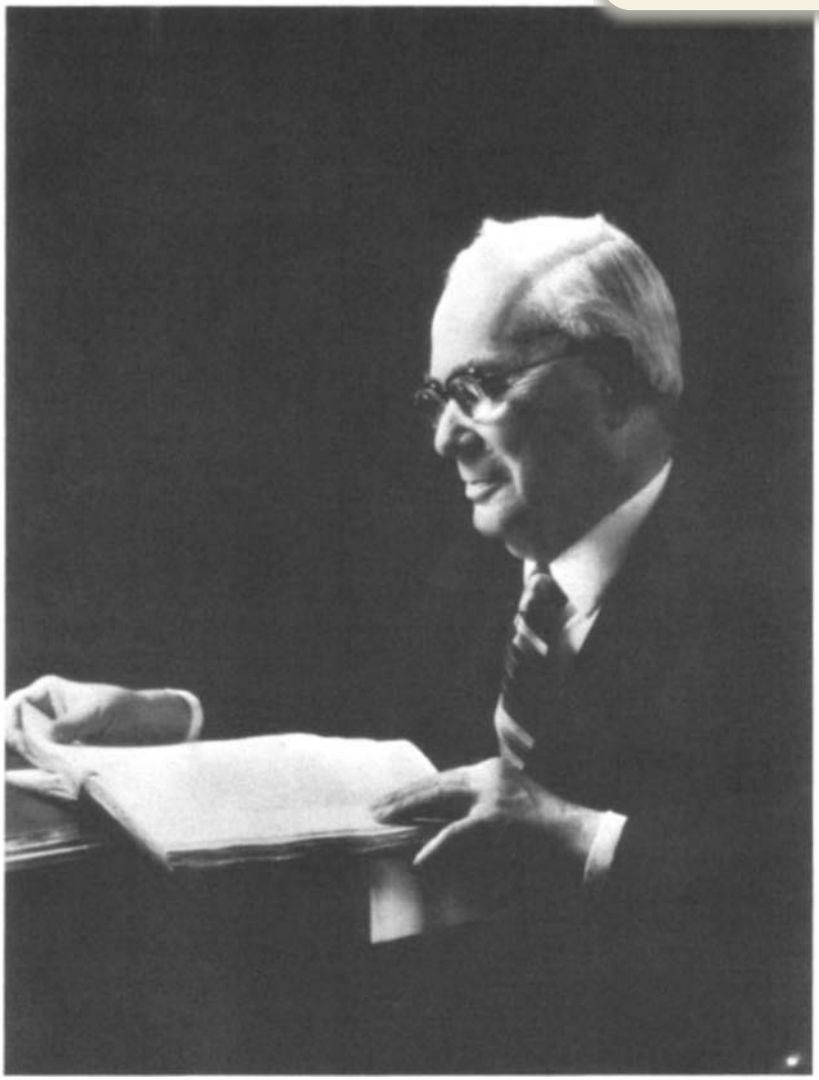

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THE BEST LAID SCHEMES O' MICE AN' MEN GANG AFT AGLEY

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Some 25 years ago I attended a symposium with about 30 participants. On the evening before the first working day, the organizers had what appeared to me to be a very original idea. At first, they dined and wined us very well; when we continued a lively conversation in comfortable surroundings they suggested that each of the main speakers give the reasons why he or she had taken up research as an important part of their career, and detail the circumstances leading to the work they were going to present at the symposium. The atmosphere was so congenial that nobody found it difficult to reveal thoughts that in other circumstances might have been considered to be rather personal. I found many of the points made of great importance to me since I have always been interested in knowing what had moved scientists whom I admired to choose a problem and approach its solution in a manner which was so often admirable. Originally, it had been planned to include these presentations in the proceedings of the symposium but this idea was eventually abandoned for a number of reasons.

When I was asked to write this prefatory chapter for the *Annual Review of Pharmacology and Toxicology* I was somewhat surprised that this honor should come to me because my pharmacological activities are of relatively recent origin. Then I remembered the events at the symposium and thought of relating the reasons for my rather unorthodox career, at least as far as I understand them.

My school and university education took place in Germany. My father was a medical practitioner and he tried to dissuade me from taking up medicine and suggested the legal profession. However, after six months during which I was fascinated by the intricacies of the legal system of the ancient Romans, I finally decided to study medicine. In those days, it was

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still possible for medical students to arrange the course in such a way that they could follow their inclinations without neglecting the formal requirements too much. Thus, I laid the basis for my future interest in basic research by spending a great deal of time in the departments of histology and physiological chemistry and learning the basic experimental techniques of these disciplines. However, the most important stimulus was the experience I had in the laboratories of Rona who had been a pupil of L. Michaelis. In my third year of medicine, I attended lectures and clinics from 8 to 11 A.M. and spent the remainder of the day, often until late in the evening, learning biochemical microanalysis and the fundamentals, both theoretical and practical, of physical chemistry in relation to biology and medicine. Rona was a hard taskmaster who would not tolerate untidiness or sloppy techniques; I have always been grateful for what I learned in his laboratories.

After I had obtained my medical degree and qualification in 1928, I worked in the first medical department of the University of Berlin, whose head was W. His of auriculoventricular bundle's fame. In those days, one was lucky to be allowed to work on the wards and laboratories of a university department and, as was usual, I did not receive any remuneration for two or three years. Although I had been involved in research during my student days, I now developed the first major research interest of my own, in the area of carbohydrate metabolism and its relation to liver disease and diabetes mellitus. I tried to imitate some of the early clinicians who combined clinical work with experiments in the laboratory designed to elucidate and analyze problems they encountered in patients. O. Minkowski was my hero at that time; in the late nineteenth century he had established that removal of the pancreas caused diabetes in dogs. I was particularly interested in the clinical observations that diabetic patients had a better tolerance for fructose and sorbitol than for glucose. I was able to confirm Minkowski's finding that fructose could form liver glycogen in diabetic dogs in the absence of insulin and extended this observation to sorbitol. I also showed that diabetics utilize galactose, which is converted to glucose in the liver, better than glucose, even to the extent that it had antiketogenic properties in severe diabetes. This finding was the beginning of my quest for the mechanism that leads to the Walden inversion of galactose to glucose.

So great was the pressure for posts in medical university departments at that time that when I obtained my first paid appointment, it was as an assistant diagnostic radiologist. The clinical and laboratory work in which I was interested had to be done in my spare time; in this effort I had the full and sympathetic support of W. His, the head of the department. I should point out that I liked the radiological work although it took up a large part of the working day; at that time, modern radiology of the gas-

trointestinal tract was in its early stages of development and, when in doubt, gastroscopy was used to verify the diagnosis.

During the five years after I had obtained my medical degree, I had acquired a basis for my later work. The two people who had the most influence on me were Rona, whom I have already mentioned, and one of my senior clinical colleagues, Petow, who supported me through all the ups and downs of the early stages of a scientific career. In particular, I had the kind of experience which is probably shared by many young people; manuscripts, which I thought I had prepared carefully and in an intelligible way, were returned to me again and again with the remarks that they were too long, that my experimental procedure would be impossible to repeat from the description, and that the discussion was too long, too speculative, and to a considerable extent irrelevant. This was a painful lesson which stood me in good stead in later days.

When I left Germany in 1934, I went to Aberdeen where J. J. R. Macleod was professor of physiology. His long experience in carbohydrate metabolism and the actions of insulin in diabetes attracted me to work in his laboratory. This was many years after the controversies which surrounded the discovery of insulin in Toronto in 1922 where Macleod was then chairman of the Department of Physiology. He made a great impression on me, both as a scientist and a person who unfortunately was already suffering from the illness that caused his death a year later. I shall be forever grateful for the encouragement he gave me in this phase of my career. There were several people in the department with a lasting influence on me. Among them were J. M. Peterson, who was my mentor through many a difficult year, and D. J. Bell, from whom I learned a great deal of synthetic carbohydrate chemistry. I took my British medical qualification and my PhD; I had also to improve my knowledge of biochemistry and physiology which I was soon asked to teach to medical students.

As far as research was concerned, I decided to attempt to resolve the problem of the conversion of galactose to glucose. By using two different strains of yeast, both of which fermented glucose but only one could ferment galactose, I could differentiate between the two monosaccharides and their phosphate ester and determine them quantitatively. It was much more time consuming than modern chromatography. By differential fractionation of the barium salts of the organic phosphates extracted from the liver, it was possible to demonstrate that after feeding of galactose to rabbits a phosphoric ester of galactose accumulated in their livers. This was shown to be galactose-1-phosphate, identical with synthetic α-galactose-1-phosphoric acid, and circumstantial evidence was adduced for the view that phosphorylation at C_1 was the first step in the conversion of galactose to glucose, as was later documented step-by-step by the brilliant investigations of Leloir.

In the early 1940s, the first chapter of my research career was brought to an end. Throughout that time, I had the encouraging support of the Coris and of F. G. Young, and I received the degree of DSc from the University of Aberdeen. However, it seemed to me that this type of work, although intellectually very stimulating, was incongruous with the efforts required by a very bitter war. One of these was the production of more medical graduates and therefore the teaching of medical students was made much more intense. As a research project I selected one that dealt with the more fundamental aspects of nutrition, namely the influence of the quality and quantity of dietary protein intake on the composition of the liver.

In collaboration with Rosa Campbell, the earlier findings of Addis and his colleagues were confirmed, that changes in protein intake cause rapid alterations in the protein content of the liver. What was surprising to us was the observation that these findings could not be explained by increases or decreases in stored protein since there were proportionate changes in the contents of phospholipids and RNA; the only constant constituent was DNA. These responses of the liver served as a basis for the bioassay of the nutritional quality of dietary protein. Of considerable interest were changes found in pregnant rats; the constituents of liver cytoplasm increased with RNA taking a prominent part. This phenomenon was only partly due to the presence of the fetuses since it still occurred, although to a lesser extent, when the fetuses were removed as long as the placentas remained intact.

During the course of this research I was elected a Fellow of the Royal Society of Edinburgh which gave me a great deal of encouragement. However, this chapter came to an end in the early fifties, partly by intent and partly by circumstances. I transferred my attention to the physiology and pharmacology of the peripheral autonomic nervous system, a subject with which I am still concerned. This interest was kindled by two factors: Ian R. Innes and I were intrigued by a paper published by W. B. Cannon in 1922 reporting that liberation of sympathin on stimulation of the hepatic nerves and its action on the denervated heart was greater in cats fed on milk and meat than in those given mainly fat and carbohydrates. In those days the nature of sympathin was unknown and Cannon could not match the chronotropic responses of the denervated heart against noradrenaline and thus exclude variations in sensitivity of the heart. In the early 1950s this had become possible mainly due to the work of U.S. von Euler and, on repeating Cannon's experiments, we found to our disappointment that diet had no influence on the amount of noradrenaline released after stimulation of hepatic nerves. If it had been true, a great deal of very interesting work could have been done.

The second factor was an invitation by O. Krayer to come to Harvard and spend a few months in the laboratories of the pharmacology depart-

ment. This invitation was accepted with much pleasure because I had known Dr. Krayer ever since his Berlin days and admired him for the strength and integrity of his personality. The five months I spent in Boston were hectic: Apart from some teaching, I accepted the challenge to analyze some of the actions of veratramine. To our great surprise this compound caused a periodic sinus rhythm in the cat heart. We thought we had made a fundamental discovery until we learned that Luciani working in Ludwig's laboratory had published a paper in 1873 in which he described a similar phenomenon when the frog heart was tied above the auriculoventricular groove and the ventricle filled with serum. We further learned that these Luciani periods attracted a great deal of attention during the last two decades of the nineteenth century. Is this proof for the saying that one of the ways of making discoveries is to search the literature of more than 50 years ago for unresolved problems? This period at Harvard had a very formative influence on me and I was very pleased and flattered when I was invited to Boston to give the Otto Krayer Lecture for 1977.

Back in Aberdeen, I started to become interested in the physiological role of the myenteric plexus. Having learned my lesson from the Luciani periods, I extended my reading more and more into the past. I came across the paper in which P. Trendelenburg showed in 1917 that morphine in very low concentrations inhibits the peristaltic reflex in the guinea pig isolated ileum mounted in an organ bath. Since the myenteric plexus is a relatively simple nervous system, we spent a great deal of work designed to elucidate the physiological mechanism of the peristaltic reflex. It was hoped that such an understanding would help in the analysis of the mode of action of morphine; it was also planned to develop a method for the assay of the pharmacological potency of morphine and its congeners. By about 1960, the work had reached a stage when it was thought to be possible to use this reflex and the inhibition of the response of the nictitating membrane of the cat to stimulation of its postganglionic fibres for this purpose. I had become more and more convinced that it would be difficult and perhaps impossible to initiate such an investigation in the central nervous system in view of its complexity and the absence of a simple pharmacological model. The action of morphine is highly selective although widespread, and therefore morphine-sensitive and morphine-insensitive neurones will occur side by side in varying proportions.

In 1962 I paid a brief visit to the United States to become familiar with the intense research efforts in the narcotic analgesics field. My first call was at the laboratory of Julius Axelrod. After a most interesting morning, he took me for lunch at the Cosmos Club in Washington to meet Dr. Nathan B. Eddy, the undisputed Nestor in the field of narcotic analgesics. During the hour or two we had together he asked me about my plans in his

inimitable searching manner, familiar to everyone who knew him. It was the beginning of a friendship between the master and a newcomer to the field who was grateful for advice and encouragement unstintingly given until Dr. Eddy's death. It gave me therefore the greatest pleasure when the Committee on Problems of Drug Dependence selected me as the winner of the Nathan B. Eddy award for 1978.

My next port of call was the laboratory of Dr. M. H. Seevers at Michigan University in Ann Arbor where I was particularly interested in the colony of morphine-dependent monkeys. I was asked to give a seminar on the work of my group on the use of the peristaltic reflex of the guinea pig ileum and of the cat nictitating membrane in the assay of narcotic analgesics. I gave my talk and at the end Dr. Seevers said in his frank manner which I learned to cherish later, "Dr. Kosterlitz, this has been very interesting but I am afraid I cannot believe that such simple models are of real use." I then asked him what I could do to convince him. "Well, if you are agreeable, I shall send you six coded samples for you to test. If you can assess the majority correctly, I shall accept that there is something in what you told us today." I was somewhat taken aback but agreed to his proposal. After my return to Aberdeen, a packet arrived and we started to assay the samples with our models. Five of them did not cause us too much trouble. One of them was likely to be morphine since it agreed in behavior and potency with authentic morphine but there was another compound which with the experience we had had up to that time, also seemed to be morphine. We sent Dr. Seevers our results and to our surprised satisfaction we were right in all but one of our findings. The exception was the second morphine-like compound which turned out to be nalorphine; until then, we had not studied compounds with dual agonist and antagonist actions. Anyhow, we had gained Dr. Seevers' confidence and he remained our friend until his recent death.

In the course of further work it was found that the action of the opiate alkaloids was difficult to express in quantitative terms. Some years earlier W. D. M. Paton of Oxford had devised a simple and ingenious method of exciting the fibers innervating the longitudinal muscle of the guinea pig ileum either by stimulating an intestinal segment coaxially or by applying field stimulation to strips consisting of myenteric plexus and longitudinal muscle. It was found that these preparations could be used as a model for the quantitative assessment of the agonist and antagonist actions of opiate alkaloids and the study of their kinetics. Over the years, many compounds were investigated, pure agonists, compounds with dual agonist and antagonist actions, and pure antagonists. From investigations on the release of the transmitter, acetylcholine, it appeared that the effect of the opiates was presynaptic and that these receptors were distinct and separate from inhibition by presynaptic α-adrenoceptors. The predictive power of these tests

was considerable and research laboratories of the pharmaceutical industry often asked us to assay newly synthesized compounds with agonist and antagonist action which frequently had potent analgesic effects in animals and man without necessarily leading to compulsive drug abuse.

Until about this time I was a member of the physiology department of Aberdeen University. In the earlier years, the teaching was mainly to medical students. Courses to science students started to develop in the late fifties, and these were attended also by the brighter medical students who wanted to become acquainted with the scientific basis of medicine. Some of these students joined me or other members of staff in ongoing research projects. One of the problems in the teaching of physiology, which has been and still is a challenge to many teachers of physiology, is the transition from the preclinical to the clinical years and the difficulty of convincing students of the clinical relevance of the basic aspects of physiology. Robert Aitken, who was professor of medicine during and for some years after the war, and I both felt that an attempt should be made to resolve this problem. So we started to teach the medical students in their last preclinical term in a joint course in which we tried to illustrate the significance of physiology on patients with classical signs of disturbance of the cardiovascular or respiratory systems. We were personal friends but this did not prevent us from attacking each other's preclinical or clinical stances without much inhibition. The more heated the discussion became, the greater was the interest and enjoyment of the students. They obviously learned something to take away because they turned out in force at 9 A.M. on a Saturday morning!

In 1968 I was asked to become chairman of the new Department of Pharmacology. For historical reasons, the Department of Materia Medica had so far taught Pharmacology; this was so in Aberdeen as in all Scottish Universities but Edinburgh. Thus, my main task during the next five years was to establish a new department and to develop new teaching courses, particularly for science students. In this effort I had the never failing support of G. M. Lees, J. C. Gilbert, and J. Hughes. One of the problems that was successfully resolved was the collaboration between the new department and the Department of Therapeutics and Clinical Pharmacology whose chairman, A. G. Macgregor, had been the driving force in the creation of the new department. In spite of additional preoccupations of an administrative nature in the university, I was able to continue with our research successfully, mainly because a number of enthusiastic young people had joined the department.

In 1972, when I still had one year before retirement from the chair, my scientific friends, particularly in Britain and the United States, suggested to me in quite forceful terms that I should continue with the research after my

retirement. Applications for funds were successful and the University of Aberdeen set aside sufficient laboratory and office space for a team of 6 to 8 academic members to establish a new Unit for Research on Addictive Drugs.

One of the problems that was to be tackled was the further assessment of new compounds and particularly the search for new antagonists. In 1972, a new model had been developed in the Department of Pharmacology. In the white mouse, transmission from the stimulated hypogastric nerve to the vas deferens was depressed by morphine due to a reduction in the release of noradrenaline from the nerve terminals. Again, pure agonists, components with dual agonist and antagonist actions and pure antagonists, and the kinetics of the interaction between agonists and antagonists were studied. In principle, the pharmacological responses of the mouse vas deferens to opiates were similar to those of the guinea pig ileum. There were, however, important differences in detail and more were to be found later. At that stage, it was shown that compounds classified by W. B. Martin of Lexington as agonists exciting κ-receptors, represented by the ketazocines, were distinct from the agonists exciting μ-receptors, represented by morphine. These differences between the two types of agonists were also present in our models. The mouse vas deferens was throughout less sensitive to κ-agonists than the guinea pig ileum, and naloxone did not antagonize the κ-agonists as readily as the μ-agonists.

The most important task of the Unit was based on possibilities which we, and certainly also other workers in the field, had been considering for a number of years. The receptors in our models, the guinea pig ileum, the mouse vas deferens, and some other tissues, e.g. the cat nictitating membrane, were so well developed that we became more and more convinced that they were not designed only to interact with the alien alkaloids of the opium poppy. This view was strongly supported by the almost simultaneous findings of E. J. Simon in New York, S. H. Snyder in Baltimore, and L. Terenius in Uppsala, that membrane fragments of brain tissue had high and specific affinities for both agonist and antagonist narcotic analgesic drugs. Therefore, it was decided to search for compounds in the central nervous system that interacted specifically with the receptors in the peripheral models and the binding sites of brain membranes.

I discussed this problem with John Hughes who was then one of my lecturers in the Department of Pharmacology. He was enthusiastic about the scientific possibilities; after the university authorities made it possible for him to retain his official status, he joined the Unit at the outset in October 1973.

In an effort of this kind, three aspects had to be considered with great care. First, it had to be as certain as possible that the concept of the presence

of endogenous opioid ligands was correct. Second, the methods of extraction had to take into account the possible destruction of the compounds. There was no preconceived idea of the possible structure of opioid ligands: The assumption was that they were probably of relatively small molecular weight with some similarities to morphine and other opiates; for example, they would have a positively charged N-atom. If they were neurotransmitters or neuromodulators, they would probably be liable to destruction by enzymes present in the central nervous system. Third, a good monitoring system was essential to ensure an efficient control of the process of purification. In this latter respect, our situation was probably particularly favorable. In the two models, we had receptor systems in which the responsive tissue, the smooth muscle, acted as a minicomputer and gave the answer in about one minute; the specificity could be controlled by specific antagonists, some of which were quaternary and therefore particularly rapid in their action. We had also pairs of isomeric synthetic antagonists and thus could test for stereospecificity of the antagonism.

John Hughes extracted large quantities of pig brain which he collected at an unearthly early hour at the local slaughter house. Within a few weeks he obtained a crude extract in which our bioassay systems indicated the presence of opioid compounds beyond any doubt. At that stage, when the nature of the endogenous opioid material was unknown, one of the great worries was contamination by one of the many opiates investigated in the laboratory. For this reason, the room set aside for the isolation of the endogenous opioids had never been used for work on opiates. It was a relief when the solubility characteristics of the endogenous compounds indicated that we did not deal with basic alkaloid-like substances. The isolation of sufficient material for analysis and the identification of the structure took about two years. It was demonstrated quite early that the compounds in question were peptides, as had also been shown by Lars Terenius who discussed this problem with us during a two-month visit to our laboratories in the spring of 1974.

The sequencing of the suspected peptide-like structure was complicated because both thin-layer chromatography and electrophoresis falsely indicated that we dealt with a single compound. The Edman-degradation gave the sequence Tyr-Gly-Gly-Phe for the first four amino acids but Linda Fothergill of the biochemistry department of Aberdeen University found it difficult to allocate positions for the methionine and leucine which were shown to be present by amino acid analysis. At this stage, we were very fortunate in obtaining the collaboration of Howard Morris of the Imperial College of Science and Technology in London, who established by mass spectrometry the presence of the two pentapeptides, methionine-enkephalin and leucine-enkephalin.

One of the most surprising coincidences occurred shortly after Howard Morris had established the structure of the enkephalins. Derek G. Smyth of the National Institute for Medical Research in London had been invited to Imperial College to give a talk on the prohormone β-lipotropin originally isolated by C. H. Li of the Hormone Research Laboratory at San Francisco who also had established its structure. Derek Smyth showed the structure of several fragments of β-lipotropin. Among those was C-fragment, now more commonly known as β-endorphin. It is an understatement to say that Howard Morris was greatly surprised when he realized that the first five amino acids of this fragment had the sequence of methionine-enkephalin. Thereafter, Derek Smyth quickly established in collaboration with his colleagues at the National Institute for Medical Research the so far unknown fact that β-lipotropin$_{61-91}$ had a high affinity for the opiate receptor. We showed that it depresses the electrically evoked contractions of the guinea pig ileum and mouse vas deferens and that this phenomenon is reversed by naltrexone.

Our paper revealing the structure of the two pentapeptides was published in *Nature* on 18 December 1975. At the beginning of December we had posted copies of the printer's proofs to our friends, competitors in the field. Some time later I received a letter from Dr. C. H. Li, in which he described some of the events that had occurred. On December 11, 1975, Dr. Avram Goldstein of Stanford wrote a letter to Dr. Li asking him for peptide samples related to the pentapeptides derived from β-lipotropin. On December 16, Dr. Li showed this letter to Dr. R. Guillemin of the Salk Institute who visited Dr. Li on that day. I learned from a recent discussion with Dr. Guillemin that he commenced the isolation of α-endorphin (lipotropin$_{61-76}$) in October 1975, and succeeded in isolating the pure peptide. He had started the sequencing when we published the structure of the enkephalins.

Thus, within a few days of our publication, intensive work on the long-chain opioid peptides present in the pituitary and, as we know now, in certain regions of the brain, began in London, San Francisco, and La Jolla. As was to be expected, many pharmaceutical laboratories also synthesized the enkephalins and began to design many analogues, for which numerous patents have been registered.

Thus started the story of the opioid peptides. Many laboratories the world over are engaged in analyzing their distribution by various methods of assay and by immunohistochemical techniques, but we still know relatively little about their physiological functions. In this respect, the best guide is an analysis of the side effects of morphine, which would suggest physiological roles in the control of respiration, motor activity, mood, endocrine control, and probably many other functions. While morphine and the opioid peptides or their analogues can be used as analgesics, it would

appear that analgesia is a pharmacological effect rather than a physiological function. It is of considerable interest that we are not dependent on the opioid peptides as shown by the lack of a withdrawal syndrome after the injection of the antagonist naloxone; on the other hand, they will produce such dependence after prolonged administration to animals. It will take many years before we shall have a real understanding of the significance of these and other peptidergic neurones. Much information will be gained from a study of their biosynthesis, metabolic inactivation, and the receptors involved. Moreover, these systems are sufficiently complex that disturbances may well lead to pathological disorders.

Before leaving the subject of the opioid peptides, I would like to put on record that this work would have been impossible without the enthusiastic and outstanding collaboration of my young colleagues and friends. John Hughes is well known for his excellent achievement when he undertook the hard work of isolating the peptides. Others, who are or have been members of the Unit, are Maureen Gillan, Graeme Henderson, Michael Hutchinson, Frances Leslie, John Lord, Alexander McKnight, Alan North, Stewart Paterson, Linda Robson, Terence Smith, Roberto Sosa, and, last but not least, Angela Waterfield. My recent election as a Fellow of the Royal Society of London may be taken as a recognition of the successful work of the team. It also gave me great pleasure when John Hughes and I were given Pace-Setter Awards by the National Institute on Drug Abuse and I received the Schmiedeberg Plakette of the German Pharmacological Society.

At this point, it may be of interest to relate a conversation I had with A. Gilman about a year ago. He thought that, apart from the scientific outcome, the work of the Unit has shown that collaboration between two people at opposite ends of their careers can be very productive, provided their personal characteristics are complementary. I fully agree with this sentiment. We have found it important that all hierarchical gradings usually associated with differences in age have to be suppressed uncompromisingly.

Our success would have been impossible without the generous support of the UK Medical Research Council, the US National Institute on Drug Abuse, the US Committee on Problems of Drug Dependence, and again, last but not least, the authorities of the University of Aberdeen.

It is generally accepted that there are mainly two possibilities regarding the selection of one's research interests. The first leads to a high degree of expertise in a well-circumscribed area in contrast to the second where the research area is changed at more or less irregular intervals. Temperamentally, the second variant suited me better, probably because such a change creates a challenge to become familiar with new concepts and then try to compete as a newcomer. This may lead to complications which can be worrying at the time but, I believe, are helpful in postponing the inevitable losses in flexibility and adaptability.

Looking back on my own professional career, I am aware that such decisions were often made for me by factors over which I had no or only very little control. I had planned to become an academic physician who would be active in clinical research. Circumstances prevented me from pursuing this goal. Then I became interested in the basic aspects of galactose metabolism and thought of following a biochemical career. It was probably lack of courage to live for a number of years on grant money which made this impossible for me. Thus I became a physiologist and was active as such for twenty-five years until about ten years ago when I moved to the new Chair of Pharmacology, from which I retired after five years. Finally, the exciting new developments induced me to continue with my research on a scale sufficiently large to make the effort viable and productive. When all these circumstances are considered it is not surprising that, when looking for a title for this prefatory chapter, I recalled the quotation from a poem with which Robert Burns addressed a mouse: "The best laid schemes o' mice an' men gang aft agley."