Bernard lecturers, Bernard Halpern and Jean Hamburger, who had lectured to us about the new phe-

nothiazine derivatives that the French pharmaceutical industry had recently made available to them and that they had found to be powerful antihistaminics (before others were to recognize their neuroleptic activities). And there were reports from Kahlson's laboratory in Lund indicating large amounts of histamine in hypothalamic extracts. My idea was to see whether one could reach a "stage of adaptation," in Selye's terminology, by repeated injections of Phenergan after it had lost its ability to induce (nonspecific) release of ACTH while still retaining its specific pharmacological activity as an antihistaminic. Thus we could ascertain whether the ultimate mechanism triggering the release of ACTH was histamine, or at least some endogenous substance specifically affected by Phenergan. I discussed this with Fortier and we started a few experiments along these lines. There was soon no doubt that repeated injections of Phenergan would each stimulate less and less ACTH release, as judged by the adrenal ascorbic acid test of Sayers, and that a time could be reached when injections of Phenergan were no longer followed by acute release of ACTH. In these animals the drug retained its normal, potent antihistaminic property and would completely prevent the acute release of ACTH normally induced by injection of enormous doses of histamine. I then observed that in such a preparation any other type of stress agent such as surgery, injection of formalin, or forced immobilization would still stimulate the normal release of ACTH. The "first mediator" of Selye's stress syndrome likely was not histamine. This was reported in a short note with Claude Fortier (*Trans. N.Y. Acad. Sci.,* Vol. 15, 138–140, 1953) and was my first contribution to the field of neuroendocrinology. I conducted similar studies somewhat later, with anticholinergic and adrenolytic drugs, with similar results. I also ascertained that the drugs were indeed acting within the central nervous system, demonstrating their ability to inhibit focal cortical seizures recorded by electrocorticography and induced by depositing, on the parietal cortex of the rat brain, small paper pledgets soaked with histamine, carbachol, or serotonin. The conclusion was thus reached that none of the classical neurotransmitters studied here (acetylcholine, epinephrine, serotonin, histamine) was the exclusive ultimate mediator, "the first mediator" of Selye, of the (stress-induced) release of ACTH. Some other (novel?) substance of hypothalamic origin had to be postulated as the ultimate mediator.

Leaving Montreal

Somehow, it was now 1952. I was still in Montreal, having enrolled in 1949 in an extraordinary series of courses in endocrinology (in which I met Murray Saffran as one of the undergraduate students) jointly offered by McGill and the Université de Montréal, and I was completing a dissertation for the Ph.D. degree in physiology (actually called experimental medicine and surgery). The experimental work for that degree dealt with the mechanisms

involved in the production of hypertension and kidney lesions by desoxycorticosterone acetate—the topic Selye had assigned to me when I arrived in Montreal. Nothing earthshaking is to be found in that dissertation, but I had learned the fundamentals of experimental endocrinology, how to design an experimental protocol, and how to be critical of oneself (and of others). They had been 4 extraordinary years and I have always been grateful to Hans Selye for having given me the opportunity. While I knew that I enjoyed the life of an investigator, I was not too sure that I had what was necessary to be a meaningful one. I knew, though, that if there were to be a way of mine, it would be different from the ways of Selye. I had come to recognize that Selye's style was absolutely unique and probably not to be emulated. He would always be dealing with a purely descriptive phenomenology, with more than a touch of the dramatic and a need to be read and/or presented as generating "unified" theories of medicine. Moreover, with the exception of a few early and elegant studies on the neuroendocrinology of the milk-letdown reflex, very classical in their approach, Selye's descriptive phenomenology, as I called it above, was the result of experimental decisions of such extremes as to make one wonder about their relevance not only to physiology but also to the causes of diseases of man. I would probably best fit as a "traditional" physiologist.

Having completed the work for and received my Ph.D. degree, I decided to leave Selye's laboratory. By that time I had married Lucienne Jeanne Billard, the nurse who had been in charge of my case when, a couple of years earlier, I had been diagnosed with TB-meningitis. Three of the young people in Selye's lab came down with rather sudden infection with tuberculosis at about the same time. The source of infection was never fully elucidated. I was thus one of the early beneficiaries of Waksman's recent discovery of streptomycin and intrathecal dihydrostreptomycin. I was also given very large doses of the even more recent cortisone for reasons difficult to understand today. But the result of it all was adequate enough so that I did not lose more than a summer in Notre Dame Hospital in Montreal. Our first daughter, Chantal, was born in October 1952.

After I visited C. N. H. Long at Yale, who had been an early proponent of an adrenergic mechanism for ACTH release, he offered me a job in his Department of Physiology, which I accepted. A few days later, through a peculiar set of circumstances connected with my having received one of the early scholarships of the John and Mary R. Markle Foundation the year before, I was asked to join the Department of Physiology at Baylor University College of Medicine in Houston, Texas, as an assistant professor to teach endocrinology. Hebbel Hoff had recently moved to Baylor from McGill as chairman of the department. I flew to Houston and met him and Michael deBakey, the cardiovascular surgeon who had just joined Baylor as chairman of surgery. At Baylor, there were space, money, an incredibly open future, and also azaleas and live oaks. Somehow, I sensed that all that

meant more than the Ivy League. I never regretted that decision. I sent a cable of apologies and regrets to C. N. H. Long, who, always the gentleman, never held it against me, as I was to know later on many occasions. In September of 1953, I joined the faculty of Baylor and started to teach endocrinology. With my money from the Markle Foundation, I also immediately started a modest research project to complete the "adaptation" studies started in Montreal on the mechanisms of ACTH release.

Baylor College of Medicine, 1953–1970

I stayed at Baylor and worked and taught physiology in Hoff's department for almost 20 years. Hoff was probably the most considerate and the most generous chairman I could ever have hoped for. He created an environment in that department where I could work with a minimum of encumbrances. His teaching load was immeasurably greater than mine for all those years. He accepted generously that I would prefer to spend much of my time and effort in my laboratory with a few graduate students and postdoctoral fellows rather than with the medical students. He shielded me from too much involvement in the internal problems of the school (thanks to him, I barely knew when there were problems at the school). Hoff also easily enrolled me in his scholarly interest in the history of medicine. All the work we published together on Claude Bernard's manuscripts, on the early time recordings of natural phenomena, on the history of blood transfusion in man, in which I was rather deeply involved at some time—all that was seeded, started, and nurtured by the prodigious energy and the encyclopedic mind of this great scholar of modern American physiology. Hoff revolutionized the teaching of modern physiology to medical students by his early introduction, with Leslie Geddes, of simple and rugged electronic instrumentation in the early 1950s (the Physiograph), to replace Ludwig's kymograph and the accompanying smoked drum that were still in use at that time. All of the current instrumentation is so obvious now that we forget it was not always like that. Hoff could have chosen to spend more of his time in his own laboratory to pursue his earlier work with John Eccles in Sherrington's department on the electrophysiology of the pacemaker of the heart or later with John Fulton on the physiology of the respiratory center. He chose instead to let me, along with other younger people in the department, spend my time in my laboratory. My debt of gratitude to Hebbel Hoff is equaled only by my feelings of affection for him and my respect for his scholarly mind.

The man who had been in charge of the department before Hoff became chairman was A. D. Keller. Keller and Breckenridge had reported a series of experiments in which they had observed persistence of hypophysial functions after "extirpation" of the pituitary stalk and also after partial hypophysectomy. Breckenridge was still in the department, and I got to know him well. A gentle man who decided to get an M.D. degree (which he

obtained when he was in his late 40s), Breckenridge was still interested in the physiology of the control of pituitary secretions. He showed me how to hypophysectomize dogs by an elegant transpalatinal approach that I showed later to one of my postgraduate fellows, Harry Lipscomb. Keller's original results, totally at odds with what Geoffrey Harris had reported, were, and still are, best explained by uncontrolled regeneration of the hypothalamo–hypophysial portal vessels.

One day, from Houston, I went to Galveston to visit the tissue culture laboratory of Charles Pomerat and also to see some of his famous watercolors of churches in Mexico. Pomerat showed me around, showed me his extraordinary time-lapse movie photography of various cells, particularly of neurons, speaking either in English or in his slow, perfect French, with always the most exquisite and exacting choice of the *mot juste.* He introduced me to a young undergraduate, Barry Rosenberg, who had been culturing adenopituitary cells as his assignment for an M.Sc. degree. Then Pomerat said, "You know these pituitary cells which grow so well, for some reasons which we don't understand, do not seem to secrete hormones." I asked which hormones and he said, "Gonadotropins. We are testing the fluids by injecting them into mice prepared as for pregnancy tests." I immediately told Pomerat, "I think I know why this is so: Your pituitary cells *in vitro* are lacking some substance of hypothalamic origin. Could one culture jointly pituitary and hypothalamus?" Pomerat was not particularly impressed. In a short discussion later that day, I described to him the rationale for my statement, but I did not convince him. I left, intrigued, and kept thinking about this strange observation and my proposal. A week or so later, I went back to see Pomerat and asked him whether I could set up some simple experiments in his laboratories to test the idea I had mentioned to him the previous week. I do not remember exactly what happened; Pomerat was no more enthusiastic than on the first occasion, but he suggested that perhaps Rosenberg could join me at Baylor in Houston (Rosenberg had applied to Baylor College of Medicine, as I seem to recall) to study that very problem. Barry Rosenberg came to Baylor a few weeks later and showed me how to do tissue cultures with the clot and coverslip method, originated by Alexis Carrel, 40 years earlier at the Rockefeller and still in use. Shortly thereafter, as I recall, Barry Rosenberg left to go to medical school in New York. A few months later, I knew that the pituitary cultures released ACTH in the culture medium, but only for the first few days following transplantation, and that they would release ACTH again if and when they were co-cultured with fragments of median eminence or ventral hypothalamus. I had set up the adrenal ascorbic acid bioassay for ACTH, Sayers's assay, as I thought that it would be more sensitive than the assay for gonadotropins used earlier by Rosenberg in Pomerat's department, and also probably because studies on the mechanisms of response to stress (the release of ACTH) were much in my mind after 4 years in Selye's

laboratory. I still remember going home the evening of the day on which I had seen for the first time the effect of adding a fragment of hypothalamus tissue to the pituitary cells *in vitro* and had observed the depletion of adrenal ascorbic acid in every animal of the bioassay, the sign of the presence of ACTH. I remember well telling my young wife, "I have made an observation today of such importance that you will never have to worry about our future in academic medicine." I also remember, during the next few weeks, the extraordinary excitement of learning the methods of pharmacological assays for vasopressin, oxytocin, histamine, epinephrine, and norepinephrine, and getting them to work in the one-room laboratory; the elating sensation of learning so many new things, getting them to work for new goals—things which had never been done by others; the thrill of discovering, of realizing the pregnant future, known only by those who have really experienced it. I am happy to see that still happening to the younger people in the laboratory when they come up with a good new idea or make an unexpected observation, confirm it, and expand it. It is certainly a different feeling from what one feels when putting the final mark on the solution of a problem which has taken years to solve. The former is all action, movement, and expectation. The latter is a feeling of achievement, of having reached the goal, of finally breathing, and of "what next."

That summer (1955) I spent a month in the Maudsley laboratories of Geoffrey W. Harris at the Institute of Psychiatry in London. I showed the early results of combined tissue cultures to Harris. He was rather skeptical. During that short stay at the Institute, I met Seymour Reichlin, Bernard Donovan, Keith Brown-Grant, and H. J. Campbell; Claude Fortier was there also. Later that fall, David Hume came to Houston for the meeting of the University Surgeons and he talked on kidney transplants, his new interest. I showed Hume the results of the combined tissue cultures with hypothalamus and pituitary. Hume was impressed and, I remember, encouraged me to go on with the *in vitro* method.

After a seminar I gave at Baylor, I was approached by Walter Hearn, another young fellow like me, from the Department of Biochemistry. He proposed that we work together to isolate the hypothalamic hypophysiotropic substances. I was delighted by the proposal. I had by that time pretty much decided that the most important contribution to understanding the mechanisms whereby the hypothalamus controlled the secretion of the pituitary was to establish the nature of the hypothalamic factors involved. Anything short of that would be beating around the bush. Once the hypothalamic hypophysiotropic neurohormones were isolated and characterized, all the real physiological as well as clinical studies could proceed with synthetic replicates of the neurohumors in unlimited amounts.

In one of our lectures at McGill, David Thompson had once generalized that all hormones secreted by cells of ectodermic origin were proteins or polypeptides, those secreted by cells of endodermic origin were proteins or

small-size derivatives of amino acids (thyroxine), and those secreted by cells of mesodermic origin were steroids. Perhaps naively, I had formed the hypothesis that the postulated hypophysiotropic hypothalamic substances would be peptides, probably small, as were oxytocin and vasopressin. Since du Vigneaud had characterized and synthesized these two, the hypothetical others would be synthesized also. Of revolutionary significance was the work led by Vincent du Vigneaud (V du V) at Cornell with Charlotte Ressner, Darrell Ward, Panayotis Katsoyannis, which had led to the separation of oxytocin and vasopressin, the establishment of their primary structure as two closely related nonapeptides, and, in 1952, the total synthesis of oxytocin, the first complete synthesis of a large and complex polypeptide endowed with hormonal activity (*J. Am. Chem. Soc.,* Vol. 75, 4879–4880, 1953). (Du Vigneaud would receive the Nobel Prize for Chemistry in 1957 for that achievement.)

Hearn and I worked for 2 years (until he left Baylor for another job at Ames, Iowa—where one of his technicians, later graduate student, would be Roger Burgus). Joined by several graduate students and postdoctoral fellows, William Cheek, Buford Nichols, Dwight (Gene) Householder, Sidney Levine, and later Harry Lipscomb, we purified extracts of hypothalamic tissues (a few fragments, maybe 10–50, collected locally from a sheep and steer kosher operation) and later of posterior pituitary tissues; both had ACTH-releasing activity. Through simple chromatographic separation, we convinced ourselves that the ACTH-releasing activity both in the hypothalamic tissues and in the pituitary extracts was due to some substance that appeared to be different from oxytocin and vasopressin, known since John Abel in 1924 (and as seen again in my bioassays) to be also in these hypothalamic extracts.[2] Why the work on extracts of the posterior pituitary? Early in 1955, a note by Don McCann and John Brobeck reported that injections of relatively large doses of Pitressin (a commercial clinical preparation of vasopressin) would release ACTH in rats having large lesions of the median eminence produced by electrocoagulation through electrodes located with a stereotaxic instrument. Such a lesion had been reported by McCann to inhibit or prevent the acute release of ACTH that takes place upon exposure to any sort of stressful situation. McCann in these early studies showed how such lesions could be produced routinely in the rat.

[2]There were already, at that time, good reasons to suspect that the hypothalamus should have neurohumoral control of all adenohypophysial secretions, not just of ACTH. Sid Levine and I tried for more than a year to observe whether the co-cultures with rat hypothalamus would lead to increased secretion of gonadotropins and/or thyrotropin as assessed by our simple bioassays. While there was always a trend of the numbers in that direction, no experiment ever yielded a statistically significant set of results. I have come back many times to these protocols and the experimental results. Nothing statistically defensible could have been interpreted in these results; nothing was ever published. The "trends" were absolutely correct, though it would take years to comfort them into facts and corresponding chemical structures.

Sam McCann was born and reared in Houston; I had met him after my early studies with Fortier in Brobeck's department during a short visit in Philadelphia and that same evening at Brobeck's house. I had just received pure synthetic lysine–vasopressin from du Vigneaud and had observed that it would not stimulate release of ACTH from the pituitary tissue cultures. I confirmed McCann's and Brobeck's reports when I observed that Pitressin would release ACTH from the pituitary tissue cultures whereas the synthetic vasopressin (LVP1) from du Vigneaud would not. My conclusion was that some substance other than vasopressin in the relatively crude Pitressin was the responsible hypophysiotropic agent. That was the beginning of an extraordinary series of experiments and spirited exchanges between McCann and myself that would last for almost 5 years. There would be the "vasopressin school," with McCann as its leader, followed by an ever-increasing number of people, pharmacologists, physiologists of sorts, and clinicians, all satisfied that vasopressin could be and was the physiological mediator of the stress-induced release of ACTH. The evidence appeared overwhelming, but was circumstantial. Against all these would be the other school (the "CRF school," as it would be named later), proposing that vasopressin could not be the physiological mediator of ACTH release (too-high doses of vasopressin were necessary), which instead was controlled by a corticotropin-releasing factor (CRF), possibly related chemically to vasopressin but different: Hearn and I had obtained by paper chromatography "fraction D" from hypothalamic extracts, which had no or little pressor or antidiuretic activity, which had a mobility different from that of pure synthetic lysine–vasopressin, and which released ACTH. Finally, both McCann and I agreed in 1959 that vasopressin was not the *exclusive* mediator of stress-induced release of ACTH after a series of experiments I reported with Buford Nichols showed that in nonanesthetized trained dogs one could totally dissociate release of ACTH, measured by plasma 17-hydroxycorticosteroid levels, from the release of vasopressin as shown by concomitant antidiuresis; also, in several hundred animals (rats) stereotaxically placed hypothalamic lesions would or would not inhibit stress-induced release of ACTH, as assessed by the new flurometric method I had devised with George Clayton to measure plasma corticosterone, with no correlation whatever with the presence or absence of diabetes insipidus.

What was the origin of that name "corticotropin-releasing factor," or CRF? In 1955 I had organized with Charles Carton, a young assistant professor in neurosurgery, and William Fields, a professor of neurology at Baylor, the Third Annual Meeting of the Houston Neurological Society, to be devoted to recent studies on the hypothalamus. The only previous meeting on that subject had been the imposing meeting of the Association for Research in Nervous and Mental Diseases (ARNMD), which had taken place in New York in December 1939. The lean volume that came from the Houston meeting (compared to the 1000-or-so-page *The Hypothalamus*

published by the ARNMD in 1940), titled *Hypothalamic Hypophysial Interrelationships,* was edited by the three of us and was the first volume ever devoted to the neuroendocrinology of the hypothalamus. To my surprise, I learned at that meeting from Geoffrey Harris, who came with John Green, whom he had been visiting at UCLA, that somebody else had been doing *in vitro* work with pituitary and hypothalamus. Harris had visited Saffran at McGill a few weeks earlier, who had shown him results of short-term incubation (a few hours vs the days and weeks of my tissue cultures) of rat pituitary. Saffran had been incubating rat adenohypophyses with fragments of hypothalamus, brain cortex, or posterior pituitary. It was the first I had heard of Saffran's work on the control of ACTH secretion. Harris also said that Saffran, too, had concluded that vasopressin was not the releaser of ACTH—rather, it was another peptide that he called "corticotropin-releasing factor," in short CRF. Harris added that Saffran had almost isolated the corticotropin-releasing factor, which was present (probably stored) more in the posterior pituitary than in the hypothalamus. All of that was in remarkable agreement with what I had independently concluded.

When I got in touch with Saffran and exchanged information and results with him, it became obvious to me that Saffran's methodology was much more quantitative than mine, with his use of rat hemipituitaries (rather than my coverslip tissue cultures) and with a well-characterized incubation medium (rather than the tissue culture fluid with chick embryo extract and calf serum). There were also some peculiar discrepancies in results. The most potent extracts Hearn and I had made to stimulate release of ACTH were of hypothalamic origin. Saffran's co-incubations with extracts of the hypothalamus were inactive in releasing ACTH or were replaceable by brain cortex and required the presence of a catecholamine; his best results (the most potent additive to stimulate release of ACTH) were obtained with rat posterior pituitary. Saffran's results (reported with Bruno Benfey and Andrew Schally) on the pituitary incubation and extracts, together with the whole thrust of the *in vivo* experimental results from McCann and my own *in vitro* observations of ACTH release with Pitressin but not with synthetic vasopressin, led to intense work on extracts of the posterior pituitary. On the appeal of the quantitative aspect, I shifted rapidly to the short-term hemipituitary incubation. Following discussions with Claude Fortier, who had just joined the staff of the Department of Physiology at Baylor, we so modified its design and calculation to make it even more amenable to sound statistical calculation—simple but powerful modifications that I was happy to learn from Saffran shortly thereafter he and Schally immediately incorporated in their subsequent studies. Hearn, Householder, and I observed corticotropin-releasing activity in fractions from posterior pituitary extracts with mobilities identical to those of the hypothalamic materials in several systems; all were different from the vasopressins or oxytocin. In studies of tissues other than hypothalamus and

posterior pituitaries, I was surprised to observe occasional release of ACTH by fractions of brain cortex extracts as well as by fractions of relatively crude substance P of brain or gut origin. In all cases, the active fractions (releasing ACTH) behaved on two or three chromatographic systems identically to the ACTH-releasing fraction of hypothalamus or posterior pituitary origin. The most purified materials (fraction D) active *in vitro* at 1 μg/ml were obtained in minute amounts (less than 100 μg); they were not homogeneous. When we tried to obtain them in greater purity by paper electrophoresis, we would regularly lose activity and peptide.

Hearn left Baylor for Iowa State at Ames. I immediately contacted some of the younger biochemists in the Medical Center in Houston to pursue the isolation of CRF. I was not a biochemist. Obviously, the isolation of CRF required knowledge of chemistry that I lacked, and the isolation of CRF was the most important thing to pursue and complete. One day, I received a letter from Andrew Schally, writing from Saffran's laboratory and inquiring about the possibility of joining me at Baylor. My first meeting with Schally was at the following Federation Meeting in Atlantic City. Schally had written that he would be getting his Ph.D. degree the next summer or fall and that he would like to come work in my laboratory without delay after that to complete the isolation and characterization of CRF and, he hoped, move on later to other suspected hypothalamic factors. The Atlantic City meeting confirmed these goals and Schally's interest. In that first conversation, I found Schally to be an intense younger man who, to me, the physiologist, appeared to be a qualified biochemist already with knowledge and technical know-how on peptides and, more particularly, on CRF from his training with Saffran. Working together appeared to be a sure bet to finish the isolation of CRF which I had started with Hearn. The same conclusion had obviously been reached by Schally on the basis of his work with Saffran and of what he had read of my papers. Somewhat surprised that he would not do all he could to keep him, I wrote Saffran to tell him of the letter and conversation with Schally. Saffran was affable as always, gave Schally a good though guarded recommendation, and told me that he (Saffran) was leaving on a sabbatical to work on projects unrelated to CRF.

Schally joined me in Houston in 1957. We worked together very well, very hard, with never an unpleasant word, on the isolation of CRF, which we saw within reach in another few months—repeatedly every 6 months for the next 4 years. There is no doubt in my mind that Selye's studies on stress and his remarkable observations of the involvement of the pituitary–adrenal axis in response to stress were powerful and persuasive incentives for the early studies by Geoffrey Harris in London; David Hume, Don Nelson, and Fran Ganong at Harvard; Claude Fortier in Montreal; Evelyn Anderson, Gordon Farrell, and Sam McCann then at NIH; and later myself. To characterize the first mediator of the whole endocrine response to stress was quite a challenge in which many were interested. Selye's concepts

made it of physiological interest, with possible clinical significance, when J. S. L. Browne and Eleanor Venning started to show in the early 1950s that the endocrine response to stress in man also involved the pituitary–adrenal cortex system. Selye, through his stress concept, had thus a major stimulating role in orienting the early efforts in neuroendocrinology toward the study of the hypothalamus–pituitary ACTH–adrenal cortex functional relationships. Strangely enough, and unwittingly on Selye's part, this is probably about the worst thing that happened to nascent neuroendocrinology. The search for CRF was to prove so complex and baffling that it was not completed until 1981 through the elegant work led by Wylie Vale, one of my former students and collaborators, as we shall see later.

The lack of an answer (isolation of CRF) after the first 3–4 years of early work—in fact, the lack of clear-cut progress toward isolation of CRF, multiple statements to the contrary—raised in the minds of many biologists, aware of the success of others in isolating biologically active substances (such as the ever-productive groups of Viktor Mutt in Stockholm or Vittorio Erspamer in Rome), grave doubts about the validity of these early concepts of neuroendocrinology; the same doubts were also directed, with concern, at the few people involved in these unsuccessful attempts at characterizing CRF. One can reasonably and musingly post-pose that, had we started to work on the hypothalamic control of thyrotropin secretion, or even of gonadotropin secretion, this most likely would not have happened. While the isolation of TRF (the thyrotropin-releasing factor) and the characterization of its molecular structure took from 1962 to 1969, the sequence of events involved was always logical and constructive and the reasons for slow progression were reasonably well understood.

In June 1960 I assumed the post of associate director of the Laboratory for Experimental Endocrinology, of which Robert Courrier was chairman, at the Collége de France, in Paris. The family—we then had six children, ages 8 years to 1 month—moved to France. We lived in the Château de Prunay, a beautiful place with 40 acres of park that belonged (and still does) to the Institut de France. On the insistence of Hebbel Hoff, I maintained my laboratory at Baylor operative, funded, and active with Harry Lipscomb and Andrew Schally. I literally commuted between Paris and Houston. Three years later, I decided to return to Houston; local circumstances in Paris had been such that I could not reconcile them with my goals or my ethics in science. My wife and all the children found the return to Texas somewhat difficult. It was in Paris, during those 3 extraordinary years, that Edouard Sakiz and I obtained our first solid evidence for the presence of a luteinizing hormone-releasing factor (LRF) in hypothalamic extracts and reported its early purification by gel filtration and ion-exchange chromatography (*C. R. Acad. Sci. Paris,* Vol. 256, 504, 1973). The very same methodology was to be used 10 years later in the final isolation of LRF by my laboratory as well as that of Schally. Sam McCann and Geoffrey Harris also had reported evidence for the existence of LRF at about the same time. I

have given in some detail a historical account of the search for LRF, its purification, and the involvement of both my laboratory and Schally's in its isolation and synthesis (see *Am. J. Obstet. Gynecol.,* Vol. 129, 214–218, 1977). The most important achievement of those 3 years in Paris was the report with Edouard Sakiz, Eichi Yamazaki, and Marian Jutisz of the first incontrovertible evidence of a thyrotropin-releasing factor in hypothalamic extract (*C. R. Acad. Sci. Paris,* Vol. 255, 1018–1020, 1962), its first purification and, with Don Gard, the early evidence of the mode of action of TRF in competition with thyroid hormones at the pituitary level (*Endocrinology,* Vol. 73, 564–572, 1963). Large-scale collection of sheep hypothalamic fragments also was started in Paris. When I returned to Houston in 1963, I carried with me half a million fragments of sheep hypothalamus, dissected, trimmed, and lyophilized, ready for work. By 1962, I had definitely concluded that enormous quantities of hypothalamic tissues would be necessary to complete the work involved in the chemistry of isolating and characterizing the hypothalamic hypophysiotropic factors. Of their existence, there was no doubt in my mind.

Back in Houston, I went to about every one of the largest slaughterhouses in the Midwest and Southwest, spending 1 or 2 days working on the floor with the local people to make clear what I wanted. Over 3 years, I collected 5 million fragments of sheep hypothalamus. There were some colorful episodes; my French accent was of little help in Paris, Texas. The summer after Wylie Vale became a graduate student at Baylor, his assignment was to work full time on the killing floor of one of the slaughterhouses in San Antonio which had agreed to donate the tissues if we could furnish the labor. That was hard labor for him, for that summer. Eventually, every one of the fragments was redissected in the laboratory to ascertain anatomical correctness of the section involved and to trim away peripheral tissues (du Vigneaud, who came to visit in Houston had once told me that, in the isolation of oxytocin, the most efficient purification step had been that of separating the pituitary from the cow). More than 50 tons of fresh frozen tissues was handled, processed, lyophilized, and extracted in the laboratory from 1964 to 1967.

When in November 1963 I returned from Paris to Houston, Schally was no longer in my laboratories at Baylor. Deeply disturbed at our inability to solve the problem of the nature of CRF in the 4 years of collaborative efforts, I had told him during one of my many commuting visits from Paris to Houston that perhaps some reappraisal of our collaborative arrangement would be necessary in the future. Late in 1962 or early 1963 Schally went to New Orleans to set up a unit of research on polypeptides at the Veterans Administration Hospital, where he pursued his own efforts on the characterization of hypothalamic peptides controlling pituitary functions.

In our working together, Schally and I had learned much about the strategy of an isolation program that would be of use to both of us in future endeavors. As I said above, it is perhaps unfortunate for neuroendocrinology

that we did not first look for a TRF or a LRF in hypothalamic tissues. The hard work that Schally and I had devoted unremittingly to the characterization of CRF had little immediate reward. But this is the usual case with hindsight. Edouard Sakiz and Eichi Yamazaki had both decided to come with me to the United States from Paris. At the last minute, Yamazaki told me that he wished to abandon science for the spreading of true Buddhism; he became (and still is) a high-ranking member of Sokagakai, a very large religious and political party in Japan. Sakiz went to Houston ahead of me by a few months to get things going at our usual pace. From Paris to Houston, Sakiz and I worked together for almost 7 years, with a bond of friendship and of intellectual commensality that I never encountered with anybody else. That warm friendship is still very much alive. Sakiz became the director of research and later the president of Roussel-UCLAF, one of the largest and most successful pharmaceutical companies in France.

Upon return to Houston, we worked hard at purifying both LRF and TRF, in consultation with Darrell Ward, who had come from du Vigneaud's laboratory to M.D. Anderson Hospital across the street, where he became chairman of biochemistry. I organized the handling and cutting of the hypothalamic fragments and their lyophilization in industrial-size desiccators. I also performed the first solvent extraction and stockpiled the products of the first steps of purification of the extract, chromatography on gigantic gel-filtration columns of 15 cm x 2 m, in batches of 100,000 fragments, followed by ion-exchange chromatography on columns also of respectable size (all methodology that we had ascertained early on small scale). Meanwhile, Sakiz was running the bioassays; he was also writing more and more sophisticated computer software for our statistical analyses in experimental endocrinology. In spite of all that hard work, efficiency, and enthusiasm, there is no doubt in my mind that the move from Paris back to Houston, with the accompanying physical and emotional strain, was a terrible drawback for our work in the laboratory.

In my historical account (mentioned above) of the isolation of LRF, I explained how the vagaries of the bioassay had led me early in 1965 to shelve the isolation of LRF until a better assay became available (that was not to arrive until 1969 from our work with Max Amoss, when Max reduced to practice a solid-phase radioimmunoassay for rat LH of exquisite sensitivity and great specificity). Because the bioassay for TRF I had designed in Paris in 1962 was so reliable, we could devote our full-time effort to characterizing TRF. With Edouard Sakiz and Pierre and Simone Ducommun, who had joined us after 3 years in Fortier's department in Quebec, and young Wylie Vale, we also conducted *in vitro* and *in vivo* physiological studies on the mechanisms of simultaneous secretion of ACTH and TSH and on the mechanisms of secretion of TSH as modified by thyroid hormones and using (sparingly) relatively crude preparations of TRF. With Wylie Vale, we showed the effects of elevated K^+ on TSH secretion, the role of Ca^{2+}, the

antagonism by thyroid hormones, and the rapid degradation in plasma of purified (though not homogeneous) TRF. From these times came the elegant study that was part of Wylie's dissertation showing the dissociated effects as a function of time of cycloheximide vs actinomycin D on thyroxine inhibition of TRF-induced release of TSH.

Soon after my return from Paris I had begun inquiring for a chemist to join our group, as Darrell Ward could not devote much of his time and efforts to this project and I knew I did not have the competence to bring to completion the isolation of TRF and its structural characterization, particularly in view of the submilligram quantities that were expected to constitute the final yield. In September 1965, Roger Burgus joined us at Baylor. While he had been working on the chemistry of cobalamines in the preceding few years, he had trained with Hearn at Ames, Iowa, and had been involved in Hearn's own efforts, after he had left Baylor, in preparing CRF from posterior pituitary powders. The arrival of Burgus was taking place at a propitious and also critical time: propitious because I had accumulated large amounts of the hypothalamic extract and Sephadex fraction containing TRF for a meaningful attack on its final isolation; critical because Sakiz, Ward, and I had recently started to wonder whether TRF was a polypeptide or at least a homomeric peptide since we could not destroy the biological activity of our preparations of TRF by incubating them with trypsin, Pronase, carboxypeptidases A and B, or leucine amino peptidase. A short note stating that question (*C. R. Acad. Sci. Paris,* Vol. 262, 2278–2281, 1966) was prepared some months after Burgus's arrival. Burgus had carefully insisted that the text clearly read that, while the results recently obtained were compatible with such a proposal, they did not exclude the possibility that TRF could be a peptide, although a somewhat unusual one (by our thinking at the time).

Then followed the series of experiments that led in 1968 to the isolation of TRF in the laboratory at Baylor. The decisions as to the chemical steps, the handling of the minute amounts of pure material generated, and the final approach with mass spectrometry of the synthetic pGlu-His-Pro-NH_2 and finally of the native ovine TRF were those of Roger Burgus, while the performance and appraisal of the bioassays and the surrounding biology were my decisions, together with the participation of Wylie Vale, who was to receive his Ph.D. in physiology in the summer of 1968. I have given, with Burgus and Vale, a careful historical account of these 4 years, of how we purified, isolated, and characterized the structure of TRF, in an extensive and critical review (*Vit. Horm.,* Vol. 29, 1–39, 1971). To this day, we have nothing to add to, remove from, or qualify in that review. It goes with great technical detail into the approach that we set and followed in what was to be a classic of the strategy used in the isolation and characterization of a natural product of complicated biological assay that is present in minute amounts (for the technology of that time, i.e., before the appearance of

molecular biology) in an unusual starting material. References to the various pertinent technical papers are extensive and exhaustive, not only to the publications of my own laboratory but also to the few other groups working on the isolation of TRF, particularly Schally's group. Of particular significance was the fact that we obtained the primary sequence of ovine TRF through low- and high-resolution mass spectrometry, a method leading to incontrovertible direct evidence. The identity of R_fs (ratio of mobility to the front of solvent) in multiple chromatographic systems, as proposed later by Schally's group, is never of concluding weight.

I will recount here only a few details in a lighter vein. With Roger Burgus, Thomas Dunn, and Wylie Vale, I submitted to *Science,* in February 1969, a manuscript describing the TRF biological activity of the protected (treatment with acetic anhydride) synthetic tripeptide (R)Glu-His-Pro-OH and showed, in the same note, the absence of TRF biological activity of all the other protected isomer tripeptides. This was without any possible argument the first evidence of a known peptide (H-Glu-His-Pro-OH) with no TRF activity in itself but showing the generation of TRF activity solely upon protection of its N-terminus as we knew native TRF to be protected. Moreover, synthesis of the series of all tripeptides composed of His, Pro, Glu, produced in record time at my request by Rolf Studer and his collaborators at Hoffman–LaRoche in Basel, had been triggered by our recently acquired knowledge that the whole of the molecule of TRF could be accounted for by the three amino acids His, Pro, Glu as we had just publicly reported—Schally and Folkers in the audience—in January 1969 at a meeting in Tucson, organized by the NIH (see J. Meites, ed., *Hypothalamic Hypophysiotropic Hormones,*[3] Williams & Wilkins, Baltimore, 1970,

[3]The published proceedings of that Tucson meeting make interesting reading for the historian of neuroendocrinology. One finds in it the extensive description by Schally and Arimura of their isolation of "GHRH" and its biological activity (pp. 208–226); the claims of FRF free of LRF activity (p. 248), both being proposed as polyamines (pp. 248–252): the claims of the isolation of MIF and MRF (rather, MSH-RIH and MSHRH) (pp. 171–183)—all from the laboratories of Schally. All turned out to be artefacts. There also is the paper with Burgus showing the isolation of ovine TRF (pp. 227–241) and the original figures showing its composition: 81.6% of the weight was accounted for in terms of the three amino acids His, Glu, and Pro; theoretical ponderal contribution of the amino acids for a tripeptide isolated as a monoacetate is 86%. Three months after our first note on the TRF activity of protected Glu-His-Pro-OH appeared, Schally was still *concluding* in 1969 that TRF was not a homomeric peptide and that the nonpeptidic moiety of the molecule of TRF (66% of its weight) was responsible for the biological activity of TRF: the ultimate proof of that conclusion, so said Schally *et al.* in *J. Biol. Chem.,* Vol. 244, 4077, 1969, was that, indeed, all tripeptides composed of Glu, His, Pro had no biological activity. Schally had had such peptides prepared by the group of Merck as early as 1966. In fact, when Burgus and I became interested in the very same compounds, I wrote to my friends at Merck asking for these, only to be told that none was left because the whole lot had been given to Schally. I then wrote Schally asking for some small aliquots of these peptides, pointing out that this request and a cooperative response on his part would be not only in good scientific spirit but also for the best use of our taxpayers' grant moneys.

pp. 21–35). That manuscript was rejected by *Science* on the comments of one referee who said "the posterior pituitary peptides [oxytocin and vasopressin] are active [in stimulating release of TSH] in the nanogram range. I mention the claims of LaBella (Franck LaBella, a Canadian pharmacologist) not to advocate the posterior pituitary peptides as physiological TRFs but to compare the relatively high doses of the synthetic material with the low doses of substances known to occur in the vicinity of the hypothalamus." Having anticipated that this might well be the fate of this revolutionary manuscript, I had sent a month later, in early March 1969, a short note to the French Academy of Sciences (which was to appear in *C. R. Acad. Sci. Paris,* Vol. 268, 2116–2118, 1969). I returned the manuscript to *Science* with what I thought was a clear and careful rebuff of such ridiculous comments, but also with new data, only to have it returned a month later with a new comment by the same referee, I suppose, that he had just read the note in French describing the same results; *Science* did not deal in repetitions. That fateful referee's knowledge of French had to be about as profound as his knowledge of and, I would guess, his contribution to the field as shown by his earlier comments: the new version sent to *Science* had now the evidence of the pyro-Glu N-terminus and other additional new data leading to conclusions proposed but not proven in the earlier note in French. There is no doubt in my mind that had *Science* published that note when we sent it, the unpleasant exchanges that were to follow later in the year with Folkers and Schally about the priority of our characterizing the primary structure of TRF would never have occurred (or who knows!).

I have said and written on several occasions that I consider the isolation and characterization of TRF the major event in the establishment of

Schally did not see fit to go along with my request, with the excuse that "the FDA did not allow such transfers across state lines" (*sic*). The data on the homogeneity of our latest batch of ovine TRF reported by Burgus at the Tucson meeting, showing it to be a peptide composed exclusively of the three amino acids, had been obtained and definitely ascertained only a few days before the meeting. In view of the results published by Schally's group, our new observation was obviously a turning point, of which Burgus and I fully realized the implications. We never considered not to reveal it at the Tucson meeting. But Burgus and I knew that time would be short for us, as soon as we had given it away. It was from Tucson that I made by phone my first request to the group at Hoffmann–LaRoche for the synthetic tripeptides since we had no knowledge at that time of how to synthesize rapidly enough even a simple tripeptide. The conclusion is inescapable that Schally and his collaborators never isolated TRF as a single entity, either in 1966 or later. I have previously said so in *C. R. Acad. Sci. Paris,* Vol. 269, 1870–1873, 1969; also in *Vit. Horm.,* Vol. 29, 1–39, 1977. I do not doubt that their porcine TRF was probably obtained as a peptide practically free of other peptides; it was, however, so contaminated with other nonapeptidic side products that Schally was led to believe that the peptide in their TRF was not the principal component. These nonpeptidic components turned out to be classical contaminants, cellulose, dextran, myrystoleic acid, leached from the equipment used in the last stages of purification as finally recognized by Schally on p. 1103 of *Biochemistry,* Vol. 9, 1970, in a footnote acknowledging that their preparation of TRF was never better than 65% pure.

modern neuroendocrinology, the inflection point that separated confusion and a great deal of doubt from real knowledge. Contemporary neuroendocrinology was born of that event. Isolations of LRF, somatostatin, the endorphins, others later, were all extensions of that major event—the isolation of TRF, a novel molecule in hypothalamic extracts, with hypophysiotropic activity, the first so characterized. I am happy that Geoffrey Harris was still alive when that happened. I have a letter from him in which he expressed in friendly terms his satisfaction with that happening. The event was the vindication of 14 years of hard work within the paradigm of a hypothalamic neurohumoral control of adenohypophysial secretions. From observation of what has happened in neuroendocrinology since 1969, the isolation of TRF was also the vindication of my early decision, as a physiologist, that the most heuristic event in neuroendocrinology would be the isolation and characterization of the first one (any one) of the then-hypothetical hypothalamic hypophysiotropic factors.[4]

After TRF, pioneering in neuroendocrinology ceased and became the harvesting of a new and expanding science. And expand, it did, in ways and concepts that were expected, so to speak in the normal science (in the sense of Thomas Kuhn) that followed but far more interestingly in the revolutionary (again in the sense of Thomas Kuhn) observations that were to follow. With the discovery of TRF several offers for chairmanship of a department of physiology came my way. One of the most intriguing was from the new campus that the University of California was creating at Irvine. While we were perfectly happy in Texas and at Baylor, I thought that one should not limit one's vision to the acquainted surroundings. Sometime in January 1969, my wife and I went to the Irvine campus as guests of the search committee for a new chair of a combined department of physiology and pharmacology. That evening, the chairman of the search committee took us for dinner to a very nice restaurant called Victor Hugo, by the ocean. For the first time, we saw pods of gray whales blowing their spouts in the annual migration from Alaska to the lagoons in the south of Baja California in Mexico, where they have their calves, before returning north in March–April. While we were having dinner, our host was called briefly to the phone.

[4] What about the cost? Over the years (from 1953 to 1969) the funding of my laboratory at Baylor College of Medicine was almost exclusively by the National Institutes of Health (NIH), more specifically the National Institute for Arthritis, Metabolic and Digestive Diseases (NIAMDD), with a few tactically important contributions from the Ford Foundation, the Population Council, and the Markle Foundation. Making rather simple assumptions based on the budget of my own laboratory and what I was reading in newspapers in Houston, where Mission Control for the moon landing Apollo program was located (assuming that all work and expenses from 1953 to 1969 were necessary for, involved in, and responsible for the isolation of the first 1 mg of ovine TRF), I once calculated that that 1 mg of native, pure, ovine TRF made 1 kg of pure, native TRF, 2.5 times more expensive than a kilogram of moon rock brought back from the Apollo XI mission. Today the cost of synthetic TRF is a few cents per milligram.

Upon his return, he said to me, "Jonas wishes to speak with you." I asked whether that had anything to do with the whales we were watching and he answered, "No, no, the real Jonas, Jonas Salk, is trying to reach you." A couple of days later, after I had for all practical purposes declined the offer from UC Irvine—the structure of the combined department as suggested was more than I felt comfortable handling and leading—we drove to La Jolla and saw the Salk Institute for the first time. We met Jonas Salk, the resident fellows, and the nonresident fellows of the institute gathered for their annual January meeting. The group of people was impressive. In addition to Jonas Salk, we met resident fellows Jacob Bronowski, Ed Lennox, Mel Cohn, Leslie Orgel, and Robert Holley, who had just received the Nobel Prize for his work on the structure of transfer-RNA and who had just joined the Institute. The nonresident fellows were Francis Crick, Jacques Monod, Salvador Luria, Warren Weaver, Steve Kuffler, and Daniel Lehrman. The president of the Salk Institute at that time was Joe Slater, who had come from the Ford Foundation. That group was the senior faculty of the institute, resident and nonresident fellows having each and all the same voting capacity in decisions involving the current and future academic programs of the institute.

I soon found out that they were interested in starting two new programs at the institute, one dealing with neurobiology and another dealing with or looking at fundamental research involved in the biology of birth control, to be expanded into means and problems of population control. In the couple of days of conversation that followed, it became apparent that the group I was dealing with was well aware of the latest literature on the purification of hypothalamic peptides, their significance in the physiological control of endocrine functions, and their potential clinical use. The discussions were friendly, fast paced, and with as much vision as technical subtleties.

But even more impressive than that impressive group was the structure, the building of the Salk Institute. Designed by Louis Kahn, in close spiritual and practical collaboration with Jonas Salk, it was positioned at the edge of a cliff opening westerly to the Pacific Ocean. The two identical buildings were separated by the plainest of travertine patios designed by Luis Barragan. Their monastic lines created in me a shock, a spiritual experience which I had never expected there or ever felt except perhaps when I first saw the Cathedral of Köln at the end of the second World War, all black and how gothic and intact, in the middle of such devastation. I was mesmerized by the extraordinary beauty of that Salk Institute and right then, decided that I could not pass the opportunity to work and live in such a unique place. To this day, 28 years later, I still have the same emotion each time I see that building.

In June 1970, the whole group moved from Baylor College of Medicine in Houston to the newly created Laboratories for Neuroendocrinology at the Salk Institute; 10,000 square feet of totally open space that I designed

in close consultation with the architects in charge of outfitting it into a highly efficient, multipurpose laboratory, one half physiology, one half chemistry, and between the two halves an island with a conference room and 10 small offices for the staff and an administrative entrance for three secretaries. All central walls were of glass so that one could see through the whole space from any one location: nobody could, or should, think they were working alone in that laboratory. There were ceiling to floor length curtains, though, in the staff offices that could be closed should one wish. The curtains were rarely drawn. I worked in that laboratory for 20 years and never had to modify that original design, except in minor details. To the "TRF group" from Houston, we added Jean Rivier, who had been a postdoctoral fellow at Rice University in Houston, where he studied NMR spectroscopy with Richard Turner; Jean would be in charge of a 100-MHz NMR to be installed in the lab; Catherine, his wife, was completing her dissertation for the Ph.D. in physiology in my Baylor lab; Nicholas Ling, who came from Stanford with the most elogious recommendation from his mentor, Carl Djerassi, and who was to be in charge of a mass spectrometry unit; Michael Monahan from UCSD, who would be in charge of a unit of solid-phase peptide synthesis; and Anne Pitzer, who came with us from Houston as our in-house computer expert. We had developed in Houston a sophisticated unit of computerized statistical analyses, modeling, etc. Nothing of that was then commercially available; we distributed free of charge, of course, hundreds of copies of the software, EXBIOL, the principles of which go back to the early days with Edouard Sakiz. When the laboratory was fully functional we were about 50 people. The move from Houston to San Diego (La Jolla) was so well organized that within 30 days of arriving at Salk, we were already generating data.

In the last year or so of the characterization of TRF, when it became obvious that that problem would soon be solved, I had decided to reopen the earlier project of the isolation of the hypothalamic factor, LRF, controlling the secretion of the gonadotropin LH (luteinizing hormone). A new graduate student, Max Amoss, had reduced to practice a solid-phase radioimmunoassay for rat LH. While still in Houston, we had brought into routine use the elegant method of short-term monolayer tissue culture of pituitary cells as originally conceived by Wylie Vale. I decided that it would be the method of choice, coupled with the radioimmunoassay for LH, for our work with LRF in the side fractions of our extracts of ovine hypothalamic tissues remaining from the TRF isolation program. By the end of the year we had isolated a few hundred micrograms of homogeneous LRF, which, based on quantitative HCl hydrolysis, yielded 9 amino acid residues. We also established that the C-terminal was amidated, as in TRF, and that the N-terminal was pyroGlu, again as in TRF. The complete and correct sequence of porcine LRF as that of a decapeptide, the 9 residues we had seen earlier plus a residue of tryptophan, with the C- and N-termini as we had seen and re-

ported for the ovine peptide, was actually first proposed in June 1971 by the group of Schally; Schally completed the isolation process, the elegant chemistry for sequencing and early synthesis to be credited to the distinguished chemists Hisayuki Matsuo and Yoshihiko Baba, both visiting scientists in the laboratory in New Orleans. Three months later, Roger Burgus in our new lab at Salk completed the sequencing of ovine LRF by classic Edman degradation, and later Nicholas Ling confirmed the sequencing using mass spectrometry with our newly activated Varian Mat CH-5. The molecule of ovine origin had the same sequence as that of the decapeptide of porcine origin reported earlier by Schally's group. The peptide of human origin, which we characterized a few years later with immunochemistry and microsequencing, is identical to that decapeptide. The sequence is well conserved throughout the vertebrates with only minor variations and the mammalian decapeptide has biological activity in all species of vertebrates studied.

That latest statement took me to China. When President Nixon and Henry Kissinger reopened relations with China 10 years later, one of the first practical results was some form of a cultural exchange program in which the United States gave China the names of 15 Chinese scholars—mostly mathematicians and physicists, as I seem to remember, whom we wished to invite here to lecture, and reciprocally, the Chinese gave the names of 15 American scientists whom they similarly wished to lecture in various centers in China. To my surprise, my name was on that list, my formal host being the Institute of Zoology from Academia Sinica. My wife and I went to China for about a month, a few months after the death of Mao as I remember it, and I lectured in quite a few places. On the opening day of my formal reception by my host agency I was given the explanation for this unexpected invitation: Chinese biologists, following a note by the group of Maurice Fontaine in Paris—which I had read, but with no further thought—had recognized that synthetic mammalian LRF would produce spawning and egg laying when injected into the coelomic cavity of the carps which the Chinese had traditionally grown in ponds as a major source of food protein. The carps never reproduced in captivity; thus, from time immemorial, farmers trekked each spring to the main rivers, sometimes hundreds of miles away, to collect smallfry, which they brought back to their ponds. The Chinese had built two large-scale synthesis facilities, one in the north and one in the south of China, in which they were producing kilograms of an analog (D-Trp^{6}-desGly^{10}-LRF) of the decapeptide originally reported by our lab as more potent than the native molecule and which they were distributing to these fish farmers. The carps could now be induced to reproduce in captivity; no more trekking to the Yang Tse or any other faraway river. Several of the Chinese biologists and chemists who had been involved in that episode came to my lab at the Salk Institute, subsequent to my visit to China. The Institute of Biochemistry, Academia Sinica, in

Shanghai where I lectured for several days was indeed home to a group of remarkable chemists (who, by the way, had been the first to synthesize insulin in 1958–1959, before Katsoyannis in New York or Zahn in Austria). They had organized the large-scale industrial synthesis of the LRF peptide, at a time when we were only able to make milligrams by solid phase! The old director, Wang Yin Li, was still there and told me some extraordinary stories about their survival through the Cultural Revolution following his meeting with Mao Tse-tung and Mao's wife when she understood that they were "synthesizing life" (insulin is indeed life-saving!), a unique feat of Chinese science that she decided must be left to proceed to show the world!

So, two novel polypeptides in the hypothalamus, unquestionably controlling secretion of pituitary hormones, had now been characterized. There was enough evidence that the decapeptide stimulated concomitant secretion of both gonadotropins LH and FSH, though with strange stimulus/response times that would not be elucidated until 10 years later by the elegant studies of Ernst Knobil on the frequency of the pulsatile release of LRF by the hypothalamus as controlled by the suprachiasmatic nucleus. Cyril Bowers in New Orleans made the unsuspected observation that TRF would stimulate the secretion not only of TSH but also of prolactin, though there was a great deal of species variation (not very active in the rat, very active in humans, even more so in bovidae). There were two more pituitary hormones in search of their hypothalamic-releasing factor: ACTH and growth hormone (GH). As soon as the nature of LRF was ascertained, we synthesized large quantities of the peptide and started doing physiological studies *in vitro* and *in vivo* with the synthetic replicate. I decided that concomitantly we should start looking for the hypothetical growth hormone-releasing factor (GRF). Seymour Reichlin had shown in 1959 that lesions of the ventromedial hypothalamus in the rat would lead to animals that were obese but shorter than their age-controls. Their long bones were shorter and their tibial plate cartilage was 30–50% thinner than that of their age-controls. Using the pituitary tissue culture method now fine-tuned by Wylie Vale and a radioimmunoassay for rat GH that a new Canadian post doc, Paul Brazeau, had reduced to practice, we started looking for evidence of a GH-releasing substance in extracts of fresh ovine hypothalamic tissues. To our surprise, the extracts would acutely inhibit the *in vitro* secretion of GH rather than stimulate it. After repeated and careful checking of everything in the experimental design and performance of the experiments, we had to conclude that the results were correct and could be interpreted only as evidence of some substance in the hypothalamic extract that would inhibit the secretion of growth hormone. There was nothing in the literature that would comfort this observation except for a short note by Lad Krulich and Don McCann that they had observed inhibition of GH secretion in the extract of some localized sampling-punches from the hypothalamus next to others that would stimulate secretion of growth hormone. In about a month

we had isolated a peptide that accounted for all the inhibitory activity of the original extract. It had 14 amino acid residues, including 2 Cys. Its potency was on the order of what we had seen for TRF or LRF (i.e., in the low nanomolar range, with an exquisite linear log-dose/response relationship). In a couple of weeks Roger Burgus had sequenced it by manual Edman degradation, one residue a day, and simultaneously Jean Rivier was proceeding with the synthesis by the Merrifield solid-phase method. Both the linear and the oxidized form of the synthetic peptide had full biological activity at $\geqslant 1$ nM. Both the native and the synthetic peptides inhibited the secretion of only growth hormone in the *in vitro* assay, not LH or TSH, and had similar activity in an *in vivo* model in which we were taking advantage of an old, though not well understood, observation that intraperitoneal injection of pentobarbital in rats elevated their plasma levels of immunoreactive GH. I then proposed the name *somatostatin* for this novel peptide (*Science,* Vol. 179, 77–79, 5 January 1973). We had, of course, realized the possible clinical significance of somatostatin, or one of its sure-to-come analogs, in the treatment of acromegaly and other pituitary adenomas and in diabetes, should the molecule be active in humans. It was active in humans, as rapidly demonstrated by Sam Yen at UCSD in lowering GH plasma levels in acromegalics; by Jack Gerich, Peter Forsham, *et al.,* in San Francisco; and by Rolf Luft and Suad Efendic at the Karolinska in Stockholm, in dramatically sparing insulin in juvenile diabetics, in complementary multiple mechanisms that became obvious somewhat later.

We then started distributing aliquots of synthetic somatostatin to whomever asked for it, with, of course, no strings attached, except that we be kept informed of whatever results were obtained. One day, Wylie Vale got a phone call from Charlie Gale, a colleague physiologist at the University of Washington in Seattle who had early requested somatostatin to use in studies on growth hormone secretion in the baboon. Charlie Gale said that their animals had responded as expected in terms of plasma GH level but that all showed signs of profound hypoglycemia during the infusion of the peptide. A few days later, Gale called again and said that they now had evidence that infusion of somatostatin in their baboons dramatically lowered levels of plasma insulin and glucagon. That was all unexpected. We had never seen anything like that in the rat. I asked Sam Yen to check the plasma levels of insulin and glucagon in the blood samples of the acromegalics in whom we had injected somatostatin. All showed decreased levels of the two pancreatic hormones during infusion with somatostatin.

By that time we had generated in rabbits several batches of polyclonal antisera against somatostatin. The antibodies were of relatively low binding affinity. They would not lead to satisfactory radioimmunoassays but would probably be good enough for immunohistology. Puzzled by the observations of Charlie Gale, I started wondering whether somatostatin or a related peptide could be made by neurons in the vagus or other nerves with

terminals in the pancreas. Simple calculations based on the known short half-life of somatostatin and circulation time and distribution volume made it unlikely that any peripheral (pancreatic) effect of somatostatin could be due to circulating levels of the peptides of hypothalamic origin. I sent some of these antisera to Maurice Dubois, at INRA (Institut National de Recherche Agronomique) in Nouzilly, France, asking him to see whether he could demonstrate somatostatin immunoreactivity in such peripheral nerve structures. A few weeks later, he called me on the telephone and in great excitement told me that the somatostatin immunoreactivity was, in fact, present not in nerve terminals but in every one of the so-called δ-cells of the islets, well known to morphologists, but for which no function or secretion had ever been attributed, in contradistinction to the β-cells (insulin) and α-cells (glucagon). This was independently observed and confirmed by Rolf Luft and Thomas Hökfelt in Stockholm. And there was soon no doubt that somatostatin could inhibit directly at the pancreas level, in *in vitro* preparations, secretion of insulin and glucagon. That was the first evidence for a peptide originally characterized in the brain, a neuropeptide, to be found in tissues other than the nervous system and showing biological activity there in relation to that peripheral source. Some years later we actually isolated and sequenced somatostatin of pancreatic origin and found it to be identical to the molecule from the hypothalamic origin. Soon thereafter, immunoreactive somatostatin was located by several groups (Besser and Hall in England) in specialized epithelial cells in the fundus of the stomach, the duodenum, the ileum, and at each and all of these locations, somatostatin could be shown to be biologically active in inhibiting whatever the local peptide or protein secretion would be. It also inhibited release of acetylcholine by the myenteric plexus, thus leading to inhibition of peristalsis. Immunoreactive somatostatin was also demonstrated in parts of the brain other than the hypothalamus, in particular, the brain cortex, as well as parts of the hippocampus. These observations led to the abandonment of the concept of neuropeptides, i.e., peptides specifically and exclusively of central nervous system origin. And it was soon recognized, primarily from the immunohistology work of Thomas Hökfelt in Stockholm, that every single biologically active peptide isolated earlier in the gut or the pancreas could be located in some immunoreactive form in the central nervous system—ubiquitous, yes, but not random. Each of these peptides has well-recognized mapping, recently confirmed by demonstration of their mRNAs by the new methods of molecular biology. Many neurons, particularly in the hypothalamus (paraventricular nucleus, supraoptic nucleus), have been clearly demonstrated to synthesize and release several biologically active peptides, along with a particular neurotransmitter such as noradrenaline, acetylcholine, or dopamine. To my knowledge, as per the writing of these pages in Spring 1997, the significance of this co-localization of peptides and neurotransmitter is still unclear, particularly as it relates

to the presence of specific peptides in specific neurons (cortex, hippocampus, amacrine cells of the retina, etc). What has also emerged from these early observations is the multiplicity of receptors, in the central nervous system as well as in peripheral tissues, for these peptide ligands. It is thus difficult not to think that these peptides must have some role in the function(s) of the neurons they come from or have receptors for. I am confident that these roles will be elucidated in the future through the combination of the reductionism of molecular biology and good physiology. In fact, we know today that practically any biologically active peptide originally identified in the gut, the lungs, the heart, the skin can also be found in the CNS with highly specific mapping and distribution. And there are high-affinity receptors, on neurons, for these many peptides. I cannot believe that these peptides, for which, as I said above, we still do not know of a clear function in the CNS, are no more than phylogenic relics, as some have proposed.

Hundreds of thousands of analogs of these hypothalamic native peptides have been synthesized by now, both in academia and in industry. "Superagonist" analogs of the decapeptide LRF 1000 times more active than the native molecule and with longer half life, thus leading to rapid down-regulation of their receptors, are now the treatment of choice for precocious puberty, a part of the treatment for uterine fibroids, endometriosis, some types of pituitary adenomas, and prostate tumors, in this case with analogs of somatostatin. The market for these molecules is currently several billion dollars world wide annually. Specific antagonists are being designed for these peptides, of experimental as well as clinical interest.

The isolation and characterization of the *endorphins* was an easy exercise, when compared to the previous years of anxiety. In the spring of 1975 I was invited to lecture on the current status of the hypothalamic peptides at the annual meeting of the Canadian Association for the Advancement of Sciences, in Winnipeg. After the lecture, my host, Henry Friesen, asked me what I thought of the recent report by that group in Scotland about a small peptide in the brain that had morphine-like activities. I had not heard of it. But I immediately perked up because: (1) we still did not know what the growth hormone-releasing factor was and (2) I knew that injection of morphine in humans as in laboratory animals was a powerful stimulant of acute GH secretion. Back at the Salk Institute, I searched the literature. There was essentially the still ongoing work of Sol Snyder and his group, Lars Terenius in Stockholm, Avram Goldstein at Stanford, and Eric Simon in New York, all dealing with their localization of opiates receptors in the brain; a statement from Goldstein's group that they had actually been looking for an endogenous ligand to these opiate receptors but with no clear-cut results; and the papers from Kosterlitz' group in Scotland who, indeed, reported purification of one or more small peptides (6 or 7 amino acid residues) which had opiate-like bioactivity in a couple of bioassays which I had never heard of (myenteric plexus of the guinea pig, contraction of the rat

vas deferens). That endogenous ligand of the opiate receptor may, indeed, be our still-elusive growth hormone-releasing factor. I immediately decided to search for such a molecule in our inexhaustible supplies of side fractions from the TRF, LRF, and somatostatin isolations. I called Avram Goldstein at Stanford, told him of my intentions, and asked him whether he would agree to teach me the bioassay with the myenteric plexus of the guinea pig ileum which I knew he was using, along with his receptor-binding assay. Even though he knew I would become a competitor in the characterization of the endogenous ligand which he had attempted himself, as I said above, Avram immediately said with elegance and generosity, of course, to come anytime. A few days later, I had set up the bioassay in my lab at the Salk Institute, and within a month I had located several side fractions from extracts of hypothalamic tissues and of pituitary powders that, indeed, had powerful activity in the opiate bioassay, reversible by Naloxone. I soon realized that there were several zones of biological activity as the purification progressed using the novel methods of high-pressure liquid chromatography that Roger Burgus and Jean Rivier had just introduced in the lab, following the more classic ion exchangers and molecular sieves columns. It also became rapidly evident that the molecular sizes of the peptides I was isolating were much larger than what Hans Kosterlitz and John Hughes in Scotland were writing about, under the name of enkephalins. I decided to use the name *endorphins* for these larger peptides, a name which had been proposed earlier by Eric Simon for the still-unknown endogenous ligand that the studies on the opiate receptors were postulating. On December 31, 1975, Roger Burgus completed the sequence of the smallest of the three peptides I had isolated in homogeneous form by HPLC,[5] a 16-residue peptide which I called α-endorphin because it was the first of three to be fully characterized. A week before, the day before Christmas, I had received the issue of *Nature* in which Hughes, Kosterlitz, Fothergill, Morgan, and Morris reported their identification of Leu^5-enkephalin and Met^5-enkephalin. They had noticed the similarity of their Met^5-enkephalin to the sequence 61–65 of the molecule called β-LPH, a 91-amino-acid residues isolated from pituitary extracts years before by C. H. Li at Berkeley and which had always remained some sort of a mystery because it had no well-characterized biological activity except for a minor lipotrophic effect, hence its name. Amazingly, the sequence of α-endorphin contained Met^5-enkephalin as its N-terminal and the extension 6–16 was identical to the sequence 66–76 of β-LPH.

I presented these latest results at the Harvey Lecture I delivered on January 8, 1976. The other two endorphins I had isolated were a 17-residue

[5] α-Endorphin was, to my knowledge, the first native peptide isolated by HPLC in its native, i.e., nonderivatized, form. That methodology, as we reported it, rapidly became routine everywhere for separating native oligopeptides and other molecules.

peptide, which turned out to have the same sequence of α-endorphin plus one Leu residue at the C-terminal, which I called γ-endorphin; and a 31-residue peptide, which turned out to have the exact sequence of 61–91 of β-LPH and which I called β-endorphin upon some (friendly) forcing by C. H. Li, who wanted to be sure that that molecule would clearly be identified with (his) β-LPH of old. That whole field of opioid peptides exploded in the ensuing months and years with the recognition of the multiple opiate receptors δ, κ, μ; the identification of several other opioid peptides, some related to the endorphins (Matsuo's neo-endorphins), the others unrelated, such as the dynorphins recognized and identified by Avram Goldstein and his group; and more recently several other molecular species, including the strange der-morphins with a D-amino acid as recognized by Erspamer and his collaborators. The nature of the precursor molecules of these opioid peptides is now totally clarified thanks to the powerful methodology of molecular biology. There was a flurry of excitement when Floyd Bloom and I injected β-endorphin in the cisterna of rat and produced dramatic catatonia, reminiscent, of course, of the clinical picture of hebephrenic schizophrenia. Early reports claimed disappearing of auditory hallucination of schizophrenics given Naloxone. Nothing of these clinical claims was confirmed. β-Endorphin, however, is undoubtedly a powerful analgesic agent in man when injected intrathecally or even by epidural location.

As soon as we had enough synthetic replicates of α-, β-, and γ-endorphins, I immediately tested whether they would stimulate the release of growth hormone, since that had been my early incentive for all that work. Indeed, they stimulated secretion of growth hormone *in vivo,* in the rat, as does morphine, but they were totally inactive *in vitro* in the pituitary monolayer cell culture assay. Endorphins were not the growth hormone-releasing factor still expected to be found in the hypothalamus.

The nature of the hypothalamic-releasing factor for growth hormone was not to be established until 1982 and in a totally unexpected way. Sometime in 1978 I decided that it was time to look again for that elusive GRF. By that time we knew about the several forms of somatostatin present in the hypothalamus, the 14-residue peptide we had first characterized, an N-terminal extended 16-residue peptide, and the 28-residue peptide first identified by Viktor Mutt in Stockholm in porcine intestinal extracts. We also had excellent radioimmunoassays for these somatostatin-related molecules so that they could easily be located in any extraction scheme of hypothalamic tissues. We also had good knowledge and practice of the bioassay, which would be the monolayer tissue culture of rat pituitary cells, combined with radioimmunoassay measurement of the growth hormone secreted in the incubation fluid. The residues/side fraction from the earlier extraction programs were now almost 20 years old; though always kept at ca. −20°C they were showing signs of proteolytic degradation. I then decided to obtain some fresh hypothalamus tissues and I signed a contract with a large

supplier of laboratory rats to obtain 250,000 rat brains shipped frozen on dry ice to the lab. We purified GRF, well separated from several zones of somatostatin-related peptides, but we had major technical problems of instability of the GRF fractions which we could not reduce to homogeneity. In the fall of 1980 I gave a plenary lecture on the physiological regulation of somatic growth at the annual meeting of the French Society of Endocrinology in Paris, in the same room of the old Ecole de Médecine where in 1885 Pierre Marie had described acromegaly. I mentioned those rare cases of acromegaly in which no pituitary adenoma can be found but in which some peripheral tumor may function as an ectopic source either of growth hormone or of a growth hormone-releasing substance. Such a case had just been reported by Michael Thorner from the University of Virginia in which removal of a small pancreatic tumor had led to a decrease to normal of the blood levels of GH in an acromegalic patient with no pituitary tumor. Bioassay of the pancreatic tumor had shown it did not contain growth hormone. I told my audience in Paris that should they recognize such a case of acromegaly with a normal pituitary and observe a peripheral tumor (carcinoid, islet cell, lung tumor, etc.), that I would be interested in obtaining that tumor as a possible source of GRF. A few months later, Geneviève Sassolas, then a young assistant professor in the Medical School in Lyon, wrote me about such a patient. Fusun Zeytin from the laboratory at Salk went to Lyon, organized the collection of the tumor in the operating room, and was back in San Diego 2 days later with a large, grossly heterogeneous tumor removed from the pancreas of that patient. Assays showed that some regions were rich in somatostatin bioactivity, and others extremely active to stimulate release of growth hormone. In a few weeks, with Peter Böhlen, Paul Brazeau, Fred Esch, and Nicholas Ling, we had isolated, sequenced, and synthesized human GRF as a 44-residue, C-terminal amidated linear peptide along with two C-terminal truncated fragments, 1–37 and 1–40, with lower specific activity. Later that year we identified GRF from human brains and showed it to be identical to GRF 1–44(NH_2) isolated from the pancreas tumor. The synthetic molecule is highly active in man to stimulate secretion of growth hormone. Immunocytochemistry by Bernard Bloch located GRF neurons in a discrete region of the ventral hypothalamus (arcuate nucleus, ventromedial and lateral nuclei of the tuber) with rich axonal projections to the portal vessels in the median eminence. An extensive series of experiments ensued, leading to a clear understanding of the mechanism of action of GRF, its noncompetitive inhibition by somatostatin, its pulsatile secretion by the hypothalamus, cloning of its message and precursor, etc. That rare tumor as an ectopic source of GRF had led to solving in a couple of months what had been in limbo since the first observations of Reichlin in the 1950s and our early, repeated failures.

A couple of years earlier, the group led by my former student, then associate, then colleague, Wylie Vale, had solved a similarly frustrating

quest for one of these hypothalamic peptides by isolating and characterizing CRF, the corticotropin-releasing factor from ovine hypothalamus. First shown to exist in 1955 by my laboratory in Houston and that of Saffran in Montreal, it was not until 1981 that CRF was identified as a 41-amino-acid residue linear peptide by Wylie Vale, Joachim Spiess, and Jean and Catherine Rivier from some of the fractions of our previous TRF, LRF, and somatostatin isolation programs. It is also to the credit of Wylie Vale's group to have subsequently done the mapping of CRF neurons and CRF receptors throughout the brain and to have shown its profound effects, all triggered by exposure to stress, on the endocrine, immune, and autonomic systems, in a series of superb physiological experiments.

But I should soon close that accounting. I must however, mention the work that went on in the laboratory at the Salk Institute that led to our first identification, sequencing, and cloning of acidic and basic FGFs (fibroblast growth factors); the first isolation and characterization through molecular biology in collaboration with Genentech of inhibins, molecules which had eluded everybody for 50 years; the recognition that differential recombination of the α- and β-chains of that heteromeric molecule produces structures with opposite biological activities and which I called activins; and the recognition and final identification of the molecules called folliculostatins by Nicholas Ling, Shao Ying, and Shunichi Shimasaki. The two FGFs are now recognized to be major growth factors for neurons, certainly during embryonic life; they are actually synthesized and utilized through autocrine pathways by some neurons and in highly specific locations. For instance, in the hippocampus only neurons of the CA2 show by *in situ* hybridization mRNA for FGF and FGF receptors. The biological significance of these growth factors in the development, differentiation, repair, and aging processes of neurons throughout the CNS and the peripheral nervous system is now a major chapter in the neurosciences. Similarly, inhibins, activins, and follistatins originally recognized in gonadal tissues or fluids have now been located in specific mappings of the brain, along with high-affinity receptors. I am now quite incapable of following all that literature.

Sometime in 1977, about a month before the traditional December date, I received instructions on how to prepare the one-hour Nobel lecture I would have to deliver; about a week before the lecture I got a note informing me that the lecture would be 45 minutes; the day before the talk, when in Stockholm, of course, I was told that since Rosalyn Yalow, Andrew Schally, and I would lecture the same day, that each talk would be no more than 30 minutes. That was undoubtedly the most stressful talk I ever gave—I still remember the dry mouth—without notes, in 30 minutes. But I had kept the title: "Peptides in the Brain: The New Endocrinology of the Neuron" (see *Science,* Vol. 202, 390–402, 1978). And a few years later when I delivered the Walter B. Cannon Memorial Lecture (see *The Physiologist,* Vol. 28, 391–396, 1985), I titled it "The Language of Polypeptides and the Wisdom

of the Body." If things had changed and expanded from 1978 to 1988, today's exponentially moving knowledge is giving us both insight and control, all the way to the genome, to realize that nervous systems, endocrine systems, and immune systems have ontogenic as well as phylogenic commonalities, to explain and lead to the integrated physiology we now recognize. Besides the wonderment as to how all that happened, how elegant so much of it is, is the additional rejoicing that so much of all this knowledge is now put to use rationally to cure diseases, to alleviate pain, and again to make us wonder at the brain—our brain.

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