

A Brief History of Great Discoveries in Pharmacology: In Celebration of the Centennial Anniversary of the Founding of the American Society of Pharmacology and Experimental Therapeutics

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Foreword

When the American Society of Pharmacology and Experimental Therapeutics (ASPET¹) Centennial Committee began considering ways to celebrate the Society's 100th anniversary in 2008, an early interest was expressed in having a publication that presented the research history of the discipline. However, the Committee recognized that such a tome would fill a very large volume and be an immense task.

Several problems were perceived. First, it would take an enormous effort, one which few authors would be willing to undertake. Second, the likelihood that a quality publication of that magnitude could be produced by 2008 was slight. Third, no matter how thorough an author might be, the work of many excellent pharmacologists would be omitted and could lead to conflicts. Finally, possibly the most important problem would be that the sheer mass of material would not attract many young pharmacologists as readers. More than anything else, the Centennial Committee wants this publication to be interesting to young scientists.

It came to the attention of the Committee that Dr. Ronald Rubin had been independently considering writing about key discoveries in the history of pharmacology. The Committee offered to sponsor the project. What follows is the outcome of that effort by Dr. Rubin. In the view of the Committee, what Dr. Rubin has written avoids the major problems noted above.

The history is written in a highly interesting vein and is of a length that can be read in a relatively short period of time. The theses chosen are of such importance and

are developed in such a style that it would be difficult to fault their selection. The lead investigators that Dr. Rubin highlights were (or are) remarkable individuals. Although each discovery discussed herein culminated in a Nobel Prize, many other familiar names are woven into the fabric, and the evolution of ideas from multiple individuals is emphasized.

The Centennial Committee is pleased to sponsor this publication and hopes that the memories of more senior scientists will be relived and that young scientists will find the stories inspiring. We give our thanks to Dr. Rubin for his efforts and for these fine results.

William W. Fleming
On behalf of the
 ASPET Centennial Committee

I. Prologue

This series of essays attempts to profile how extraordinary individuals have shaped our concepts in various areas of biomedical research, particularly as they relate to the discipline of pharmacology. These gifted researchers broke through the shibboleths of scientific thought that dominated their time because of their cogent analysis and the accuracy of their hypotheses. Many of the discoveries that are chronicled in these essays are not only timeless in terms of their impact on the human condition but are also inspirational and embody certain virtues espoused by Winston Churchill, including "a constancy of mind and persistence of purpose." These essays will also attempt to illustrate how the collaboration of colleagues not only contributed immeasurably to the success of a project but may also have led to controversies that even extended into the courts. In perusing a number of documents on a given subject, one is often presented from different perspectives. To deal with this situation, I have tried to explore as many diverse accounts that I felt were needed to paint as accurate a picture as possible.

¹ Abbreviations: ASPET, American Society of Pharmacology and Experimental Therapeutics; ACh, acetylcholine; NYU, New York University; NIH, National Institutes of Health; SSRI, selective serotonin reuptake inhibitor; EPP, end-plate potential; ICI, Imperial Chemical Industries; DCI, dichloroisoproterenol; 6-MP, 6-mercaptopurine; ACTH, adrenocorticotrophin; NSAID, nonsteroidal anti-inflammatory drug; PKA, protein kinase A; NO, nitric oxide; EDRF, endothelium-relaxing factor.

Although pharmacology is a discipline with a rich and enduring heritage, present-day pharmacology is quite a different discipline than the more traditional subject I studied as a graduate student in the early 1960s. The discipline is now deeply rooted in molecular biology and molecular genetics, both of which provide powerful tools for the study of pharmacodynamics. In addition, the development of more sophisticated methods has allowed researchers to make important conceptual advances that may have eluded them for many years. Another aspect that sets present-day science apart from the past is how rapidly it progresses. The number of publications continues to grow to such an extent that many investigators now consider it an inefficient utilization of time to devote their attention to the older literature in their respective fields. However, analogous to the study of history in any format, the recollections of past events are key to understanding the discipline as it exists today and how it may evolve in the future.

There are several other reasons to have perspective on past work. Although scientific progress is viewed by some as being configured by the building of knowledge onto knowledge, I prefer to look upon science as an entity that is constantly permutating, fluctuating, and even vacillating. As a result, basic concepts are constantly reevaluated and modified. In essence, these perturbations make the pursuit of science such an interesting and intriguing endeavor. Furthermore, an historical perspective may enable one to profit from a review of previously missed opportunities to make fundamental advances and thereby avoid the pitfalls experienced by even the most gifted among us. It is also apparent that although molecular biology represents a focus of much of our present day research, the pendulum has recently been swinging back toward integrative and translational research. The genesis of this swing resides in the idea that when experimentation at the subcellular and molecular level is channeled back to the whole animal and ultimately to the patient, the etiology of disease is better understood and the effectiveness of its treatment is enhanced. And finally, it may just be worthwhile to devote time to reflecting upon the development of scientific thought, because it enables one to view his/her own research from a different perspective. This approach may lead to greater insight into present-day problems because "in science, as in life, conceptual progress once achieved sometimes turns out to be the rediscovery of the past" (Hechter, 1978).

In authoring this series of essays, I have omitted the work of some of our most distinguished scientists. This was done to prevent the essays from becoming an overwhelming chore to digest. So I have attempted to limit detailed discussion to selected examples of discoveries that I feel have had important and direct implications for pharmacological research and pharmacotherapy. In addition, each discovery has been selected for inclusion

because it had the broadest of implications for humankind. I have also limited the number of references cited in order not to detract from the concepts and/or ideas that I hoped to convey. The personal anecdotes and vignettes embedded in these essays are meant to express a reverence for the gifted scientists with whom I have had the fortune to interact. But my overall objective relates to the fervent hope that the reader will achieve deeper insight into the cultural heritage of present day science, and of pharmacology in particular.

II. Major Discoveries in Pharmacology

A. Thomas Renton Elliott: Elaboration of the Concept of Chemical Neurotransmission

Neurotransmitters mediate the transfer of information from one nerve cell to another or from nerve cell to effector by the process of synaptic transmission. The genesis of the concept of chemical synaptic transmission has been attributed to John Newport Langley (Fig. 1), a heralded British figure in the annals of physiology/pharmacology. He determined in 1901 that adrenomedullary extracts (which contained both epinephrine and norepinephrine) elicited responses in different tissues that were similar to those induced by sympathetic nerve stimulation. In the wake of these findings, Langley proposed in 1905 that a "receptive substance" was the site of action of chemical mediators liberated by nerve stimulation. At about the same time, in Germany, Paul Ehrlich developed his own receptor theory of selective binding of toxins and nutritive substances. Drugs were initially excluded because they could be readily extracted from tissues and were therefore not deemed to be firmly bound to the cell. By 1907, Ehrlich revised his concepts to include the binding of drugs to receptors that he



FIG. 1. John Newport Langley (1852–1925) is credited with postulating a "receptive substance" in nerve stimulation, although the concept of chemical transmission can be traced to Thomas Renton Elliott (1877–1961; not shown), Langley's brilliant and perhaps under-encouraged graduate student. Courtesy of the National Library of Medicine.

called *chemoreceptors*. The revised concept became the theoretical basis for his subsequent work, culminating in the discovery of the arsenical Salvarsan, the first chemotherapeutic agent used for the treatment of syphilis.

However, a more rigorous line of research was needed to develop a basic understanding of the fundamental mechanism by which nerves communicate with other nerves or with diverse effectors. A young graduate student named Thomas Renton Elliott was responsible for providing the experimental results and the conceptual advances in our understanding of this most fundamental physiological process. Despite the fact that attributions to Elliott are for the most part buried in the annals of scientific history, the remarkable story of this brilliant young student should be recounted, because scientific lore has unjustifiably assigned Elliott an ambivalent role at best in the early development of scientific thought. He has generally been portrayed as a potentially gifted researcher who failed to follow up on his very promising findings and then summarily abandoned experimental research to pursue a relatively obscure and pedestrian career in clinical medicine. As you will see, my reexamination of his story invites a radically different interpretation.

This story had its origin in 1895 in the UK when George Oliver, a rural medical practitioner, paid a visit to Professor Edward Schaefer at University College London. Oliver's own experiments had yielded a pressor effect of adrenal extracts on various animals, and Oliver wanted to verify his findings. After Schaefer agreed to the collaboration, the two men conducted a series of experiments to examine the effects of adrenal extracts on the systemic circulation. Because of the therapeutic potential of this work, the publication of these findings prompted the search for a purer extract of the active principle. Two years later, John Jacob Abel of Johns Hopkins University, together with A. C. Crawford, isolated and purified the active principle from the adrenal medulla, which Abel later named "epinephrin" (no "e"). Abel, the Father of American pharmacology, would make another important contribution some 30 years later when he crystallized insulin.

Because Abel's extracts did not exhibit strong physiological activity, an industrial chemist named Jokichi Takamine sought to develop and patent a further purification step of the active principle a few years later. Takamine then arranged for Parke, Davis & Company to market the pure crystalline substance as "adrenaline." Takamine's work stimulated much academic and commercial interest, and soon "adrenaline" was recognized as the active principle of the adrenal gland. Because of the availability of this substance (now called epinephrine in the United States), Thomas Elliott, a student in the Department of Physiology at Cambridge, was able to conduct an extensive analysis of the comparative effects of medullary extracts in the form of epinephrine and sympathetic nerve stimulation.

After examining a variety of smooth muscle preparations and glandular tissues in a large number of animal species, Elliott became cognizant of the similarity between the pharmacological actions of epinephrine and the effects of sympathetic nerve stimulation. In May 1904 in a preliminary communication to the British Physiological Society, Elliott introduced the concept of chemical transmission into scientific lore. "But since adrenalin (epinephrine) does not evoke any reaction from muscle that has at no time been innervated by the sympathetic, the point at which the stimulus of the chemical excitant is received, and transformed into what may cause the change of tension in the muscle fiber, is perhaps a mechanism developed out of the muscle cell in response to its union with the synapsing sympathetic fiber, the function of which is to receive and transform the nervous impulse. Adrenalin might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery" (Elliott, 1904).

A total of four publications were authored by Elliott, all dealing with the comparative effects of epinephrine and sympathetic nerve stimulation. In a 68-page treatise published in 1905 (Elliott, 1905), Elliott provided numerous examples of this relationship by demonstrating that the effects of sympathetic innervation and exogenous epinephrine on the bladder exhibited a similar variability among diverse species, which depended upon the density of sympathetic innervation. Armed with this comprehensive evidence, Elliott offered the postulate that the "effector" stimulated by epinephrine was the "myoneural junction" and not the nerve endings or muscle fibers.

Although this study dealt mainly with epinephrine, it was also prophetic in its analysis concerning what is now known about the functions of acetylcholine (ACh) at postganglionic parasympathetic nerves, synapses in autonomic ganglia, and the neuromuscular junction. Lacking convincing experimental evidence, Elliott nonetheless correctly speculated that these other components of the autonomic nervous system possessed a different type of junction. His intuitive recognition of a biochemical link among the three sites of cholinergic transmission would be substantiated by experimental evidence a decade later.

Elliott's last article on this subject reflected a remarkable breadth of knowledge regarding the physiological implications of his findings. However, the basically correct concept of chemical transmission that Elliott delineated in principle and reported in his preliminary note in 1904 was not reaffirmed in his subsequent publications, despite the fact that the scientific establishment failed to offer alternative explanations for his findings. One can only conjecture about the factors that contributed to the growing ambivalence in Elliott's perception of his own original hypothesis. In making no further reference to his original theory in future publications, Elliott never recanted and in fact eventually renounced

his proposed theory during his presentation at the Sidney Ringer Memorial Lecture in 1914. In his remarks, he stated that "It is always a pleasure, and therefore a temptation, to accept a theory which harmonizes all the facts into a close-fitting plan. But the evidence at present does not justify us in welcoming this simplification" (Elliott, 1914).

Realizing his singular attributes as an experimentalist, a few associates unsuccessfully tried to dissuade Elliott when he decided to terminate his research activities and resume his clinical training. After fulfilling his medical commitments, Elliott served as a medical officer during World War I, where he eventually rose to the rank of colonel. When the war ended, Elliott returned home to occupy the first of London's full-time Chairs of Clinical Medicine at University College Hospital. During his career in medicine, he continued to publish research articles on clinical topics until 1930. Elliott also won many awards for his service over the years. Most notably, he was elected to the very prestigious Fellowship of the Royal Society of London. When he retired as Chair of Clinical Medicine at University College Hospital in 1939 at the age of 62, his associates paid tribute to his wisdom, high standards, and keen vision. So although Thomas Elliott failed to consolidate his early scientific contributions into a lasting legacy, he was nonetheless remarkably successful in pursuing a distinguished administrative career in clinical medicine.

Some years later, after the evidence became overwhelming that chemical transmission was operative at synaptic sites, the legendary Sir Henry Dale, disregarding his own involvement, attributed the reluctance of Elliott to promote his theory to the perceived lack of interest in his work exhibited by the elite of the scientific establishment. In particular, John Langley, Elliott's mentor and department chair, was known as an individual who disapproved of speculative theories, especially those proposed by relative neophytes working under his direction. So Langley was apparently unwilling to give Elliott's transmitter concept an honest evaluation. In addition, the formulation of the concept of "receptive substance" first proposed in 1905 by John Langley (Langley, 1905) has, at least in part, been attributed to ideas expressed by Elliott about how a muscle cell responds to a chemical stimulus. However, in his publication, Langley failed to give any consideration to Elliott's ideas, which may have further discouraged the young investigator and diverted attention away from what probably was the most important advance in neurobiology up to that time.

Nevertheless, Elliott's contributions to neuroscience, although transient and incomplete, proved to be enduring. His seminal work became a paradigm for later studies, which would ultimately lead to the elucidation of the basic processes involved in nerve function. In 1907, Walter Dixon, a pharmacologist working at Cambridge, attempted to extend Elliott's findings by arguing that

parasympathetic nerves similarly liberate a neurotransmitter to activate effector sites (Dixon, 1907). To document his theory, Dixon demonstrated the release of this putative neurotransmitter from the mammalian heart following stimulation of the vagus nerve. After making an extract from canine heart following inhibition of contractility produced by vagal stimulation, Dixon found that the extract produced a depression of contractility of an isolated frog heart. The inhibition produced by the extract, like that caused by vagal stimulation, was blocked by the muscarinic antagonist atropine; however, due to the limitations in methodology and basic knowledge that existed at the time, this study was not continued. As a result, conceptual advances in this field were further delayed.

In his later writings, Sir Henry Dale suggests that the active substance in Dixon's experiment was probably choline, the product of ACh degradation (Dale, 1934). However, at the same meeting of the Physiological Society at which Dixon presented his results, Reid Hunt, an American pharmacologist, reported that the adrenal gland produced a hypotensive substance that was too robust to be attributed to choline. This experiment provided the impetus for Hunt to examine a series of related compounds that were synthesized for him by Rene de M. Taveau. In reporting his findings, Hunt proposed that either a precursor or derivative of choline was the main hypotensive principle. One of the esters investigated was ACh, which was found to be several orders of magnitude more active than choline in producing a drop in blood pressure. However, the transient nature of the hypotensive action exhibited by ACh and other choline analogs argued against any further experimentation to assess their significance as possible therapeutic agents. In a more detailed analysis carried out in 1914, Sir Henry Dale identified the muscarinic and cholinergic actions of ACh (Dale, 1914). While acknowledging the possible physiological significance of the resemblance between the actions of choline esters and the effects of certain elements of the parasympathetic nervous system, Dale felt that any further consideration of the physiological implications of these results should be deferred due to the limited amount of background knowledge on the subject that was available at the time.

Still, the experiments carried out by Dixon, Hunt, and Dale gave credence to the interpretation of Elliott's earlier work and would ultimately vindicate his research. However, Dixon and Hunt did not continue to explore this problem much further; so the attribution of Dixon's and Hunt's role, like Elliott's role, was relegated to brief references in certain historical accounts. One may argue that the scientific community might be forgiven its disinterest in this line of research, since the limitations in methodology made it difficult, if not impossible, to employ a more direct experimental approach to the problem at the time. However, it also seems fitting to conclude that Elliott, Dixon, and Hunt did not possess the burn-

ing interest and passion needed to overcome the obstacles presented by this fundamental biological problem (Maehle, 2004).

B. Sir Henry Dale and Otto Loewi: Chemical Transmission of Nerve Impulses

1. Sir Henry Dale. Although additional pertinent information derived from experiments of the type carried out by Elliott and Dixon would not be forthcoming for another 15 years, further insights into the mechanisms involved in synaptic transmission were fueled by the “applied research” carried out by Sir Henry Dale (Fig. 2) for Wellcome Laboratories from 1904 through 1914. The original firm had been established in 1894 by Henry Wellcome, an American-trained pharmacist, to produce serum antitoxins for clinical applications. Then, in 1895, Wellcome established the Research Laboratories. The second branch of the firm was dedicated to conducting original research and was to be divorced from the commercial subdivision.

In 1904, Dale, a Cambridge-trained biologist, was offered the position at Wellcome Laboratories to carry out experimental research. Despite the admonitions of academic colleagues against accepting a position in an institution that was tainted by commercialism, Dale needed the job for personal reasons, and so he accepted the position only reluctantly (Tansey, 1995). The appointment of Dale was profoundly significant, not only because it was responsible for guiding biological research into new directions but also because the dual approach inaugurated by Henry Wellcome enabled productive research to develop in concert with a successful

business enterprise. In future years, this dual program would be duplicated by other pharmaceutical firms.

However, at the time a schism between academia and industry existed. As an example, when Dale first met John Jacob Abel in 1909, Dale noted that Abel was rather suspicious of him because of his connections with a commercial enterprise. Abel epitomized an academican of the time. Having trained in Germany at the Pharmacology Institute headed by Oswald Schmiedeberg, Abel had returned to the United States to occupy the first Chair of Pharmacology at the University of Michigan in 1891. Then, in 1893, he assumed the Chair of Pharmacology at Johns Hopkins University. He also played a key role in the founding of ASPET in 1908. By such efforts, Abel was responsible for fashioning pharmacology into a discipline primarily concerned with the study of drugs from a systematic and mechanistic perspective, with implications for therapy. His dedication to the discipline of pharmacology also made him wary of anyone whom he believed would sully its reputation by engaging in commercial endeavors. But Abel was eventually persuaded by academic colleagues that Dale did possess strong scientific principles and ultimately accepted him as a colleague (Tansey, 1995).

Wellcome Laboratories had a strong interest in the properties of derivatives of the rye fungus ergot, and Dale was assigned this project. Dale justified this undertaking to himself by reasoning that one component of ergot extracts, ACh, was probably a naturally occurring compound, and therefore its study was of potential physiological significance. In their comprehensive pharmacological analysis published in 1914, Dale and Laidlaw found that the actions of ACh on cat blood pressure and exocrine glands, as well as rat smooth muscle, resembled those of the alkaloid muscarine. They also observed that the pharmacological effects of exogenous ACh exhibited a striking similarity to the effects of parasympathetic nerve stimulation, which was also comprised of muscarinic (blocked by atropine) and nicotinic actions (mimicked by nicotine).

In reporting the transient nature of the action of ACh, Dale suggested that an esterase in tissues or blood was probably responsible for its rapid metabolism. In this article, Dale alluded to the possible presence of ACh in humans and its potential biological significance. Although the key physiological implications of his work seemed to elude Dale at the time, this study did provide the theoretical basis for defining the pharmacology of autonomic drugs. The physiological relevance of ACh would be established by the classic experiments performed by Otto Loewi a few years later.

In 1910, Dale also published a detailed account of the sympathomimetic actions of a number of biogenic amines synthesized by George Barger. By demonstrating that several structurally diverse amines reproduced the effects of sympathetic nerve stimulation, Dale provided support for the hypothesis elaborated several



Fig. 2. Sir Henry Dale (1875–1968). Copyright Nobelstiftelsen.

years earlier by Thomas Elliott that epinephrine, or some other catecholamine, transmitted the response elicited by sympathetic nerve stimulation to the postsynaptic effector site (Barger and Dale, 1910). The luxury of hindsight enables us to conclude that by unwittingly excluding from their investigations the epinephrine (adrenaline) series of sympathomimetics, Dale and Barger overlooked the most physiologically relevant derivative, norepinephrine (noradrenaline). The fact that at the time norepinephrine was available commercially and did not require its synthesis by Barger made Dale's oversight even more vexing. As a result, the correct identification of the putative neurotransmitter of postganglionic sympathetic nerves would be delayed for many more years.

In reflecting on the reasons why he did not initially champion the concept of chemical neurotransmission as elaborated by Thomas Elliott, Dale noted that exogenous administration of epinephrine produced several inhibitory actions on sympathetically innervated end organs that were not duplicated by sympathetic nerve stimulation. This inconsistency suggested to him that some alternative process was operative. Years later, Dale tried to rationalize his missed opportunity by noting that even if he had suggested that norepinephrine was the putative neurotransmitter, because of the limited technologies available at the time (c. 1915), it would have been very difficult to identify each of the various catecholamines that might be present. So, until 1921, the physiological mechanisms involved in the transmission of signals across synapses were a subject of intense debate. In fact, certain distinguished scientists of the time gave credence to the hypothesis that synaptic transmission was an electrical event, brought about by transmission of the activation wave from the nerve ending to the effector. All of that began to change at the beginning of the 1920s, when the classic demonstration of chemical transmission was finally achieved by a simple, yet ingenious experiment carried out by Otto Loewi.

2. Otto Loewi. Otto Loewi (Fig. 3) had been trained as a pharmacologist at the University of Marburg in Germany at the beginning of the 20th century. Fortunately for Loewi, the conditions that prevailed during the early 1900s in Germany were most favorable for the development of scientific thought, with no government intervention (Loewi, 1961). Loewi took advantage of these positive conditions to learn to view scientific theory through a wide lens. As a result, his ideas were not constrained by existing dogma. After he was invited to accompany his superior Hans Meyer to Vienna, Loewi accepted the Chair of Pharmacology at the University of Graz (Austria) in 1909, where he conducted his classic experiments.

Although Loewi had professed long-term interest in the concept of chemical transmission, he eventually decided to actively participate in the development of this idea. The sequence of events leading to the establish-



FIG. 3. Otto Loewi (1873–1961). Courtesy University Archives of Vienna (UAW Fotoarchiv: 106.I.1533).

ment of chemical transmission as a basic biological concept began the night before Easter Sunday in 1920. After awakening from a sound sleep, Loewi formulated an idea for testing the hypothesis of chemical transmission and scribbled a few notes on a pad before going back to sleep. The next day he found his scrawls unintelligible. Fortunately, however, early the next morning at 3 AM, the idea returned to him; so he went to his laboratory and performed the now-classic experiment that was to revolutionize concepts of nerve function.

After Loewi placed two frog hearts into a single bath, the vagus nerve of one heart was stimulated, thereby slowing it, while causing the rate of the second heart to also diminish. From this experiment, Loewi reached the obvious conclusion that a substance liberated from the first heart was responsible for causing inhibition of the second heart. He termed the unknown substance *vagusstoff*, which was later identified as ACh. Subsequent articles by Loewi provided additional evidence favoring the similarity of this substance to ACh, including its characteristic sensitivity to destruction by an esterase that Loewi had extracted from heart muscle.

Loewi also used the frog heart preparation to demonstrate that sympathetic nerve stimulation caused the liberation of a substance, which he called *acceleransstoff*. He showed that it shared many of the properties of epinephrine in that it could be destroyed by alkali, fluorescence, and UV light. In addition, its activity at adrenergic effector sites was blocked by ergotamine and augmented by cocaine. Loewi also observed that the effects of sympathetic nerve stimulation and epinephrine on the heart declined very slowly, in contrast to the transient effects of ACh. These findings that suggested different modes of inactivation were operative for the two putative neurotransmitters would be substantiated

by the work of Julius Axelrod and his colleagues some 40 years later. On the basis of these experiments, Loewi proposed that parasympathomimetic effects were mediated by ACh and sympathomimetic effects were transmitted by epinephrine.

Despite the potential far-reaching implications of this work, Loewi faced some formidable challenges from colleagues concerning the validity of his conclusions. Their skepticism was based primarily on the technical limitations of Loewi's experiments, which were deemed responsible for contradictory results obtained by other investigators. Most importantly, the frog heart preparation was widely considered to be an unpredictable experimental model, with regard to the reproducibility of responses that various stimuli were able to elicit. In addition, because the preparation used by Loewi functioned as a hypodynamic heart, it was viewed by some as nonphysiological in terms of its functionality. Unfortunately for Loewi, the hypodynamic preparation yielded the most favorable results in support of his theory.

Thus, progress in this field was shackled by the controversy that Loewi's experiments and conclusions engendered among his colleagues. However, decisive evidence in favor of Loewi's hypothesis was eventually produced when the liberation of vagus-stoff was observed in a nonhypodynamic heart. Moreover, much of the conflicting data obtained by various laboratories was discounted because of the known instability of vagus-stoff, which Loewi had identified as ACh. Dale had already proposed in 1914 that the rapid breakdown of ACh was due to the presence of esterases in blood and tissues. This idea was confirmed in 1926 by Loewi and Navratil, who reported that extracts of frog heart tissue rapidly degraded ACh, presumably by a form of acetylcholinesterase (Loewi and Navratil, 1926). They also found that eserine could not only inhibit the enzyme but could also markedly enhance the inhibitory effects of ACh and vagus-stoff on the frog heart. So vagus-stoff could now be defined pharmacologically as a substance whose action was inhibited by atropine and enhanced by eserine. Because the properties of vagus-stoff were identical to those exhibited by the muscarinic actions of ACh, this work left little doubt that the neuronal stimulus was transmitted to the postsynaptic effector by chemical means rather than by electrical transmission.

Although one might argue that Loewi's original experiments were not very convincing, he did possess the tenacity of purpose to doggedly pursue his theory, until it was ultimately confirmed in its most basic form. In 1926, after Loewi reproduced his basic experiment 18 times on the same frog heart preparation at the famed Karolinska Institute in Sweden, his colleagues began to comprehend what he had accomplished. His work and conclusions were finally vindicated in 1933, when the introduction of the leech muscle preparation for bioassay enabled Wilhelm Feldberg and Otto Kraye to demonstrate definitively that the stimulation of the vagus

nerve liberated ACh into the coronary vasculature of mammals. A major advantage of the leech muscle for the bioassay of ACh was that it was extremely sensitive to very low levels of endogenous ACh but was not responsive to catecholamines. So, by the early 1930s, it was generally accepted that the autonomic nervous system was regulated by two substances with antagonistic actions: an ACh-like agent liberated by parasympathetic fibers and an epinephrine-like substance released by nerve fibers of the sympathetic system.

At this point, evidence was needed to assess whether the substance released from parasympathetic fibers might be a choline ester with pharmacological properties similar to ACh. Dale and Dudley made progress on this issue in 1929, when they reported the extraction and identification of ACh as a natural product of oxen and equine spleen (Dale and Dudley, 1926). By this time, Dale, now working as Chief Pharmacological and Biochemical Officer at the National Institute for Medical Research in London, was a strong proponent of the chemical theory, and he coined the terms *adrenergic* and *cholinergic* to describe the actions of autonomic and motor nerve fibers.

The hypothesis that described an ACh-like transmitter was later extended by Dale and his colleagues to synaptic transmission at autonomic ganglia. He and his distinguished associates, including Wilhelm Feldberg, Sir John Henry Gaddum, and Marthe Vogt, demonstrated by bioassay the presence of ACh in isolated perfused cat sympathetic ganglia following nerve stimulation. Not surprisingly, the detection of ACh in the venous effluent of perfused ganglia was predicated upon the presence of *eserine* in the perfusion solution. These findings and those previously made by Loewi suggested that a fundamentally similar process was operative in synaptic transmission of excitatory effects at all autonomic ganglia and postganglionic parasympathetic effector sites.

Despite the mounting evidence in support of the theory of chemical transmission, debates still raged during the 1930s concerning the general applicability of this theory. Dale and his colleagues maintained their important role in endorsing this concept, despite being challenged by colleagues who continued to argue in favor of electrical transmission. The most renowned proponent of this latter view was the Nobel Laureate Sir John Eccles, who continued to perpetuate this outdated theory. It was reported that rather harsh words were sometimes exchanged between Dale and Eccles on this issue. But by the 1950s, when overwhelming evidence finally resolved the argument, the debates finally ended in mutual respect between the two Nobel Laureates, exemplified by their frequent correspondence of more than 20 years.

Decisive experiments were also conducted by Dale and his colleagues on the neuromuscular junction in the 1930s, which established that the action of ACh was not

confined to the autonomic (involuntary) nervous system. Together with Wilhelm Feldberg and Marthe Vogt, Dale published two articles that not only provided a clear demonstration that ACh was released from motor nerve endings following nerve stimulation but also embellished their results by showing that when injected close to the muscle, ACh produced a depolarizing effect similar to that of nerve stimulation (Dale et al., 1936). The fact that ACh release was detectable even when the responsiveness of the motor end-plate was impaired by curare represented an analogous result to that obtained by Loewi on postganglionic parasympathetic effector sites. Loewi had already shown that atropine blocked the postsynaptic action of ACh on cardiac muscle but did not modify its release elicited by vagal nerve stimulation. Thus, incontrovertible evidence eventually persuaded Dale to lend his unequivocal and influential support for the theory of chemical transmission. One cannot overemphasize the importance of Dale's endorsement, because many of the *cognoscenti* at the time still firmly believed that the data were not sufficiently strong or convincing to incontrovertibly validate the new concept that would ultimately alter the scientific world's view of neuronal function.

However, final validation of the concept of chemical transmission had to await studies that would conclusively identify the agent involved in synaptic transmission at postganglionic sympathetic nerves. Although Elliott had shown that the effects of epinephrine were similar to those of sympathetic nerve stimulation, innumerable experiments, including those of Dale, showed that the *inhibitory* effects elicited by injected epinephrine were not prominent following nerve stimulation. A few years later, the renowned Harvard physiologist Walter Cannon observed quantitatively different effects on the chronically denervated and supersensitized pupil of the cat, when he compared the effects of exogenous epinephrine with those induced by hepatic or cardiac nerve stimulation. To address such disparities, Cannon and Rosenblueth proposed in 1933 that sympathin, a hypothetical mediator elaborated by sympathetic nerves, combined with either excitatory or inhibitory substances at the postsynaptic site, forming either sympathin E (excitatory) or sympathin I (inhibitory). These two substances were then released into the blood stream, leading to either a stimulatory or inhibitory response (Cannon and Rosenblueth, 1933).

The apparent conundrum resulting from the analysis of the comparative effects of epinephrine and sympathetic nerve stimulation was eventually resolved by a less complex and more physiologically relevant explanation. In 1948, Raymond Ahlquist (Fig. 4) at the Medical College of Georgia reasoned that if the rank order of potency of a series of catecholamines was the same in all tissues, then the variation in their relative activities must be due to differences in their chemical structure. However, if the rank order of potency varied from tissue

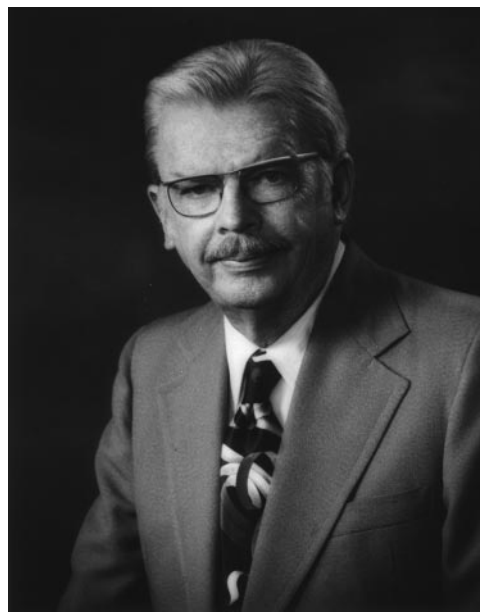


FIG. 4. Raymond Ahlquist (1914–1983). Courtesy of the National Library of Medicine.

to tissue, the observed variations must be due, at least in part, to inherent differences in the receptors. To test this postulate, Ahlquist compared the relative potencies of several sympathomimetic amines (including epinephrine) with sympathetic innervation on several isolated mammalian preparations. Only two orders of relative potency were observed with regard to inhibitory actions such as vasodilation and bronchodilation (isoproterenol > epinephrine > norepinephrine). For the excitatory actions such as vasoconstriction and pupillary dilation, the rank order of potency observed was epinephrine = norepinephrine > isoproterenol. The differential sensitivity of the various tissues to the agonists could not be readily explained by the theory of Cannon and Rosenblueth, which centered on two types of transmitters. Rather, the different patterns of relative efficacy more likely represented a preferential affinity of each agonist for one of two types of adrenoceptors.

Capitalizing on the additional discovery in 1946 by Ulf von Euler that norepinephrine was the adrenergic neurotransmitter, Ahlquist postulated in 1948 that the action of norepinephrine on postsynaptic sites was mediated by two types of adrenergic receptors, which he called α and β . It is of interest to note that the original manuscript submitted by Ahlquist was rejected by the *Journal of Pharmacology and Experimental Therapeutics*, despite the fact that it contained strong evidence to support Ahlquist's concept. Eventually, with the help of a friendly colleague, Ahlquist's manuscript was published in the *American Journal of Physiology*. But the scientific community was reluctant to accept this concept because of its novel approach to pharmacology and the mathematical modeling that Ahlquist used to explain his theory.

All of that began to change, however, when in 1954 Ahlquist was invited by Victor Drill to write the chapter on adrenergic pharmacology in Drill's *Pharmacology in Medicine*. As author of this chapter, Ahlquist took advantage of the opportunity to promote his theory, which ultimately enabled it to gain general acceptance. The concept not only prompted fresh thinking about adrenergic receptor pharmacology, it also vaulted scientific research into new directions that would guide future drug development. In particular, Ahlquist's ideas presented in Drill's textbook were adopted by Sir James Black in his quest to develop an agent that would reduce the demand for oxygen by the heart. In fact, Black maintains that Ahlquist's concept provided the conceptual framework for the development of β -receptor blockers, which was to earn Black the Nobel Prize (see section II.F.1.). Moreover, this fundamental concept led to the identification of adrenergic receptor subtypes, which subsequently spawned the development of more selective and useful therapeutic agents such as the β -1 receptor-blocking agent atenolol, the β -2 agonist terbutaline, and the α -1 antagonist prazosin.

Despite the irrefutable and overwhelming evidence favoring chemical transmission at synapses of the autonomic nerve system, during the 1950s, a few members of the scientific establishment maintained an obdurate refusal to relinquish their outdated theory. Sir John Eccles resolutely remained a dissenting voice, arguing that transmission at the neuromuscular junction was too rapid to be mediated by a chemical event. To quash these dissenters once and for all, Sir Bernard Katz and his skilled associates took it upon themselves to develop precise and sophisticated intracellular recording techniques to conduct comparative studies on the effects of exogenous ACh and nerve stimulation at the motor endplate. As a result of their work, the proposition that synaptic transmission at the neuromuscular junction involved a chemical process was finally rendered indisputable once and for all. The final resolution of this elusive question was obviously important from many perspectives. However, it should be emphasized that by establishing the concept of chemical transmission in peripheral nerves, Dale, Loewi, and Katz provided the foundation for further experimentation by others to probe the mechanisms of synaptic transmission in the central nervous system. As a result, major progress in our understanding of cell signaling mechanisms in the nervous system has led to better treatment and management of neurological and psychiatric disorders, which plague a major segment of our population.

In recognition of their extraordinary achievements, Dale and Loewi shared the Nobel Prize in 1936 for their work on chemical transmission of nerve impulses. Dale's contributions to pharmacology were legion, but it is important to single out his unique ability to distinguish, characterize, and classify drugs by virtue of their selective actions. In this way, Dale made fundamental and

lasting contributions to the development of pharmacology as a discipline. The legacy of Loewi also endures, although his later career was tainted by intrigue and politics. Two years after becoming a Nobel Laureate, Loewi was jailed by the Nazis for his religious beliefs. However, eventually Loewi was granted safe harbor to leave Austria, but only after he transferred his prize money to a Nazi-controlled bank and was deprived of all properties and belongings.

For the next few years, Loewi experienced a rather nomadic existence. He first sought haven at the Université Libre in Brussels, followed by another move to the UK in 1939. Once in the UK, he then traveled overseas in an attempt to establish himself at Harvard under the auspices of Walter Cannon. However, because of the massive influx of European scientists at that time, Harvard was reluctant to offer even a Nobel Laureate a faculty position. So, in 1940, Cannon contacted Homer Smith, his former research fellow and now a famed renal pharmacologist/physiologist, to offer Loewi a professorship in pharmacology at New York University (NYU) School of Medicine. Like so many other expatriated scientists of that era, Loewi expressed his gratitude by embracing his new country. For the rest of his life, Loewi worked at NYU during the winter and at the Marine Biological Laboratory in Woods Hole in the summer, where he continued to conduct research until his death in 1961. The life of Otto Loewi clearly illustrates how one unique individual envisioned and proved a scientific truth that had been ignored and even ridiculed. The full importance of Loewi's contributions can be better understood from the perspective that his breakthrough discovery led to the recasting of ideas about how nerves function.

C. Daniel Bovet: Synthetic Compounds That Inhibit the Action of Certain Body Substances, and Especially Their Action on the Vascular System and Skeletal Muscle

By identifying the physiological roles played by biogenic amines in cell function during the 1920s and 30s, Otto Loewi, Sir Henry Dale, and their colleagues set the stage for pharmacologists and chemists to pursue drug development in a structured and systematic manner. Toward this end, Daniel Bovet (Fig. 5), noting that drugs such as arsphenamine and sulfonamides had been introduced into therapeutics empirically, decided to take a more rational approach toward synthesizing and testing new pharmacological agents. Bovet based his strategy on the principles inherent in the antimetabolite theory of Woods and Fildes (Fildes, 1940; Woods, 1940). This theory explained the bacteriostatic action of sulfa drugs by virtue of their ability to competitively antagonize the normal cellular utilization of *p*-aminobenzoic acid, a metabolite with a chemical structure very similar to that of the sulfonamides.



FIG. 5. Daniel Bovet (1907–1992). Courtesy of Professor Egidio Miele, University of Sassari, Sassari, Italy.

Adopting this general approach for studying pharmacological activity, Bovet examined a series of chemical derivatives through several steps to determine which chemical groups were responsible for the antagonist or agonist activity in question. Bovet defined the steps as 1) an analysis of the structure of parent compounds possessing a specific pharmacological activity followed by 2) the synthesis and testing of various chemical derivatives or analogs for agonist or antagonist activity. In this way, Bovet applied the concept of competitive interactions to pharmacodynamics in the hope of providing clues as to the nature of the receptor site and possible mechanism of drug action. These advances, in turn, might then lead to the development of more useful and effective therapeutic agents.

Bovet favored the view that substances of natural origin such as ergotoxin, curare, and atropine could serve as useful models for the development of more selective receptor antagonists. Thus, he initially directed his attention to the ergot alkaloids, a fecund resource of pharmacological activity that was investigated by Sir Henry Dale many years before. In carrying out his work, first at the Pasteur Institute in Paris and then after 1947 at the Istituto Superiore di Sanità in Rome, Bovet initially sought the identity of the active principle of the ergotamine moiety. Toward this end, Bovet and his colleagues synthesized and characterized a voluminous number of compounds whose

actions would mimic or inhibit those of the naturally occurring ergot. However, the search for the active principle proved formidable, since the structure of ergot was quite different from that of epinephrine and other derivatives of phenylethylamine. Nevertheless, by using the autonomic nervous system as the test model, Bovet observed a gradual reduction in sympathomimetic activity and the emergence of antagonistic activities of compounds with structures of increasing complexity. However, when Bovet observed a lack of correspondence between the results obtained in animals and those found in humans, he discontinued the work with adrenergic blocking agents and redirected his attention to agents that acted at cholinergic sites.

Bovet realized that the study of anticholinergic agents would also prove to be a daunting task because of the multiple systems on which ACh acted, including postganglionic parasympathetic effector sites, autonomic ganglia, and the neuromuscular junction. Noting that ACh antagonists differed structurally, depending upon their site of action, Bovet decided to confine his investigations to an examination of the neuromuscular junction. Identifying the synapse as the primary locus of action of curare had been accomplished during the 1850s by the classic experiments of Claude Bernard. The specific mechanism of action of *d*-tubocurarine and other competitive neuromuscular blocking agents was elucidated more than a century later by Sir Bernard Katz and his colleagues using intracellular recording and microiontophoretic techniques.

Curare was a generic term for various South American arrow poisons. The crude preparations that were initially available consisted of a thick, black, gelatinous-like substance obtained from various remote sources in South America. These crude preparations made any analysis of the pharmacological properties of curare very difficult. In addition, to become effective therapeutically, a stable preparation had to be developed that possessed pharmacological actions that were free from undesirable side effects. So the study and therapeutic use of curare was thwarted for almost a century until the pure alkaloid, *d*-tubocurarine, finally became available in the 1940s. The alkaloid then was used as an adjuvant during general anesthesia (West, 1984).

With the advent of the pure alkaloid, Bovet and his colleagues could now use diverse methods of biological testing to compare the pharmacological properties of *d*-tubocurarine with analogs that were less complex structurally. Bovet's overall strategy was based upon the principle that manufactured pharmacological agents would prove more useful than naturally occurring substances, because they were more selective in their sites of action, relatively free from side effects, and had a shorter duration of action. Concentrating on phenolic esters containing quaternary ammonium activity, Bovet's team synthesized gallamine (Flaxedil). This drug had a more rapid onset of action than tubocurarine, as well as a

more rapid recovery time. Although the clinical use of gallamine was limited by its positive chronotropic effect and its proscription in patients with kidney disease, the rigorous analysis of structure-activity relationships identified several anticholinergic compounds that were less complex structurally and more useful than their naturally occurring counterparts in terms of specificity and absence of undesirable side effects.

At about the same time, Bovet initiated a study of tubocurarine analogs by varying the distance between the two quaternary ammonium moieties. He found that there were two key characteristics that defined the activity of such bis-quaternary derivatives, the distance between the quaternary groups and the size of the substituents added to the molecule. When the chain between the quaternary groups contained 10 carbon atoms (decamethonium), maximum pharmacological activity was observed. Coincidentally, William Paton and Eleanor Zaimis made the identical discovery of decamethonium in the UK at about the same time. They demonstrated that this agent acted by depolarizing the end-plate and thereby prevented it from responding to ACh. However, because it resembled curare in not being degraded by cholinesterase, decamethonium produced a muscle paralysis that was excessively prolonged and not reversible by an esterase inhibitor (Paton and Zaimis, 1949).

Among the drugs tested for their paralytic effects at the neuromuscular junction was succinylcholine, which was composed of two molecules of ACh attached end-on-end. Bovet's meticulous analysis of the properties of this agent was perhaps his most celebrated discovery. Its development provided a major contribution to pharmacotherapy, when it was shown that the drug was rapidly hydrolyzed by pseudocholinesterase. As a result, succinylcholine possessed a short duration of action as a muscle relaxant compared with *d*-tubocurarine and decamethonium. It therefore could be employed as an adjuvant in the form of a drip to more precisely titrate the level of muscle relaxation during general anesthesia. In this way, the potential hazards of surgical anesthesia were reduced.

It should be noted that many years earlier, Reid Hunt and Rene de M. Taveau reported on the pharmacological actions of a number of choline derivatives they had synthesized, including succinylcholine (Hunt and Taveau, 1906). However, these investigators failed to identify the neuromuscular-blocking properties of the agent, because they employed curare-pretreated animals as their model for drug testing. As a result, the introduction of succinylcholine into clinical use was delayed until 1942. So, just as Dale had shown that atropine blocked the action of ACh at muscarinic sites and ergot alkaloids annulled the effects of epinephrine and norepinephrine at postganglionic sympathetic sites, Bovet and his associates were responsible for providing the conceptual framework for analyzing the pharmacology of cholinergic antagonists at the neuromuscular junction.

Because of perceived similarities thought to exist among epinephrine, ACh, and histamine with respect to their pharmacological properties, Bovet extended the scope of his work in 1937 to include histamine antagonists. Pharmacologists had long understood that the development of drugs that were capable of blocking the actions of histamine would not only help to provide deeper insights into various aspects of physiological and pharmacological mechanisms but would also be of inestimable value in the treatment of allergic disorders. However, 25 years elapsed between the articles by Sir Henry Dale and Patrick Laidlaw (1910, 1911) on the pharmacological actions of histamine and the genesis of Bovet's work to develop drugs with histamine-blocking activities. But when histamine was identified as a constituent of the body, intense interest in this autacoid was created. Daniel Bovet was among the researchers who initiated efforts to produce antagonists of histamine. He first examined the adrenoceptor-blocking agent piperoxan, which was known to possess inhibitory activity against histamine in isolated intestine. Although Bovet found that several related compounds afforded some protection against the effects of histamine, they proved too toxic for clinical use.

Bovet and Anne-Marie Staub were able to obtain a better grasp on histamine antagonists when they investigated thymoxyethyl-diethylamine (Bovet and Staub, 1937). This agent protected guinea pigs against lethal doses of histamine, antagonized histamine-induced spasm of smooth muscle, and diminished the symptoms of anaphylactic shock. Although this compound, like several others initially developed, was found to be relatively ineffective and too toxic for clinical use, Bovet and his team persevered and in 1944 produced Neo-Antergan (pyrilamine) (Bovet et al., 1944). This drug is still used today as a selective H₁ antagonist in treating symptoms associated with acute allergies such as urticaria, rhinitis, and conjunctivitis. In describing the selective antagonism of responses to histamine, Bovet and Staub made it possible to construct certain general criteria for developing histamine antagonists, which are now mainstays in the treatment of a variety of allergic disorders. In addition, the contributions made by Bovet and Staub formed the basis for future structure-activity studies on histamine antagonists employed by other investigators, most notably Sir James Black.

In addition to publishing more than 300 articles that documented his work on drugs affecting the autonomic nervous system, the neuromuscular junction, and the actions of histamine, Bovet also probed various aspects of the pharmacology of the central nervous system. His observations with lysergic acid and its derivatives exerted a marked influence in the field of psychopharmacology, and in particular psychedelic drugs. By demonstrating that relatively simple molecules can modify changes in perception and mood, Bovet's work helped to

shape scientific thought regarding psychoactive drugs that are used in therapy today.

Perhaps Bovet's greatest legacy, however, is that he advanced the evolution of pharmacology as an established discipline and helped to promote the era of pharmacodynamics and a more mechanistic approach to pharmacology. In applying his expertise as an organic chemist to therapeutics, Bovet also helped to advance the discipline of pharmaceutical chemistry by conveying how chemical structure relates to pharmacological activity. Yet Bovet possessed the depth of understanding to realize that, because of the diversity of the chemical classes to which pharmacologically active agents belong, experimental findings cannot always be explained in terms of predictable scientific paradigms. For him, such atypical results could only be characterized as empirical findings. In honor of his groundbreaking contributions leading to the discovery of synthetic compounds that selectively inhibit the action of endogenous substances, Daniel Bovet was awarded the Nobel Prize in 1957.

D. Ulf von Euler, Julius Axelrod, and Sir Bernard Katz: Humoral Transmitters in the Nerve Terminals and the Mechanism for Their Storage, Release, and Inactivation

As previously chronicled, chemical transmission as a concept was attributed to a British student named Thomas Elliott, who in 1904 reported that there was a striking similarity between the action of epinephrine (which he called adrenaline) and sympathetic nerve stimulation. In 1910, Barger and Dale coined the term *sympathomimetic amine* to characterize the actions of a large series of amines that elicited physiological responses similar to those exerted by sympathetic nerve stimulation. Many years later in a volume composed of a compilation of several of his many articles, and having the advantage of hindsight, Dale laments the "opportunities missed" in the second decade of the 20th century to examine the analogs of epinephrine (adrenaline) (Dale, 1965). This oversight permitted a major discovery (chemical transmission) to elude him. It was only later that Dale realized that Elliott had been correct in principle and erroneous only with regard to the actual identification of the mediator. To profit from Dale's experience of a "missed opportunity," the reader may find it worthwhile to dust off his book and read his justification for not reaching what we now know is an obvious conclusion. Dale's comments seem generic for all who are engaged in scientific research.

1. Ulf von Euler. During the 1930s, certain investigators alluded to the possibility that norepinephrine might be the neurotransmitter liberated at adrenergic nerve endings. However, it was not until the mid-1940s that Ulf von Euler (Fig. 6) used various pharmacological and chemical assays to correctly identify the major catecholamine as norepinephrine (noradrenaline) in extracts of adrenergic nerves from different species. When it became necessary to differentiate epinephrine from

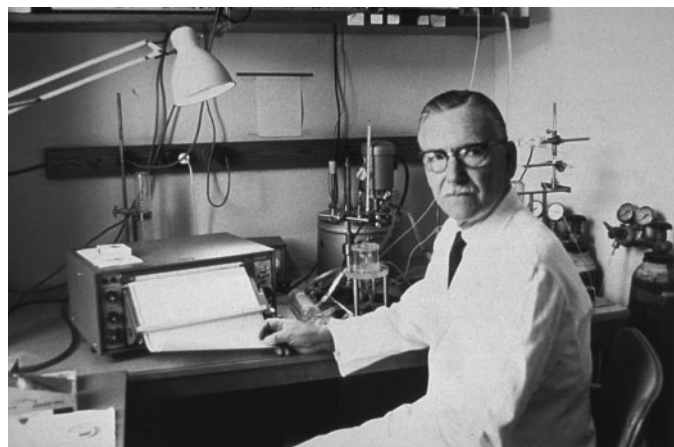


FIG. 6. Ulf von Euler (1905–1983). Courtesy of the National Library of Medicine.

norepinephrine, von Euler used two bioassays with different sensitivities to the two amines, such as the cat blood pressure and hen rectal caecum. A fluorometric technique for independently measuring epinephrine and norepinephrine, which was developed in von Euler's laboratory, also helped to raise the bar of research in this field.

The discovery of norepinephrine as the neurotransmitter at postganglionic sympathetic nerve endings positioned von Euler at the frontier of research in biogenic amines. In addition to demonstrating the presence of norepinephrine in almost all sympathetically innervated tissues of mammals, von Euler and his colleagues built upon these findings by later showing that adrenal glands of various mammalian species not only contained varying amounts of epinephrine and norepinephrine but also released them differentially, depending upon the mode and duration of stimulation. Ulf von Euler and Nils-Ake Hillarp also showed that a particulate fraction isolated from a homogenate of adrenergic nerve tissue sequestered a disproportionately large amount of norepinephrine (von Euler and Hillarp, 1956). Electron microscopy revealed that this particulate fraction was composed of granular structures that were later found to sequester biogenic amines. These studies placed a great deal of emphasis on events taking place at adrenergic nerve endings during synaptic transmission and complemented the key investigations relating to the synthesis and metabolic fate of the adrenergic neurotransmitter subsequently conducted by Julius Axelrod.

2. Julius Axelrod. Julius Axelrod (Fig. 7) was arguably one of the most beloved Nobel Laureates, who possessed all of the qualities that represent the best in our profession. His professional and personal attributes were characterized by systematic thinking, diligent effort, and humanity. Being one of the pioneers of neurotransmission and drug metabolism, Axelrod was responsible for developing treatments for the relief of pain and depression. But just as importantly, his determination



FIG. 7. Julius Axelrod (1912–2004).

in the face of the many obstacles that he encountered represents a shining example to anyone who is committed to fulfilling his/her life's ambitions.

Axelrod was born in 1912 on the east side of Manhattan and during his early education expressed a keen interest in attending medical school. In 1933, after graduating from City College of New York with a bachelor's degree, Axelrod's application to medical school was rejected. He lamented later that in those days religious bias may have played a role in the negative decision rendered to him. Because of the Great Depression, Axelrod found it difficult to obtain a suitable position in a scientific field, although for a brief time he worked as a laboratory technician at NYU at a starting salary of \$25 per month. He then went on to work for 10 years at the New York City Department of Health, where he was tasked with modifying methods that were used for evaluating the amounts of vitamin supplements added to foods. Although his work was tedious and uninspiring, the experience he gained in modifying methods for assaying vitamins proved invaluable in his later research. It was at NYU that Axelrod lost sight in one eye when a bottle of ammonia exploded in his face. This disability made Axelrod unfit for military duty, which enabled him to obtain his master's degree at NYU in 1941.

In 1946, while working at the Laboratory of Industrial Hygiene, Axelrod reached a crossroads in his scientific career when he became involved in studies concerned with the toxicity of the analgesic acetanilide. To prepare for this new project, Axelrod's supervisor suggested that he consult with Bernard Brodie (Fig. 8), Professor of Pharmacology at NYU. Brodie was also carrying out research at Goldwater Memorial Hospital, which had been established during World War II to test the clinical utility of various antimalarial drugs. Axelrod's meeting with Brodie spawned an 8-year association, first at Goldwater Memorial Hospital and then at the National Heart Institute (a branch of the NIH). Brodie, a man of



FIG. 8. Bernard Brodie (1909–1989). Courtesy of the National Institutes of Health.

extraordinary energy and an infinite source of ideas, would be responsible for directing Axelrod's early scientific career and for fostering Axelrod's long-term commitment to pharmacology.

Relying on his previous laboratory experiences, Axelrod developed methods for analyzing acetanilide. He soon discovered that it was a prodrug and exerted its actions by being metabolized to *N*-acetyl-*p*-aminophenol (acetaminophen) (Axelrod, 1948). In the early 1970s, Johnson & Johnson marketed the drug as Tylenol, which proved to be an effective and profitable alternative to aspirin. As a result of this work, Brodie invited Axelrod to remain at Goldwater Memorial Hospital to study the metabolic fate of other analgesics. As time passed, Brodie became ever more aware of Axelrod's prodigious talents. So when Brodie was recruited to the NIH as the Chief of the Laboratory of Chemical Pharmacology in the early 1950s, he invited Axelrod to accompany him.

Using in part the knowledge gained from his studies on acetanilide, Axelrod's first project at the NIH involved determining the metabolic processes by which ephedrine and amphetamine were metabolized (Axelrod, 1954). After only 1 year, Axelrod identified an enzyme localized in rat liver microsomes that deaminated amphetamine in the presence of NADPH and oxygen (Axelrod, 1955). At about the same time, Axelrod also found that ephedrine was demethylated to norephedrine by microsomal enzymes (Axelrod 1953). These studies disclosed the extraordinary talent that Axelrod pos-

essed as a research scientist, even though he had no doctoral degree.

During this period, several colleagues attempted to persuade Axelrod to leave Brodie's laboratory and continue his education. However, Axelrod always contrived reasons for not breaking the close ties with his overbearing mentor. In addition to not having the independence that he desired, Axelrod, after publishing 25 articles, many of them independently, was denied a promotion at the National Heart Institute because he did not have a doctoral degree. By the mid-1950s, the situation reached a climax when Brodie usurped major credit for the discovery of the microsomal enzyme system that is responsible for metabolizing drugs and other foreign substances. As time passed, Axelrod became more and more resolute in his feeling that Brodie had denied him primary credit for his discovery of the microsomal enzyme system. Although there was some difference of opinion regarding the validity of Axelrod's feeling of betrayal, the ill will that it generated caused a lasting schism between him and Brodie. Although Axelrod was ultimately afforded recognition for helping to lay the foundation of modern drug metabolism, he had become sufficiently disillusioned that he decided to take a leave of absence from the NIH and enrolled in the Department of Pharmacology at George Washington University. His tenure as a predoctoral student was unusually brief, because he had taken a number of requisite courses while pursuing his master's degree, and the doctoral thesis represented a virtual compilation of reprints of his previously published articles.

So, by 1955, at the rather advanced age of 42, Julius Axelrod had earned a doctoral degree and was now in a position to set up an independent research program (Axelrod, 2003). Because of his extensive experience working at Goldwater Memorial Hospital and then at the Laboratory of Chemical Pharmacology at the NIH, Axelrod soon was appointed Chief of the Section of Pharmacology at the National Institute of Mental Health. Fortunately for Axelrod, Seymour Kety was appointed the first Director of the Intramural Program at the National Institute of Mental Health and established a world-class program in which Axelrod would thrive.

Axelrod intended to launch an extensive study relating to the metabolism of biogenic amines. In searching for a specific project to pursue, Axelrod contemplated a biochemical analysis of the central nervous system and psychoactive drugs. In this context, Axelrod was apprised of an article that reported that epinephrine would turn pink when exposed to the air for several hours. This pink material, called adrenochrome, elicited great interest from the new independent investigator because it produced hallucinations when injected into animals. This action of adrenochrome led Axelrod to speculate that abnormal metabolism of catecholamines might provide an important clue to explaining the biochemical

basis of schizophrenia. However, very little was known at the time about how norepinephrine was normally released and metabolized so that its physiological action could be rapidly terminated.

Fortunately, Axelrod had the expertise to address this question, since he had previously investigated the disposition of sympathomimetics and other drugs with similar chemical structures. However, Axelrod understood that it was essential to first define the normal characteristics of catecholamine metabolism if he was to determine whether anomalous metabolism was responsible for symptoms of schizophrenia. Although initially unsuccessful in finding an enzyme responsible for converting epinephrine to adrenochrome, in 1957 Axelrod came across a brief abstract by Armstrong et al. (1957). This abstract reported the excretion of large quantities of an *O*-methylated product, 3-methoxy-4-hydroxy-mandelic acid, by patients with pheochromocytoma, a tumor of adrenomedullary chromaffin cells. To verify that this compound was a metabolite of norepinephrine, Axelrod launched a study to identify an enzyme that would *O*-methylate catecholamines. He found that when an extract of rat liver was incubated with *S*-adenosylmethionine, catecholamine was metabolized to metanephrine, the *m*-*O*-methylated product of epinephrine.

On the basis of this work, Axelrod postulated the existence of a pathway by which norepinephrine is converted to a methylated metabolite, with *S*-adenosylmethionine serving as an obligatory cofactor. Axelrod went on to find that other catechols, such as epinephrine, dopamine, and isoproterenol could also be converted to *O*-methylated products. Despite initial trepidation about introducing enzymology into his research program, Axelrod proved successful in isolating and purifying the enzyme, which he named catechol-*O*-methyl transferase (Axelrod, 1959). Fortunately, a colleague, Bernard Witkop, helped to advance the project even further by synthesizing crystals of the enzyme. So only 2 years after gaining independence as a scientific investigator, Axelrod made a fundamental discovery that is now included in textbooks of pharmacology, biochemistry, and physiology. The success that he achieved in carrying out these studies provided him with fresh thinking for pursuing research on the mechanisms involved in adrenergic neurotransmission.

For many years, it was believed that the actions of neurotransmitters were terminated by enzymatic transformation, with ACh being cited as the classic example. While realizing that norepinephrine must somehow be inactivated for the nerve to successfully transmit a subsequent stimulus, Axelrod was also cognizant of the fact that the mechanism involved in the termination of adrenergic transmission was a much slower process than that of ACh, which was very rapidly degraded by cholinesterase. These facts suggested to him that alternate mechanisms for inactivating catecholamines might exist.

Confirming that neurochemical transmission in the sympathetic nervous system was still extant when enzymatic metabolism (by oxidative deamination and *O*-methylation) was blocked, Axelrod was now confronted with the basic question of how the body dealt with the nonenzymatic disposition of norepinephrine. Because of the low endogenous levels of catecholamine in urine, Axelrod was aware that he would have to employ radiolabeled catecholamine for this study. Coincidentally, at about the same time Seymour Kety had ordered from New England Nuclear a batch of [³H]norepinephrine of relatively high specific activity. His intent was to investigate possible alterations in the metabolism of biogenic amines among schizophrenics. To add to the high cost of this endeavor, Kety had also purchased an early version of a liquid scintillation counter to quantitate radioactivity.

When Axelrod made the case for using a small aliquot of the expensive radioactive material for his experiments, Kety exhibited a lack of enthusiasm about donating the valuable isotope tracer for Axelrod's experiments in which he had no interest. However, Axelrod would not be deterred. After finally prevailing upon Kety to provide him with some of the radioactive compound, Axelrod injected it into a cat and examined various organs. Significant dividends were achieved by these experiments, since the high specific activity of the tritiated catecholamine enabled Axelrod to administer physiological amounts of the drug and then examine its localization and metabolism.

In sharp contrast to earlier studies demonstrating the rapid hydrolysis of ACh by cholinesterase, Axelrod found that the heart, spleen, and blood vessels, as well as salivary, adrenal, and pituitary glands sequestered large amounts of *unmetabolized* radioactive norepinephrine. His incisive analysis determined that the distribution of unmetabolized radioactivity followed a certain pattern: the greater the density of sympathetic innervation in a given peripheral organ, the greater the amount of radioactivity accumulated in that organ. Furthermore, he also observed that when an organ was chronically denervated, its ability to take up catecholamine was dramatically curtailed.

On the basis of these and other experiments, Axelrod postulated that the liberation of norepinephrine from sympathetic nerve endings led to an interaction of the catecholamine with its postsynaptic receptor to produce a physiological response. Following this interaction, norepinephrine was restored to the presynaptic neuron by an active uptake system, sequestered into vesicles, and subsequently released again. Axelrod further proposed that if the reuptake system was rendered nonfunctional, either by surgical or pharmacological means, norepinephrine remained at the receptor site, thereby producing an enhanced response. Axelrod extended these findings by observing that while the uptake system was dominant within the synapse, circulating catecholamines were inactivated

by an *O*-methylation reaction taking place in the liver and at certain postsynaptic sites.

So, by characterizing a novel and efficient uptake process for terminating the action of transmitter at sympathetic nerve endings, Axelrod made giant strides in our understanding of how the action of catecholamines is terminated. Although such a mechanism was not known to exist anywhere else in the nervous system, the novel and fundamental concept of catecholamine uptake first proposed by Axelrod was substantiated by a multitude of experiments performed in other laboratories, as well as by Axelrod and his many talented colleagues, including Richard Crout, Jacques Glowinski, Georg Hertting, Leslie Iversen, Irwin Kopin, and Lincoln Potter.

To prove that the mechanisms that governed catecholamine turnover in brain were similar to those established in the periphery and to circumvent the problem of the blood-brain barrier, during the 1960s Axelrod and Jacques Glowinski developed methods for injecting radioactive catecholamine directly into the brain and carrying out biochemical analyses. Not only did they find that the brain dealt with catecholamines in a manner similar to that of peripheral nerves, they also discovered that certain psychoactive drugs, such as cocaine and desmethylinipramine, could block catecholamine uptake, thereby creating an increase in unbound norepinephrine in brain, just as was demonstrated in the periphery (Glowinski and Axelrod, 1966).

Thus, on the basis of the work of Axelrod and his colleagues, the principles attributed to catecholamine metabolism that existed in peripheral nerves were generally extended to the central nervous system. From a broader perspective, they also had a particular salience for subsequent studies by others that led to important insights into the relationship between the action of certain drugs and the alteration in nerve function. In this way, Axelrod enhanced our understanding of the biological basis of human behavior. In addition, in presiding over a laboratory that was an active training ground for many of today's leaders in pharmacological research, Axelrod also provided the foundation for the advances made in the treatment of anxiety and depression. For example, there was no effective treatment for depression until imipramine, a classic inhibitor of norepinephrine uptake, was proven useful in the treatment of schizophrenia. These findings led to the screening of drugs that block the uptake of both catecholamines and serotonin [selective serotonin reuptake inhibitors (SSRIs)], thereby providing the pharmacological basis for developing potential antidepressants and antianxiety agents.

Except for the important finding by Ulf von Euler that norepinephrine served as the neurotransmitter of the sympathetic nervous system, virtually nothing had been known about the metabolism of biogenic amines prior to Axelrod's foray into the field of neuroscience. In carrying out his groundbreaking experiments, Axelrod used a simple strategy: 1) find a way to measure the amine in

question and then 2) trace its metabolic fate. While speaking at his alma mater George Washington University in 1971, 1 year after being awarded the Nobel Prize, Axelrod offered this general rule to new investigators: "Essential to research success is neither outstanding scholarship, nor exceptional intelligence, but rather motivation and commitment. This does not mean working in the laboratory day and night, but you think about the problems you are currently working with all the time, no matter what other activity you are engaged in" (Kanigel, 1986).

Over the years, Axelrod continued to make major discoveries in various areas. For example, together with Richard Wurtman, he became a pioneer in pineal gland research, making important contributions concerning its key hormone melatonin and the biosynthetic pathways involved in its metabolism. Because he always felt that science should be fun, Axelrod still frequented his laboratory as an unpaid guest researcher until the end of 2004, just prior to his death. His scientific philosophy, which he shared with anyone who would listen, encompassed a positive work ethic, a reverent and optimistic view toward science even in the face of discouragement and failure, and a broad view of science to explore fundamental problems. To implement these values, Axelrod maintained a relatively small laboratory so that he could interact closely with associates, whom he felt were to a considerable extent responsible for his accomplishments. Although Axelrod's superb acumen as a researcher cannot be overstated, he was also aided in his endeavors by the many valuable human and technical resources that were at his disposal at the NIH. These state-of-the-art methodologies helped Axelrod to combine his innate skills as a researcher with the technological and human resources available at the NIH to produce discoveries of monumental significance to neuroscience.

Finally, I would like to end this narrative of Julius Axelrod on a personal and rather self-serving note. In the early 1960s, I was pursuing my doctoral degree in the laboratory of William Douglas at the Albert Einstein College of Medicine in New York City. I was tasked with measuring adrenomedullary catecholamines by bioassay using Robert Furchgott's preparation of the isolated rabbit aortic strip. One day, Dr. Axelrod happened to pay a visit to our laboratory. After noting our arduous efforts to assay a few samples that took almost an entire day, he chided Douglas about still using the bioassay to measure catecholamines. As a result of his interaction with Axelrod, Douglas was goaded into purchasing an Aminco-Bowman spectrophotofluorometer. This newly developed instrument expedited the assay process using chemical means rather than the much slower analysis by bioassay and could detect low levels of biogenic amines. After that, I was able to carry out catecholamine analysis with much greater rapidity and efficiency. So, to Dr. Axelrod, I owe an eternal debt of gratitude for

expediting the attainment of my degree and for facilitating my future studies on medullary catecholamines.

3. *Sir Bernard Katz*. In his Nobel Laureate presentation, Sir Bernard Katz (Fig. 9) identified the common denominator between himself, Julius Axelrod, and Ulf von Euler in sharing the Nobel Prize in 1970. It had to do with their respective contributions that encompassed "discoveries relating to chemical transmission of nerve impulses." Although Sir Henry Dale and his many expert colleagues had shown years before that the transmission of a motor nerve impulse to the neuromuscular junction involved a chemical substance liberated by presynaptic nerve endings, Sir Bernard Katz and his co-workers Paul Fatt, Jose del Castillo, and Ricardo Miledi made an additional number of major conceptual advances in this field during the 1950s and 60s. By employing then novel electrophysiological techniques that included intracellular recording and microiontophoresis on single cells, they described the mechanistic basis of the local depolarizations at the chemosensitive motor end-plate (Fatt and Katz, 1952; del Castillo and Katz, 1956; Katz and Miledi, 1965a,b,c,d).

Using arduous and precise experimentation, Katz and his colleagues were able to propose that the resting nerve liberates a single packet of ACh (~1000 molecules), which is sufficient to produce miniature end-plate potentials. They further postulated that the arrival of an action potential at the presynaptic nerve terminal leads to a synchronous discharge of a number of ACh packets that can summate to trigger an evoked end-plate potential (EPP). When the ACh-evoked EPP is adequate in size, it will lead to generalized depolarization of the muscle membrane, calcium entry,



FIG. 9. Bernard Katz (1911–2003). Copyright Nobelstiftelsen.

and muscle contraction. The fact that the end-plate potential represented a statistical fusion of quantal components that were identical to spontaneously occurring components made it possible for Katz to conclude that the regulation of synaptic transmission could not be explained by electrical events but provided convincing evidence that favored the view that chemical processes regulate synaptic transmission.

In addition to unraveling the sequence of events associated with neuromuscular transmission, another major aspect of Katz's work related to demonstrating the pivotal role of extracellular calcium in making depolarization effective by regulating the amount of ACh released from presynaptic nerve endings. This most fundamental finding led to studies by others, including Bill Douglas and myself, confirming the pivotal role that calcium plays in exocytotic secretion, both in electrically excitable (nerve and muscle) and nonexcitable tissues (certain exocrine and endocrine glands). The work pioneered by Katz and his associates led them to propose the vesicle hypothesis (or quantal theory) to account for the liberation of neurotransmitter. According to this concept, synaptic vesicles containing ACh undergo frequent random collisions with the outer surface of the postsynaptic membrane at rest. The arrival of an action potential at the nerve ending would produce a nonselective increase in membrane permeability to physiological cations, including calcium. The entry of calcium then elicited the synchronous release of many quantal units of ACh, which would summate to depolarize the end-plate.

The work of Katz and his associates was far-reaching because in addition to describing the steps involved in transmission at the neuromuscular junction, this body of work also contributed immeasurably to our understanding of the interplay involved in drug-receptor interactions at other nonexcitable membranes. Recent publications by John Nicholls (2007) and George Augustine and Haruo Kasai (2007) succinctly describe the elegant work of Katz and his team and analyze its significance. They recount how Fatt (1950) and Fatt and Katz (1951) developed new tools and techniques to demonstrate that ACh caused an increase in the permeability of the end-plate membrane to allow ions to flow passively down their electrochemical gradients. From these findings, Fatt and Katz learned how a chemical signal generated by the interaction of an endogenous or exogenous agent with its receptor is converted to an electrical signal in the adjacent skeletal muscle fiber. Then, using the EPP to monitor ACh release from the motor nerve ending, del Castillo and Katz (1954) concluded that the EPP consisted of multiple, mini-like quanta of ACh and that calcium regulated the probability of a given quantum being released. These landmark articles remain the fundamental basis of our current understanding of the mechanism involved in the release and actions of neurotransmitters. Prior to these discoveries, knowledge of the mechanisms involved in synaptic transmission was

quite limited. The subsequent development of other novel approaches to the study of synaptic transmission then made it possible to investigate the release and actions of other neurotransmitters in peripheral nerves and neurotransmission in the central nervous system. Widely referenced in textbooks of neurophysiology, the work of Sir Bernard Katz and his talented colleagues still serves as a beacon for contemporary studies on synaptic mechanisms. The broad applicability of their findings is unconditional testimony to their enduring significance (Augustine and Kasai, 2007).

Due to the combined achievements of von Euler, Axelrod, and Katz, the scientific establishment finally embraced without equivocation the concept of chemical transmission of nerve impulses, and the discredited theory of electrical excitation finally faded from the scene. Moreover, because of their work, not only were the basic neurotransmitters of the adrenergic and cholinergic nervous systems finally identified, but von Euler, Axelrod, and Katz also helped in a major way to elucidate the processes involved with the biosynthesis, release, actions, and inactivation of neurotransmitters. These convergent findings incalculably enriched our fundamental understanding of a basic neurochemical process. In addition, by providing significant insights into the causes and treatment of such diseases as depression, anxiety, hypertension, Parkinsonism, Alzheimer's disease, and myasthenia gravis, the contributions of these ultra-talented investigators had an indelible influence on the subsequent development of drugs effective in combating psychic, neurological, and cardiovascular disorders. In 1970, Julius Axelrod, Ulf von Euler, and Sir Bernard Katz shared the Nobel Prize in honor of their prodigious contributions to fundamental knowledge regarding the transmission of chemical messages in neuronal systems.

E. Arvid Carlsson: Signal Transduction in the Nervous System

By the 1950s it had been firmly established that nerve cells communicated at synapses by chemical transmission, mainly involving ACh and norepinephrine. However, dopamine was perceived primarily as an intermediate in the biosynthetic pathway, originating with tyrosine and culminating in the formation of norepinephrine. Arvid Carlsson's (Fig. 10) interest in dopamine began in 1955 during a brief collaboration with Bernard Brodie, Chief of the Laboratory of Chemical Pharmacology at the National Heart Institute of the NIH. Although knowing very little about brain function, Brodie and his colleagues had the good fortune to gain access to reserpine, which had already been shown to be an antipsychotic and antihypertensive drug. In probing a possible link between changes in brain chemistry and its pharmacological actions, Brodie and coworkers established that reserpine produced an almost complete depletion of brain serotonin (5-hydroxytryptamine) levels. This finding was considered to be of major signifi-



FIG. 10. Arvid Carlsson (1923–). Copyright Nobelstiftelsen.

cance, since it disclosed for the first time an apparent link between certain biochemical changes and important brain functions (Carlsson et al., 1957c).

After Carlsson became knowledgeable about the new analytical methods for measuring biogenic amines, he suggested to Brodie that they investigate the effects of reserpine on brain catecholamines in light of their chemical similarity to serotonin. However, when Brodie showed no interest in extending the scope of these experiments, Carlsson sought a collaborator who had expertise in the field of catecholamines. Fortunately, Nils-Ake Hillarp was working at Carlsson's home institution at the University of Lund in Sweden and agreed to participate in a collaborative effort. This productive association would last until Hillarp's premature death in 1965.

Whereas Carlsson's expertise spanned the broad area of pharmacology, Hillarp's experience resided mainly in histology and histochemistry. In 1956, they published their first article, which described the depletion of catecholamines (epinephrine and norepinephrine) from the adrenal medulla of rabbits following reserpine treatment. Carlsson and Hillarp also found catecholamine depletion in brain and heart and observed that sympathetic nerves innervating these organs could no longer respond to nerve stimulation following reserpine treatment. They also showed that the administration of DOPA to reserpine-treated animals reversed the drug-induced effects. DOPA, a precursor of dopamine, was employed because, unlike the catecholamines (epinephrine, norepinephrine, and dopamine), it could penetrate the blood-brain barrier (Carlsson and Hillarp, 1956; Carlsson et al., 1957a,b).

However, when Carlsson and coworkers found that the restoration of norepinephrine levels was not observed in brains of reserpine-pretreated animals who were subsequently administered DOPA, the major focus of the project turned to dopamine, the intermediate in the conversion of DOPA to norepinephrine. This digression was prompted in part by experiments using monoamine oxidase inhibitors that had demonstrated that a biogenic amine other than DOPA was responsible for the observed behavioral effects. The fact that the repletion of serotonin levels by treatment with its precursor 5-hydroxytryptophan did not reverse the behavioral effects of reserpine also discounted serotonin as a key element in this process (Carlsson et al., 1958).

Although dopamine had generally been considered to be of limited physiological significance because of its modest activity on various smooth muscle preparations, Carlsson and Hillarp developed a biochemical method for quantitating catecholamines and showed that brain levels of dopamine actually exceeded those of norepinephrine. They also found that dopamine levels were depleted by reserpine, and the antireserpine action of DOPA closely correlated with the repletion of dopamine levels in brain. The discovery of the protracted depletion of catecholamines produced by reserpine, which took place in the late 1950s, was one of the most important findings in pharmacology up to that time. Not only did these findings enhance our understanding of both peripheral and central adrenergic mechanisms, they also laid the groundwork for other investigators to examine the actions of diverse drugs on brain amines.

Despite the convincing evidence accumulated by Carlsson, Hillarp, and their associates that favored chemical transmission in the central nervous system, a number of the most prominent figures in pharmacology, including Sir Henry Dale, Sir John Henry Gaddum, and W. D. Paton, were very skeptical about a link between biogenic amines and brain function. The recalcitrance exhibited by Dale in making this connection was surprising in light of the fact that he had championed the cause of chemical transmission in peripheral nerve for many years. In fact, at a meeting in London in 1960, Dale's strong reservations about associating biogenic amines with brain function were cogently expressed by his pronouncement that L-DOPA was a poison (Carlsson, 2003). Such views remained flashpoints for opponents of Carlsson's work and did little to validate his theories about synaptic transmission in brain. So the ardent challenges encountered by Carlsson from many of his peers were in some ways similar to those sustained by Otto Loewi some 40 years earlier. However, Carlsson, unlike Loewi, lacked support from the renowned and influential Sir Henry Dale. The general disinterest in Carlsson's work at that time was further exemplified by a statement made by Hugh McLennan in his 1963 monograph entitled *Synaptic Transmission* that there was "no evidence favoring chemical transmission in the central nervous system" (McLennan, 1963).

Since specific criticisms of his experimental evidence were not forthcoming, Carlsson became perplexed by the negative responses his work engendered and by the unwillingness of the scientific establishment to embrace his findings or at least offer alternative ideas. Nevertheless, Carlsson was not disillusioned and, together with Hillarp and a number of other collaborators, decided to augment his efforts to convince the scientific community that brain function involved chemical transmission and that dopamine served as a central neurotransmitter.

After Hillarp joined Carlsson full-time in the Department of Pharmacology at Göteborg University (Sweden), where Carlsson had been appointed Professor and Chair, the trihydroxyindole method was developed to detect catecholamines histologically by their ability to yield fluorescent products. Although this technique proved useful for studying the adrenal medulla, it lacked the sensitivity to detect catecholamines in adrenergic nerve endings. So Hillarp and colleagues devised a more sensitive method, using formaldehyde as the primary agent. They were then able to visualize norepinephrine by fluorescence microscopy in various adrenergic nerve preparations, including the iris.

Because of their efforts in establishing the requisite methodology to localize dopamine, norepinephrine, and serotonin in the central nervous system, Carlsson and Hillarp were able to provide irrefutable evidence to counter the criticisms raised by their colleagues. As a result, the tide began to turn, and putative roles of the biogenic amines in brain function began to be accepted as fact. During these studies, Carlsson also observed that in depleting catecholamine stores, reserpine produced Parkinson-like symptoms, i.e., rigidity and an inability to react to external stimuli. The administration of L-DOPA to animals not only repleted dopamine levels but also produced an abatement of the Parkinson-like symptoms. From these studies, Carlsson concluded that the etiology of Parkinson's disease involves a selective depletion of dopamine levels in the substantia nigra of the brain, which leads to a failure in dopamine release. On the basis of the elegant work by Arvid Carlsson and his team, the use of L-DOPA in the treatment of Parkinson's disease was instituted (Carlsson, 1959).

Carlsson's achievements put the development of a number of psychotropic drugs on a fast track. He was able to take advantage of Axelrod's earlier work by demonstrating that the antidepressant imipramine, a prototypical blocker of norepinephrine uptake₁, inhibited the reuptake of serotonin, as did a number of other antidepressants. Recognizing the important role that serotonin could play in brain function, Carlsson and his associates also developed an agent that would differentially block the neuronal uptake of serotonin without affecting norepinephrine uptake. This investigation would ultimately be responsible for the development of SSRIs (Carlsson, 2001).

The first such useful agent with antidepressant activity produced was called zimelidine. After a rare but serious side effect led to its withdrawal from the market, several other SSRIs were developed, including sertraline (Zoloft), fluoxetine (Prozac), and paroxetine. Since these drugs are not only useful in depressed patients but can also alleviate symptoms of anxiety, the SSRIs represent a major breakthrough in pharmacotherapy by virtue of their ability to alter the personality of individuals with psychological problems. These widely prescribed drugs thereby enhance quality of life and allow patients to function in a more productive manner.

Many of the early studies of drug action on peripheral nerves were arbitrarily extrapolated to the central nervous system because of the observed similarities between the synthesis, metabolism, and release of neurotransmitters in both systems. Carlsson provided hard evidence to justify this notion by demonstrating that certain pharmacological agents could produce chemical changes in the brain that correlated with changes in behavior. Carlsson and his associates were also responsible for spawning subsequent studies that revealed that certain major neurological and psychiatric diseases are associated with aberrations in dopamine signaling. We have already considered Parkinson's disease, which is now treated by repleting brain dopamine levels. In addition, currently used antipsychotic drugs block a subclass of dopamine receptors, and Attention Deficit Disorder is effectively treated with Ritalin by enhancing dopamine release. Thus, Carlsson's invaluable contributions demonstrated how a deeper understanding of the complex functions of the brain can lead to the development of more selective and effective drugs for treating various neurological diseases. For these fundamental scientific contributions, Arvid Carlsson was awarded the Nobel Prize in 2000.

F. Sir James Black, Gertrude Elion, and George Hitchings: Important Principles for Drug Treatment

1. *Sir James Black.* This interesting story had its genesis during the mid-19th century, when physicians found that nitroglycerin reduced the pain of angina pectoris (coronary insufficiency) by causing vasodilation of the coronary arteries. However, over a century later, Sir James Black (Fig. 11) became convinced that the therapy of this disorder could be improved after he became aware that all coronary vasodilators were not clinically effective. So in the early 1950s, while working at the Imperial Chemical Industries (ICI) Pharmaceutical Division in the UK, Black proposed that a better therapeutic strategy to treat coronary artery disease might prove to reduce inotropic action of epinephrine on the heart. Such an effect would reduce the demand of the heart for oxygen, thereby leading to the relief of anginal pain. His rationale was based upon the knowledge that heart rate was depressed by the blockade of sympathetic nerve activity and that the actions of catecholamines were



FIG. 11. James Black (1924–). Copyright Nobelstiftelsen.

associated with a decrease in the efficiency of the myocardium. To conduct these studies, Black preferred to use basic physiological and pharmacological principles to attenuate the actions of the sympathetic nervous system rather than by arbitrarily modifying natural products and testing results empirically.

Although the formulation of the word “receptor” was independently attributed to Paul Ehrlich and John Langley at the end of the 19th century, during the first half of the 20th century only a small group of researchers were advocates of the view that ligands bound to receptors. Being generally interested in the quantitative relationship between dose and response, these pharmacologists, who included Ariens, Furchgott, Clark, Stephenson, and Schild, probed the concept of “receptors” from a theoretical perspective and in terms of mathematical equations. By designing experiments that employed selective drugs, these pharmacologists were able to demonstrate the validity of their equations. For example, the Schild plot, which analyzed the binding of a competitive antagonist to its receptor, helped in large measure to define the characteristic properties of competitive antagonism. By contrast, Sir James Black attempted to explain in more mechanistic terms differences between the expression of different types of receptors with regard to both agonists and antagonists (Black, 1993). His ultimate goal was to develop a β -adrenoceptor-blocking agent that would reduce the work of the heart and thereby diminish the likelihood of cardiac insufficiency producing angina pectoris.

By the 1950s, adrenergic-blocking drugs were a well recognized class of pharmacological agents, and they

showed a pattern of action that was similar to that described for ergot alkaloids by Sir Henry Dale in the early 1900s. Adrenergic-blocking agents reversed the rise in blood pressure produced by epinephrine but did not block the associated tachycardia. Black was also aware that isoproterenol, the isopropyl derivative of nor-epinephrine, produced tachycardia, vasodilation, and bronchial dilation. The fact that these actions were not blocked by known antagonists of adrenoceptors indicated the involvement of an alternate cellular pathway.

To formulate a rationale for developing a β -receptor-blocking drug, Black decided to review his knowledge of autonomic pharmacology by reading the chapters in Victor Drill's *Pharmacology in Medicine*. In these chapters, Raymond Ahlquist proposed the existence of a dual adrenergic receptor system, in which the physiological effects of the catecholamines were mediated by α - and β -receptors. According to this classification, ergot alkaloids were α -receptor antagonists, and isoproterenol was a selective activator of β -receptors. Black would use this information to design his experimental protocol to develop a β -receptor antagonist.

In carrying out his initial studies, Black and his chemist John Stephenson first increased the size of the isopropyl group of isoproterenol on the amine nitrogen. However, this strategy proved unsuccessful until Black became aware of dichloroisoproterenol (DCI), an analog of isoproterenol. This agent had been synthesized by Eli Lilly by substituting chlorine atoms for the hydroxyl groups on the catechol ring. It inhibited the bronchodilator activity of isoproterenol and reversed the inotropic effects of epinephrine on the heart. As a result, DCI was classified as a β -receptor antagonist.

Not surprisingly, the advent of DCI generated a great deal of interest among pharmacologists. I remember the senior Dr. Gilman presenting a small sample of the material to faculty members at Albert Einstein College of Medicine who were preparing Otto Kraye's famous heart-lung preparation for demonstration to the medical students. I do not specifically recall the resultant findings, except to note that the responses to DCI did not engender great excitement as a new pharmacological tool. At about this time (in the late 1950s), Bill Fleming, now an Emeritus Chair of the Department of Pharmacology and Toxicology at the West Virginia University School of Medicine, also carried out an investigation of the effects of DCI on the dog heart-lung preparation in Kraye's laboratory while a postdoctoral fellow at Harvard. Unaware of Black's work, but noting that Neil Moran and M. E. Perkins had observed both stimulatory as well as inhibitory actions of DCI in response to catecholamines in isolated heart (Moran and Perkins, 1958), Fleming and another postdoctoral fellow, Dennis Hawkins, found that the application of experiments based upon E. J. Ariens's receptor theory to the action of DCI on the heart met the criteria for a

partial agonist rather than a pure antagonist (Fleming and Hawkins, 1960).

Sir James Black and his team at ICI also found that DCI possessed strong β -agonist activity in certain bioassays, including the classical Langendorff preparation (isolated guinea pig heart). During this investigation, Black became intrigued with the idea that the expression of the pharmacological properties of a substance could vary greatly, depending upon the specific bioassay used. Therefore, he decided to develop a new *in vitro* assay system that employed a guinea pig cardiac papillary muscle preparation to measure strength of contraction independently of rate changes. Using this preparation, Black and his colleagues were able to confirm that DCI possessed the properties of a sympathomimetic amine rather than those of a pure antagonist of β -receptors. These experiments finally persuaded Black to discontinue his studies with DCI and to search elsewhere.

Using his expert knowledge as a chemist, John Stephenson concluded that the synthesis of the naphthyl analog of isoproterenol would enable Black to achieve his goal of producing a relatively pure antagonist of β -receptors. The resulting agent, which was given the name compound ICI 38174, but eventually called pronethalol (nethalide), exhibited antagonist activity with only modest agonist activity in both atrial and ventricular preparations. The administration of pronethalol enabled a patient suffering from angina pectoris to perform a greater amount of work prior to the onset of chest pain. However, the agent elicited several unpleasant side effects in humans and was eventually withdrawn from clinical trials when it produced thymic tumors in mice (Black and Stephenson, 1962).

To acquire a safer and more effective drug, Black, Stephenson, and their coworkers synthesized and screened a large number of additional compounds. This screen provided a new dimension to the field of antiadrenergic drugs by the discovery of propranolol, which possessed 10 times greater blocking activity on β -receptors than pronethalol and at the same time exhibited minimal agonist activity. The competitive nature of the antagonism was proven by bioassay when propranolol produced a rightward displacement of the dose-response curve. The linearity of the dose-response curves and the slope of the Schild plot indicated that catecholamines (isoproterenol or epinephrine) competed with propranolol at the same site. These convergent findings gave credence to the proposition that propranolol was a relatively pure β -receptor antagonist. Black's advocacy of drug selectivity had finally yielded major dividends.

In the pursuit of developing more effective and less toxic pharmacological agents, Black eventually concluded that all β -receptors were not pharmacologically similar, in that norepinephrine was an effective cardiac stimulant but produced only modest vasodilation in skeletal muscle. Although propranolol blocked all β -receptors to a similar extent and therefore was later classified as a nonspecific blocker of β -receptors, by the early

1960s, Black succeeded in developing clinically useful agents that selectively annulled the cardiac effects of epinephrine and norepinephrine. By developing drugs that produced a selective blockade of adrenergic receptors, Black and his coworkers laid the groundwork for formulating the concept of β -receptor subtypes and were responsible for the explosive developments that were to occur in cardiovascular pharmacology in the ensuing years. These developments have contributed immeasurably to the relief of the pain of angina pectoris, the reduction in blood pressure in hypertensives, and an increase in the survival rate of patients following a myocardial infarction.

In 1964, Black moved to SmithKline & French Laboratories to pursue another project that had profound physiological, pharmacological, and therapeutic implications. Black had previously studied the factors that regulated gastric secretion, which he knew involved histamine. He was also aware that excessive secretion of gastric acid was associated with peptic ulcers and that the antihistamines available then could not ameliorate the symptoms of ulcers, although they did blunt the allergic effects of histamine. Based upon this knowledge and his previous studies on β -adrenoceptors, Black postulated that histamine binds to two different types of receptors in the body.

At the time, antacids and anticholinergic drugs (which were sometimes accompanied by serious side effects) provided some amelioration of the symptoms of ulcers, but only surgery was deemed curative. Black decided to build upon previous observations that patients with ulcers elicited an exaggerated response to histamine. In fact, the enhanced response to histamine was the basis of a diagnostic test for peptic ulcers. Realizing that effective inhibitors of histamine action would be indispensable in basic research and pharmacotherapy, Black embarked on an entirely new pharmacological approach in the mid-1960s to define a more favorable treatment for peptic ulcers. The objective was to develop a drug that would obtund gastric acid secretion but would possess limited effects on other effectors subserved by histamine, such as smooth muscle contraction. In accomplishing this feat, Black would have to address the question as to why antihistaminics available at the time could not curtail the production and release of gastric acid (and vasodilatory actions), despite the fact that they blocked the allergic responses to histamine (contractions of gut smooth muscle).

As early as 1937, Daniel Bovet and his colleagues produced the first antihistamine, thymoxidiethylamine, at a time when the classification of histamine receptors had yet to be established. Although this drug prevented anaphylactic shock in animals, it proved too toxic to be useful in patients, and the project was discontinued. During the 1940s and 50s, antihistamines were composed of a structurally diverse group of agents that were powerful inhibitors of histamine-induced smooth muscle

contractility. Following the proposal by Ash and Schild (1966) that H_1 receptors mediated mepyramine-sensitive antihistaminic responses, Black envisioned the existence of a second type of histamine receptor. In devising bioassays for his experiments, Black chose the guinea pig ileal smooth muscle, the classic system for studying antihistamine activity. He also selected guinea pig atrial tissue (pacemaker frequency) for examining histamine responses resistant to mepyramine and a luminal perfusion assay for analyzing gastric secretion.

Meanwhile, recognizing the obvious market value of a drug that could block acid secretion, the chemists at SmithKline & French set out to synthesize a host of derivatives of histamine, focusing on modifying the imidazole ring end of the histamine moiety. This program evolved over several years of negative screening by monitoring acid output. The discovery of 4-methyl histamine as a selective histamine receptor agonist by eliciting acid output in the absence of muscle contraction finally provided an important clue and the impetus that would ultimately propel this project to a successful completion.

Revitalized by the discovery of 4-methylhistamine, Black and his associates used several different *in vitro* and *in vivo* bioassays and a new experimental design to identify compounds that would exhibit antagonism. Using this approach, they found that guanylhistamine produced a modest inhibition of gastric secretion, thus classifying it as a partial agonist and functional analog of DCI. However, unlike DCI, the pharmacological activity of histamine was diminished by modifying the side chain rather than the ring structure. Chemists at SmithKline & French then lengthened the histamine side-chain to four carbon atoms and replaced the basic guanidine group with a methyl thiourea group to produce burinamide. This drug became the first antagonist of histamine-induced acid secretion with low agonist activity.

On the atrial bioassay, burinamide exhibited surmountable antagonism, shown by rightward parallel displacement of the histamine dose-response curve. Taken together with its relative ineffectiveness on the ileum, which is an H_1 system, Black postulated in 1972 that burinamide was an antagonist of a new histamine subtype, which he called the H_2 receptor. This newly discovered subtype was deemed to be responsible for mediating acid secretion and cardiac stimulation, whereas smooth muscle contraction and various allergic and inflammatory responses were expressed through H_1 receptors. However, enthusiasm for this drug waned when it was found that burinamide exhibited low potency and bioavailability. As a result, the search for an antagonist had to continue (Black et al., 1972).

In 1975, Black's perseverance was eventually rewarded when he and his team developed a more clinically useful H_2 receptor antagonist called cimetidine. Perhaps the vital significance of Black's contribution may be best perceived by the realization that cimetidine, which was marketed under the brand name Tagamet,

became the world's first billion-dollar drug. In addition to the extraordinary contributions made by Black to pharmacotherapy by introducing a drug effective in ulcer healing and gastroesophageal reflux disease, Black's finding that H_2 receptor antagonists blocked acid secretion (histamine-, meal-, and gastrin-stimulated) reinforced the relevance of histamine as a physiological mediator of acid secretion. Finally, by characterizing the histamine receptors as H_1 and H_2 subtypes, Black was also instrumental in developing new approaches to the treatment of allergic and inflammatory disorders.

In conclusion, Sir James Black judiciously exploited the analytical power of competitive antagonists to develop drugs that diminished the oxygen requirements of the heart. This advance made it possible to produce more effective and useful agents for the treatment of coronary insufficiency, myocardial infarction, angina pectoris, and hypertension. In addition, Black's emphasis on drug selectivity culminated in the safe use of H_2 receptor antagonists, which ultimately led to their availability over the counter. Although the development of the more efficacious proton pump inhibitors have somewhat curtailed present-day use of H_2 receptor antagonists, Black has been cited as responsible for developing "among the most successful agents in the history of medicine" (Burks, 1995).

2. *Gertrude Elion and George Hitchings.* The personal story of Gertrude Elion (Fig. 12) and her development as a superstar in the field of drug discovery is a very interesting one and merits consideration in some detail. Like the early professional life of Julius Axelrod previously chronicled, Elion's entry into the world of research was preceded by a series of disheartening events. However, her experiences with unchallenging, temporary, and even nonpaying jobs did little to dampen



FIG. 12. Gertrude Elion (1918–1999) and George Hitchings (1905–1998). Courtesy of the Estate of Gertrude B. Elion.

her enthusiasm and motivation to accomplish her goals, which were in her own words “to become a scientist, and particularly a chemist, so that I could go out there and devise a cure for cancer” (<http://www.nap.edu/html/biomems/gelion.html>). Her perseverance in the face of discrimination and disappointment is an enduring legacy to anyone who aspires to a more challenging and rewarding existence.

Gertrude Elion earned her bachelor’s degree from Hunter College in New York City in 1937, with a major in chemistry. Because of the Depression, the requisite financial resources to allow her to continue her education were lacking, and so she sought employment. After quickly learning that job opportunities for a female with a science degree were very limited, she attended secretarial school, worked as a receptionist, taught at a high school, and had a 3-month job teaching biochemistry to nurses. She also succeeded in finding a position as a chemist, working without pay so she could keep busy and learn from the experience. After a year and a half, she began earning \$20 per week and accumulated sufficient funds to obtain her master’s degree from New York University.

Still, Elion’s career goals were elusive. In fact, prospective employers were querulous about why she wanted to be a chemist, since at the time laboratory work was not considered suitable for women. With the advent of World War II, her opportunity finally came, when it became imperative to hire women because many men were assuming military obligations. Initially, Elion was employed by a major food company as an analytical chemist to test the acidity of pickles and the color of mayonnaise, among other responsibilities. After some time, she found the quality control work repetitive and unchallenging, and despite the fact that she learned a great deal about instrumentation, her probing mind mandated that she seek a more meaningful occupation.

An intriguing opportunity finally surfaced for Elion at Burroughs Wellcome, an international pharmaceutical firm that had a branch located in Westchester County, New York. At this facility, George Hitchings (Fig. 12) was attempting to develop clinically useful pharmacological agents by producing antagonists to nucleic acid derivatives. Elion’s appointment as a research assistant in Hitchings’s laboratory would mark the beginning of one of the most productive collaborations in science. Elion was originally hired in 1944 and was gradually promoted through the ranks until 1967, when she became head of the Department of Experimental Therapy, a position she would hold until her retirement in 1983.

Elion admitted that prior to her first meeting with Hitchings she had never heard of purines and pyrimidines. But the idea of successfully treating a variety of diseases by interfering with DNA synthesis seemed to be a very exciting challenge. Despite her chemically oriented background, Elion became deeply immersed in the biological effects of the compounds she synthesized.

Over the ensuing years, she also broadened her general knowledge of pharmacology, biochemistry, immunology, and eventually virology. Shortly after assuming her new position, Elion also began taking evening courses at Brooklyn Polytechnical Institute in pursuit of a Ph.D. degree. However, she terminated this venture after 2 years because the school required that she spend 1 year as a full-time student. In addition to Elion’s reluctance to temporarily abandon her exciting new challenges, Hitchings advised her that an advanced degree was not necessary to accomplish the work that they were going to carry out. So Gertrude Elion would become one of the few Nobel Laureates who never obtained a doctoral degree. Although Hitchings’s motives regarding Elion’s education may have been somewhat self-serving, Elion never expressed any regrets about abandoning her quest for the doctoral degree. Even without a degree, Gertrude Elion was fully funded by Burroughs Wellcome to carry out her research, elected to the prestigious National Academy of Sciences in 1990, and awarded honorary doctoral degrees from George Washington University, the University of Michigan, and Brown University. Her status as an elite scientist would be established.

It was in the early 1950s that Gertrude Elion and George Hitchings proposed that “with the aid of drugs, it should be possible to selectively inhibit the synthesis of nucleic acids used by microorganisms and neoplastic cells” (http://nobelprize.org/nobel_prizes/medicine/laureates/1988/presentation-speech.html). Their hypothesis was based upon the antimetabolite theory separately published by Donald Woods and Paul Fildes in 1940 to explain the mechanism of action of the recently developed sulfa drugs. Woods had observed that the inhibition of bacterial growth by sulfonamides could be competitively antagonized by *p*-aminobenzoic acid, a normal metabolite that bears a very close structural similarity to sulfonamides and is used by bacterial and neoplastic cells for folic acid synthesis. Hitchings and Elion postulated that a resulting deficiency in folic acid production caused by antimetabolites would lead to aberrations in the synthesis of purines and pyrimidines and therefore of DNA. Extending this line of reasoning, Hitchings theorized that it therefore might be possible to retard the growth of rapidly dividing cells, such as neoplastic cells and bacteria, with antagonists of nucleic acid bases. This strategy was based upon the premise that the *de novo* synthesis of folic acid could be preferentially inhibited in neoplastic and bacterial cells, since normal human cells lack the capacity to produce folic acid and must obtain it from the diet. The concept of differentially interfering with DNA synthesis would form the basis of our present understanding of antimetabolites as anti-neoplastic agents and immunosuppressants.

Although Elion was responsible for examining systems that were composed of purines and pyrimidines, at the time basic knowledge about nucleic acids was quite meager. The basic sequences of nucleic acids were un-

known, and the helical structure of DNA had not yet been formulated by Watson and Crick. In addition, the anabolic pathways involved in the utilization of purines for nucleic acid synthesis had not yet been mapped. Moreover, techniques available for investigating this general area were very limited in that paper and ion-exchange chromatography were still unavailable. In addition, Geiger counters, rather than liquid scintillation counters, were still employed to count radioactivity.

To carry out their studies, Hitchings and Elion first had to designate an appropriate method for determining whether a compound could substitute for purine or thymine and/or inhibit their utilization for nucleic acid synthesis. So Hitchings and Elvira Falco (Hitchings et al., 1945) developed an assay system for screening antibacterial activity using *Lactobacillus casei*. The advantage of using *L. casei* resided in the fact that they could grow in a mixture of thymine and a purine and could also synthesize purines if provided with a source of folic acid. In 1948, Elion used this assay system to discover that 2,6-diaminopurine markedly inhibited the growth of *L. casei*, an effect that was specifically reversed by adenine but not by other naturally occurring purines (Elion and Hitchings, 1950; Hitchings et al., 1950).

Thereafter, 2,6-diaminopurine was one of several compounds sent to the Sloan-Kettering Institute for testing in cancerous mice. This drug was shown to inhibit growth of mouse tumors and tumor cells in culture and induce remissions in chronic granulocytic leukemia. However, the incidence of severe nausea and vomiting, as well as bone marrow depression, precluded further consideration of this drug as a therapeutic agent. But by that time collaborations with the Sloan-Kettering Institute and other laboratories had expedited the expansion of drug testing for antitumor activity.

By 1951, after examining over 100 purines in the *L. casei* screen, Elion discovered that the substitution of oxygen by sulfur at the 6-position of guanine and hypoxanthine produced effective inhibitors of purine metabolism. Two of these compounds, 6-mercaptopurine (6-MP) and 6-thioguanine, displayed significant activity against a wide variety of rodent tumors and leukemias when tested at Sloan-Kettering. The promising animal studies led to a clinical trial at Sloan-Kettering Memorial Hospital in New York, which highlighted the relative effectiveness and safety of 6-MP as an antileukemic agent. The additional finding that a remission was later induced in a patient administered 6-MP, who had relapsed after treatment with a folic acid antagonist, indicated that there was no cross-tolerance between 6-MP and the folic acid antagonist (Burchenal et al., 1951). This represented a most significant finding, because it established the regimen of combination chemotherapy that is commonly used today in many areas of pharmacotherapy (Elion, 1993).

Because 6-MP could produce complete remission in children with acute leukemia, although relapses were

frequent, the Food and Drug Administration approved its use only 10 months after clinical trials began and before all of the data defining its effectiveness and toxicities were made available. The addition of 6-MP to the short list of antileukemic drugs available then increased the average survival time in children from 3 to 4 months to more than 12 months. Gertrude Elion was only 32 years old when she synthesized 6-MP and thioguanine, the drugs that revolutionized the treatment of leukemia. Today, as a consequence of the combined efforts of Elion and Hitchings in developing 6-MP, most children with acute leukemia can anticipate a remission when the drug is used in combination with two or three other agents, including methotrexate, and some patients can even be cured. Similarly, 6-thioguanine is of particular value in the treatment of acute granulocytic leukemia when employed in combination with cytarabine.

Any immoderate enthusiasm concerning the clinical efficacy of 6-MP was tempered by the fact that in 1952 little was known about its mechanism of action, particularly as it related to its differential effect on neoplastic cells. So, following up on their previous work, Elion and Hitchings carried out experiments aimed at providing a biochemical explanation for the actions of 6-MP. However, it was not until the mid-1950s that the pathways for purine synthesis and utilization were mapped, and the complex reactions involved in 6-MP metabolism and the site(s) of action of nucleotides derived from 6-MP were defined. Several approaches to develop more effective pharmacological agents were then undertaken to improve the therapeutic effectiveness of 6-MP, which led to the production of azathioprine. This drug, which serves as a prodrug for 6-MP, possesses a therapeutic index comparable to 6-MP in patients with leukemia.

The clinical utility of 6-MP began to enjoy additional significance in the late 1950s, when Robert Schwartz and William Dameshek in Boston investigated the effects of the drug on the immune response. This investigation was prompted by their astute observation that immature lymphocytes generated during the immune response closely resembled leukemic lymphocytes. The validity of this approach was affirmed by Schwartz's demonstration that the administration of 6-MP to rabbits suppressed the immune response to a foreign antigen (Schwartz et al., 1960). The additional finding by Roy Calne in the UK that 6-MP and azathioprine prevented the rejection of canine kidney homografts led to the successful use of a combination of azathioprine and prednisone for organ transplantation in humans beginning in 1962 (Calne, 1960). Today, azathioprine still remains a key pharmacological component of drug combinations used in renal transplantation and in the treatment of autoimmune diseases, such as rheumatoid arthritis. In addition, 6-thioguanine is still used as an immunosuppressant, especially in patients with nephrosis and collagen-related vascular disorders.

At about this time, the synthesis of 2,4-diamino-5-phenoxyrimidine enabled Falco and Hitchings to initiate studies on selective inhibitors of dihydrofolate reductase, again using *L. casei* as the assay system. This screening test for antibacterial activity not only determined whether a compound could serve as a thymine or purine analog but could also assess whether a compound was a folic acid antagonist. The earliest evidence concerning the mechanism of action of dihydrofolate reductase inhibitors emerged from the finding that the folic acid-induced growth of a streptococcal strain could be readily inhibited by a diaminopyrimidine. However, when tetrahydrofolate was employed to induce growth, 2 to 3 orders of magnitude higher concentrations of the diaminopyrimidine were required to produce the blockade. These findings suggested that the inhibition of an unidentified enzymatic reaction was responsible for the reduction of folate to tetrahydrofolate.

By 1950, it was apparent to Hitchings and his team that the enzyme involved in these reactions was dihydrofolate reductase, which was subsequently isolated and characterized from different sources. As a result of this work, Hitchings and coworkers were credited with developing methotrexate, a structural analog of folate. This drug soon became a key component in the regimen for treating acute leukemia and is still of primary importance in cancer chemotherapy today. Hitchings and his colleagues also developed the antimalarial drug pyrimethamine and the antibacterial agent trimethoprim. By demonstrating that the antibacterial actions of each agent were enhanced by combining them with sulfa drugs, Hitchings and his associates further promoted the concept of combination therapy (Hitchings, 1993).

In early 1970, Elion, Hitchings, and many of the other Wellcome employees moved from facilities in Tuckahoe, NY to those in Research Triangle Park, NC. It was there that the focus of research turned to the development of the first antiviral drug, acyclovir. This agent still occupies a prominent place in the treatment of Herpes-related diseases. Finally, the collaborative work of Hitchings and Elion culminated in the addition of allopurinol to the pharmacological armamentarium. This drug represents another example of how a rational chemical strategy can be used to develop a valuable pharmacological agent. Originally synthesized and screened as a possible antineoplastic agent, allopurinol elicited an increase in the action of 6-MP by inhibiting its oxidative metabolism. However, this increase in pharmacological activity was accompanied by a proportional increase in toxicity. Since xanthine oxidase not only catalyzes the oxidation of 6-MP but also the conversion of hypoxanthine and xanthine to uric acid, allopurinol was found to produce a dramatic reduction in serum and urinary uric acid. These findings led Hitchings and Elion to

recommend that allopurinol would be effective against gout and other forms of hyperuricemia.

The innovative ideas and new insights stemming from the work of Elion and Hitchings are a testament to the dramatic results that can accrue by the pooling of expertise. Whereas the team led by Hitchings focused on selective inhibitors of dihydrofolate reductase, purine analogs occupied a key role in Elion's work. In both cases, their extraordinary contributions led to the development of drugs that have been invaluable in treating such diverse medical disorders as leukemia, organ transplant, gout, and bacterial and viral infections. Meanwhile, Black used a novel strategy to develop drugs that were more effective and useful agents for the treatment of coronary insufficiency and other vascular diseases. In addition, Black's emphasis on drug selectivity culminated in the development of over-the-counter drugs that are now used for the treatment of gastric disorders. The resonating theme intrinsic to the strategy used by all three of these gifted scientists speaks to achieving selectivity of drug action by using basic physiological and pharmacological principles. The discoveries of important principles relating to drug treatment made by Black, Elion, and Hitchings earned them the Nobel Prize in 1988.

G. Paul Ehrlich: The Magic Bullet

The successful treatment of infectious and other inflammatory diseases has been a major challenge to researchers and clinicians since the earliest of times. For several centuries, the *cinchona* bark was used as a remedy against malaria. Salts of mercury were also employed as an important constituent of antibacterials and antiseptics, although safer and more effective modes of therapy eventually superceded them. During the late 1870s, as part of the growing interest in the germ theory of disease, anyone who harvested bacteria was destined to observe even by chance the production of contaminants that were antagonistic to microorganisms. It was therefore not surprising that the concept of microbial antagonism and its potential significance for pharmacotherapy was frequently addressed in the scientific literature during the latter part of the 19th century. For example, Joseph Lister, while pioneering the development of antiseptic surgery, observed alterations in bacterial activity when fungi were also present (Crellin, 1980). In the 1870s, Louis Pasteur, in describing the inhibition of anthrax bacilli after inoculating them with common microorganisms, proposed that common bacteria have the ability to kill other bacteria, both in vitro and in vivo (<http://www.fordham.edu/halsall/mod/1878pasteur-germ.html>). Although Pasteur did not pursue these studies, he did suggest the possibility of using the concept of microbial antagonism for clinical purposes.

The early development of antibacterial agents was in principle predicated upon Paul Ehrlich's (Fig. 13) idea of the selective interaction of chemical substances with

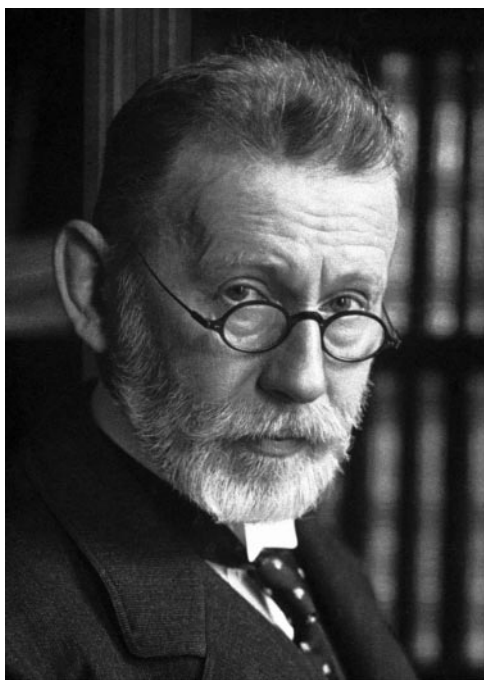


FIG. 13. Paul Ehrlich (1854–1915). Copyright Nobelstiftelsen.

receptors (or side chains) expressed by pathogenic microorganisms. In the 1890s, after discovering that methylene blue could stain malaria-producing plasmodia, Ehrlich administered the dye to two patients suffering from a mild form of this disease. The publication of the resulting cures represented the first report of a synthetic drug being used successfully to treat a specific disease. However, Ehrlich was unable to continue this work because of his inability to infect animals with malaria. Moreover, at the time he was working in Robert Koch's laboratory in Berlin, where his primary task was to transform diphtheria antitoxin into a clinically useful preparation (Ehrlich, 1913).

Ehrlich made further advances in chemotherapy early in the 20th century, when he introduced arsenicals for the treatment of syphilis. At the time, this disease was a rampant, world-wide affliction. Ehrlich predicated his approach to the problem on the premise that an infection caused by a microorganism could be cured if the drug of choice was selectively taken up by the invading microbes. After intensive screening of more than 600 arsenicals, compound 606 (the 606th compound to be tested) was found active in rabbits against *Treponema pallidum*, the microorganism that caused syphilis (Ehrlich, 1912).

Although Ehrlich has been acclaimed as the first investigator to develop a synthetically produced drug that could target and impair microbial activity, it is ironic to note that compound 606 was originally recorded by one of Ehrlich's assistants as "negative" during the original screening procedure. But when Sacachiro Hata, who originally developed the method for infecting rabbits with syphilis, joined Ehrlich's

laboratory in 1909, the analysis of compound 606 yielded positive results against syphilis in rabbits (Ehrlich and Hata, 1910). After the drug was tested on hospital patients in 1910, it was marketed as "Salvarsan", and later given the name arsphenamine. Arsphenamine was later replaced by neoarsphenamine, which contained only 19% arsenic and therefore was less toxic than other arsenicals.

At the time, syphilis was a chronic debilitating and ultimately fatal disease, just as AIDS is today; so the introduction of arsenicals had an enormous impact world-wide. Within 5 years, the incidence of syphilis in several European countries decreased by 50%. However, the search for an antibacterial agent with a broader spectrum of action and fewer side effects continued. Although the effectiveness of various metals such as bismuth, gold salts, and antimony proved variable at best, by developing arsphenamine Ehrlich served as an influential advocate for using systemically administered drugs to treat infectious diseases. In this way, Ehrlich provided the foundation for modern chemotherapy. In 1908, Ehrlich was awarded the Nobel Prize in Medicine for "outlining the principles of selective toxicity and for showing preferential eradication of cells by chemicals" (Ames, 2001).

Although interest in microbial antagonism was maintained throughout the first 3 decades of the 20th century, the futile search for more effective and less toxic antibacterial agents indicated that a more fruitful line of research was needed. In the mid-1930s, a breakthrough finally came from I.G. Farbenindustrie Aktiengesellschaft (I.G. Farben), a giant conglomerate founded in 1926 by a merger of eight leading German chemical manufacturers, including Bayer and Hoechst. The breakthrough would lead to the development of the sulfa drugs.

H. Gerhard Domagk: Antibacterial Effects of Prontosil

The interesting story that portrays the development of sulfa drugs had its genesis in 1909 and was associated with politics and intrigue. Heinrich Hoerlein, who was later to become Director of the Medical Division of I.G. Farben, began working at Bayer's facility at Wuppertal-Elberfeld to develop dyes that were particularly color-fast. Based upon the premise that there would be a strong interaction of the sulfanilamide moiety with proteins of wool, Hoerlein found that adding the sulfonamide to azo dyes increased the ability of the dye to bind to wool. Although he patented sulfanilamide in 1909, Hoerlein never viewed the compound as an antibacterial agent. Thus, the early discovery of sulfanilamide illustrates the axiom that researchers sometimes make important observations without knowing they have made them.

Another milestone in this story came in 1915, when Jacob and Heidelberger at the Rockefeller Institute in New York decided to synthesize and test a number of agents in the hope of finding one that was bacteriocidal

against streptococcal and pneumococcal infections. During their comprehensive analysis, Jacob and Heidelberger synthesized *p*-aminobenzene sulfonamide (sulfanilamide). However, they failed to conduct animal experimentation, because they were unable to envision that a compound as simple as sulfanilamide could directly combat bacterial infections. Many years later in 1972, Heidelberger expressed his regrets about dismissing the opportunity by writing "As slaves to an idea, we missed the boat in 1915, losing the chance to save many thousands of lives, and the development of the sulfonamides was delayed twenty years" (Comroe, 1976).

In 1927, Gerhard Domagk (Fig. 14) was hired by I.G. Farben as Research Director of the Institute of Experimental Pathology. He took over the group housed in the facility at Elberfeld-Wuppertal, which was tasked with identifying antibacterial activity in azo dyes. Domagk, a clinician with experience in treating war wounds, was strongly committed to finding an effective treatment for infectious disease. Although earlier investigators had demonstrated that certain acridine dyes possessed broad-spectrum antibacterial activity, virtually hundreds of such dyes that had been made by the chemists at Hoechst failed to produce a useful antibacterial agent. One of the difficulties encountered by early researchers working in this area was the lack of reliable tests for antibacterial activity. So, for this particular study, Domagk developed an ingenious method for screening the survival of mice that had been inoculated with *Streptococcus pyogenes*. This method used a highly virulent strain of hemolytic *Streptococcus* that had been isolated from a patient who had died from septicemia. Because its virulence had been enhanced by repeated subcultures in mice, the assay would identify only the most effective compounds.

After first confirming the reliability of his assay system by using compounds known to have antibacterial properties and those that were deemed ineffective,



FIG. 14. Gerhard Domagk (1895–1964). Courtesy of Bayer Health-Care AG.

Domagk then tested various gold compounds and acridine dyes with little success. But more positive results were forthcoming when Domagk and his colleagues directed their attention to dyes that were derivatives of sulfonamide containing the sulfonamide group in the *p*-position relative to the nitrogen. These dyes were found to exhibit a protective effect on animal survival. As a consequence of these favorable results, a large number of the sulfonamide-containing dyes were synthesized by chemists at I.G. Farben and submitted to Domagk for evaluation of their protective effects. In 1932, a red dye was produced by Fritz Mietzsch and Josef Klarer and given the name Prontosil *rubrum*. This dye was found to be the most effective of these compounds in protecting mice from a lethal dose of *H. streptococci*. In fact, in 1935 Domagk proclaimed a 100% success rate, as assessed by the mouse protection assay, when Prontosil was administered prior to a challenge with the potentially lethal microorganisms.

After Domagk successfully employed Prontosil to treat rabbits infected with *H. streptococcus*, it was not long before I.G. Farben began supplying physicians with the drug for treating patients with life-threatening streptococcal infections. In fact, in February, 1935, the month in which Domagk's first publication appeared, he had sufficient confidence in the drug to treat his 4-year-old daughter Hildegard, who had developed normally fatal septicemia after pricking her finger with a needle. This work understandably evoked world-wide interest when it was published (Domagk, 1935). Although additional studies confirmed the efficacy of Prontosil in the treatment of several forms of streptococcal and staphylococcal infections, the fact that pneumococcal infections responded less favorably to this agent tempered enthusiasm about its overall effectiveness.

So Prontosil did not gain wide spread acceptance by the scientific establishment until 1936, when President Franklin Roosevelt's son Franklin Jr. was successfully treated with the drug for tonsillitis, and *The New York Times* reported that "A new control for infections had been discovered" (Amyes, 2001). In addition, Leonard Colebrook of Queen Charlotte's Maternity Hospital in London, an expert on the chemotherapy of streptococcal infections, successfully treated 60 women who had contracted the dreaded puerperal (postpartum) fever, and only three of them died (Colebrook and Kenny, 1936). This report, among others, alerted the medical establishment to the potential benefits of antibacterial chemotherapy, despite the prevailing dogma that chemotherapeutic agents would only render minimal beneficial effects against generalized bacterial infections. However, the continuing reports of positive outcomes resulting from treatment with Prontosil finally led to the acceptance of the drug as the first effective chemotherapeutic agent for treating systemic bacterial infections.

The observation that Prontosil was ineffective against microorganisms in culture gave credence to the possibility that an active component released from Prontosil was responsible for the drug's action. Indeed, soon after Domagk's first publication appeared, members of Fournau's laboratory at the Pasteur Institute in Paris (which included Daniel Bovet) found that the azo link of Prontosil could be broken in tissues to yield sulfanilamide. But perhaps more significantly, they later demonstrated that sulfanilamide was the sole active moiety of the red dye and that other components of the complex were completely superfluous. Because the patent on sulfanilamide had long expired, there was at the time an unsubstantiated suspicion that I.G. Farben (and perhaps Domagk himself) had "rediscovered" sulfanilamide and spent the next several years transforming this relatively simple compound into a complex, and most importantly, now patentable dye. Advocates of a conspiracy theory would argue that the 3-year hiatus between the synthesis of Prontosil by Klarer and Mietzsch in 1932 and the initial clinical report 3 years later was a consequence of efforts by the directors of I.G. Farben to safeguard their discovery from other manufacturers. The giant conglomerate addressed this accusation by arguing that a careful and complete validation of the drug's therapeutic properties was necessary prior to making it available to clinicians. It is unfortunate that Heinrich Horlein, who patented the sulfonamide in 1909 while working at Bayer, and then later as Director of the Medical Division of I.G. Farben, and was responsible for hiring Domagk, would never reveal the truth about the actual events that surrounded the development of Prontosil. He was the only person associated with Prontosil who could have shed light on this controversy.

Confirmation that sulfanilamide was the active moiety in Prontosil spawned the synthesis of a large number of sulfonamides by the chemists at I.G. Farben. These compounds were in turn tested by Domagk on a variety of infectious diseases, with varying success. Since sulfanilamide could not be patented, any company could produce its own formulation, and in 1937 an elixir of the drug was marketed in the United States. However, after more than 70 people died from the effects of the solvent diethylene glycol, the U.S. Congress enacted the Food and Drug Act to prevent similar events from reoccurring.

Meanwhile, reports of failures using sulfanilamide began to emerge, particularly when the drug was used to treat staphylococcal infections. Tuberculosis was also a major health problem throughout the world, and sporadic reports appeared that described only modest success in treating this chronic and insidious disease with sulfa drugs. These reports prompted Domagk to express reservations about the use of sulfonamides for the long-term treatment of certain infections. The limitations in the effectiveness of sulfanilamide prompted changes in the basic molecule, which yielded numerous derivatives of sulfanilamide that were effective against staphylo-

cocci, as well as pneumococci, meningococci, and gonococci. In 1938, a publication from the UK reported the synthesis of sulfapyridine, which possessed a wide spectrum of action and was particularly effective against pneumococcal infections. In 1939, sulfathiazole was synthesized in the United States and eventually became the preferred sulfa drug because of its relatively high therapeutic index. From these initial observations arose a wealth of literature on the subject of sulfa drugs, and by the late 1940s over 5000 sulfonamides had been produced, although many were not effective clinically. As a consequence of Domagk's valuable contributions, sulfonamides were extensively employed as therapeutic agents well into the 1980s. But increasing bacterial resistance and the availability of more effective and less toxic antibiotics have gradually led to a decline in their popularity as antibacterial agents.

Since sulfonamides could be synthesized by anyone who cared to do so, Domagk profited little from his discovery. He was, however, honored for his contribution by being awarded the Nobel Prize in 1939. Although Domagk was naturally gratified to receive the award, he was coerced by the Nazi government to decline it. A personal letter from the Nobel Committee to Hermann Goering to allow an exemption for Domagk received no reply, and Domagk was even denied permission to travel to Sweden just to express his appreciation for receiving the award.

The action taken by the leaders of the Third Reich was a consequence of moral stands taken by dissidents in Germany during the 1930s against the militaristic policies of the Nazi government. One of the antimilitarists was Carl von Ossietzky, a journalist and author who had been incarcerated twice for his outspoken views against the fascist regime. In 1935, while still a concentration camp inmate and grossly mistreated, von Ossietzky was awarded the Nobel Peace Prize. The announcement of Ossietzky's award so infuriated the Nazi leaders that Hitler issued a formal decree forbidding any German to accept a Nobel Prize. So, despite the fact that Domagk had been recognized for his scientific achievements, and not because of his political beliefs, Hitler's petulant edict delayed the formal presentation of the award to Domagk until 1947. Although by this time the prize money had reverted to the Nobel Foundation, the award rendered an enduring tribute to an investigator who was responsible for a revolution in the treatment of infectious diseases.

Despite the accolades attributed to I.G. Farben and Domagk, tensions still developed among the group at Elberfeld-Wuppertal. Although the Nobel Prize had been awarded for the discovery of the clinical efficacy of Prontosil and not for its synthesis, Fritz Mietzsch and Josef Klarer were disappointed and even embittered by the lack of notoriety that they had received for their success in synthesizing the drug. Domagk himself always argued both verbally and in writing that Metzsch and Klarer should have been given a greater share of the credit. Still,

it is Domagk alone who occupies a hallowed position in the annals of science for this major contribution.

After World War II ended, another controversy surrounding I.G. Farben emerged when 23 directors of the conglomerate were tried by the Nuremberg War Crimes Tribunal. The directors were indicted for plundering property in invaded countries and using slave labor to support the wartime efforts of the Axis regime. The Farben case, which was the third largest of all of the Nuremberg trials, surpassed in scope only by the Trial of the Major War Criminals and the so-called Ministries Case, ended with the conviction and imprisonment of 13 defendants. Domagk was certainly not complicit in these crimes. In fact, he often made incisive and acerbic comments about the political situation that existed in Germany at the time, which did little to placate the Nazi leaders. During the war, Domagk remained at the facility in Elberfeld-Wuppertal, pursuing his crusade against infectious disease, despite being exposed to incessant Allied bombing until the war ended. Nevertheless, it is ironic that a company that had done so much to improve the human condition by sponsoring programs that would effectively combat infectious diseases could also serve a master that was responsible for the brutality and devastation that will forever remain a blight on mankind.

Prior to Prontosil, the idea that a systemic bacterial infection could be cured by administering a substance systemically was considered untenable and even ludicrous to both researchers and clinicians. However, Domagk and his colleagues provided the impetus for changing these perceptions. An interesting perspective on the importance of Domagk's discovery may be gleaned from the insightful remark made by Alexander Fleming, the discoverer of penicillin: "Without Domagk, there would be no sulfonamide! Without sulfonamide, there would be no penicillin! And without penicillin, there would be no antibiotics" (<http://www.dutly.ch/domagk/dom.html>). Although Fleming's endorsement may be construed as hyperbole, it certainly has merit, because Domagk's work prompted other investigators to search for new and more effective antibacterial agents. Therefore, despite the fact that Domagk's discovery was less celebrated than many others and not often revisited, there was no greater impact on human health and mortality than antimicrobial chemotherapy, and Domagk's contributions represented the beginning of this new age.

I. Sir Alexander Fleming, Cecil Paine, Harold Raistrick, Ernst Chain, and Sir Howard Florey: Penicillin and Its Curative Effects in Various Infectious Diseases

1. Sir Alexander Fleming. The story of the discovery of penicillin has been frequently chronicled and the subject of intense debate, particularly as it relates to the significance of the relative contributions made by each protagonist. At the time of his monumental discovery,

Alexander Fleming (Fig. 15) was already an eminent microbiologist, who had just been appointed Professor of Bacteriology at the University of London. Even though several serendipitous events seemed to conspire to bring about the discovery, it cannot be described as purely accidental, because Fleming was able to draw on his life-long interest in the field of bacterial lysis to bring about a positive outcome.

Fleming, a native of Scotland, spent World War I as a physician working in a military hospital in France. The prevalence of secondary staphylococcal infections from wounds pervaded his attention and fueled his future interest in antiseptics. However, Fleming was fully aware of the limitations and dangers associated with the use of antiseptics in treating wounds. So he spent a major portion of his research career studying a variety of diverse substances that interfered with bacterial growth. In 1922, he isolated an antibacterial substance he called *lysozyme* from the nasal passages of a patient suffering from acute rhinitis. An interesting property of this substance was that it could protect against certain nonpathogenic microorganisms from becoming virulent. Another interesting aspect of this finding was that the mode of action of lysozyme on microorganisms was very different from that of known immune reactions, such as antibody-induced lysis or phagocytosis. But most importantly, it was Fleming's discovery and characterization of lysozyme in lacrimal fluid and saliva that motivated him to seek other natural substances that exhibited antibacterial activity. Yet it is ironic to note that even though the interest created by the disclosure of lysozyme led to the accidental discovery of penicillin, the mechanism of action of penicillin was eventually found to be quite different from that of lysozyme (Fleming, 1922).

As a staff member at St. Mary's Hospital, Fleming ran an ill-organized and rather untidy laboratory, which was littered with contaminated Petri dishes and other detritus for extended periods of time. The disorganized state of Fleming's laboratory would have an important bearing on the discovery of penicillin. As the story goes, one day in the summer of 1928, a spore from a mold pro-



FIG. 15. Alexander Fleming (1881–1955). Courtesy of the National Library of Medicine.

duced in the laboratory located on the floor below drifted upward. The mold floated into Fleming's laboratory and settled in a Petri dish containing agar impregnated with staphylococci. As was his habit, Fleming had left this culture plate along with a number of others on a bench and departed for a holiday that would last for several weeks. As a result, exposed to air, the contaminant mold had ample time to grow.

Upon returning, Fleming randomly inspected the Petri dishes scattered throughout his laboratory. In the corner of a dish on which he had grown a strain of staphylococci, he happened to observe a small mold. But he was startled to find that around the mold, the colonies had almost completely disappeared. Fleming was intrigued by this observation because of his particular interest in lysis and because staphylococci were known to be notoriously resistant to lysis. Because the mold could attack pathogenic microorganisms, Fleming considered the possibility that the contaminant in the mold might have clinical utility.

So Fleming spent the remainder of 1928 studying the properties of the unknown substance. Although lacking expertise in chemistry, he identified the mold with the aid of C. J. Latouche, a mycologist whose laboratory was located directly below his own. In all probability, Latouche's laboratory was the source of the mold that contaminated Fleming's culture dish. Because the mold belonged to the genus *Penicillium*, Fleming coined the word *penicillin* in referring to the antibacterial substance. He found that penicillin killed streptococci, pneumococci, gonococci, meningococci, and diphtheria bacilli. He also observed that penicillin was nontoxic to animals, was more effective in combating gram-positive cocci than gram-negative bacilli, and did not interfere with neutrophil function.

Fleming also identified microorganisms against which penicillin might not be effective. He observed that enterococci, tubercle bacillus, influenza, and typhoid bacilli were insensitive to penicillin, thereby suggesting a selective action of the drug on certain microorganisms. Although Fleming was unable to reproduce the lytic effect of the mold, he did demonstrate that his batch of penicillin could be diluted 800 times before it lost activity against staphylococci. Fleming's findings were published in a 1929 article in the *British Journal of Experimental Pathology* and constitute almost the entire output of his work related to the development of penicillin. However, he did briefly refer to penicillin in one or two other publications over the next several years (Fleming, 1929).

After reading Fleming's classic article and having the benefit of hindsight, I was disappointed to find it written in a rather straightforward and nonanalytical manner, with little or no broad interpretation of the results. Its descriptive nature mirrored a report that might have been published in a pathology journal of the time. The results are described very succinctly, the discussion is lim-

ited to a single page, and the list of references is scanty. Most significantly, there was a relative paucity of references related to microbial antagonism, about which a great deal was known at the time. Fleming's argument that the rules of the journal limited his analysis loses credibility when one considers that a scientist of Fleming's renown could have published his work in a journal that allowed a more extended interpretation of his findings.

In Fleming's defense, however, the bacterial lysis that he observed was a chance observation rather than the result of a designed experiment. As a result, the implications of his findings were still a matter of conjecture. Moreover, sufficient knowledge was not available at the time that could explain the processes that mediate lysis. Finally, the isolation of an unstable product, such as penicillin, would probably have been a herculean task because of the lack of appropriate methodologies available at the time. All of these mitigating circumstances seemed to be responsible for the limited interest that this article generated from colleagues and the scientific community.

An added factor that contributed to the torpor was the fact that Fleming was a taciturn and rather introverted man who did not possess the charisma and communicative skills needed to publicize his work. Even if he had the desire to provoke interest in penicillin, his superior, Almroth Wright, was strongly against the idea of any therapeutic value of penicillin and expressed his disfavor to Fleming. So, probably influenced by Wright's strong persona and forceful arguments, as well as by the criticisms and doubts expressed by many of his colleagues, Fleming made little attempt to promote penicillin as a curative agent for systemic infections. However, Fleming did employ penicillin as an "antiseptic" for the treatment of surface infections such as carbuncles, eye and sinus infections, and leg ulcers. The utilization of the drug for these purposes yielded mixed outcomes. As a result, Fleming preferred to direct his attention to authoring several articles on the less compelling property of penicillin to facilitate the growth of certain microorganisms whose isolation had proven difficult. So, because Fleming opted to become a bystander in the development of this extraordinary drug, the unique effectiveness of penicillin would not be recognized until a decade later, when another fortuitous sequence of events culminated in the emergence of systemically administered penicillin as a curative agent for infectious diseases.

Although Fleming is credited with disclosing most of the basic properties of penicillin, including its high degree of microbial specificity, its relative lack of toxicity, and its ability to produce drug resistance, he never performed an animal experiment to demonstrate that the mold was capable of eradicating an iatrogenically induced infection. It was in this context that Ernst Chain respectfully chided Fleming for not pursuing his studies with penicillin. Chain, the codiscoverer of penicillin,

speculated that since a single injection of undiluted penicillin into a mouse failed to elicit any toxic effect, Fleming should have repeated these injections two or three times at appropriate intervals. He would then in all probability have obtained a sufficiently positive curative effect to persuade a number of chemists to isolate the active principle (Chain, 1979).

Fleming was often questioned about why he so abruptly terminated his work with penicillin, and he usually gave three rather unconvincing reasons for doing so: 1) the instability of the drug, even though a method for preserving it was found in the early 1930s; 2) the lack of a purified solution of penicillin, despite the fact that two assistants named Stuart Craddock and Frederick Ridley, in their final experiment before leaving the laboratory, produced a purified preparation that Fleming failed to recognize and use; and 3) the purported lack of cooperation from clinical colleagues to provide patients for a clinical study. In this way, Fleming excused his own lack of faith and passion in his discovery by casting aspersions on his clinical colleagues for their reluctance to have their gravely ill patients subjected to treatment with a mysterious drug that possessed unknown side effects.

Despite the mixed legacy left by Fleming's work with penicillin, history ultimately concluded that Fleming was deserving of all of the tributes that he received for his discovery. In recognition of his accomplishments, when Fleming died in 1955, a crypt in St. Paul's Cathedral became his ultimate resting place. As an enduring tribute to Fleming's work, his memorial in the majestic cathedral resides in close proximity to that of Admiral Horatio Nelson, the greatest naval hero in the history of the UK.

2. Cecil Paine. Aside from Fleming's few modest attempts to employ penicillin in treating infected surface wounds, the first person to obtain effective cures with the drug was a surgeon named Cecil Paine. In 1930, by administering crude penicillin topically, Paine successfully treated patients with eye infections at Sheffield Hospital. Using Fleming's method, Paine had grown the *Penicillium notatum* on meat broth made from cultures obtained from Fleming. Although as a medical student Paine had heard about penicillin from Fleming's lectures and had read his 1929 publication, he never consulted with Fleming during the course of successfully treating eight patients topically with penicillin. Although he found that crude penicillin was effective against eye infections caused by pneumococci and gonococci, it failed to alter the course of eye infections caused by staphylococci. Perhaps discouraged by the chemical instability of penicillin, Paine terminated his treatment of patients with the crude preparation and moved on to another institution and a different area of research. Like Fleming, Paine's failure to pursue this work represented an egregious example of a lost opportunity and was based upon at least two factors. First, his departure from the Sheffield Royal Infirmary caused him to alter his field of interest; at Jessop Hospital

for Women, he began work on the etiology and treatment of puerperal fever, which culminated in the awarding of his medical degree and the publication of several papers. Second, the fact that there was no technique available at the time to preserve the active component of the unstable filtrates probably contributed significantly to Paine's loss of interest in penicillin.

In retrospect, it now seems incredulous that Paine never published his work or at least presented his findings at a scientific meeting. Paine's contribution was revealed only after Sir Howard Florey used his influence as a Nobel Laureate to have it included in the second volume of *Antibiotics* published in 1949 (Florey et al., 1949). Although Paine proclaimed himself to be "a poor fool who didn't see the obvious when it was stuck in front of him" (Wainwright and Swan, 1986), he did recognize the therapeutic potential of penicillin, although only as an antiseptic. Furthermore, the concept of "antibiotics" was not seriously entertained in the early 1930s, even by Howard Florey, who had been apprised by Paine about the clinical cures he might achieve with the drug. Nevertheless, it took almost another 10 years before Chain and Florey succeeded in purifying penicillin and attaining the phenomenal successes that are associated with the systemic use of this remarkable agent.

3. Harold Raistrick. There was another significant player in this story prior to the advent of Florey and Chain. In 1934, Harold Raistrick, a British chemist with an international reputation in fungi, began an investigation of the properties of penicillin. After confirming that penicillin was soluble in several solvents, he found that it disappeared from an ether extract after the solvent was evaporated to dryness. Because of its apparent instability, Raistrick immediately and without further forethought concluded that the production of penicillin for therapeutic use would be an insurmountable task and terminated the project.

Some historians would argue that, because of his expertise as a fungal biochemist, Raistrick, rather than Fleming, should be held accountable for failing to purify penicillin (Wainwright, 1990). Moreover, the fact that an expert biochemist had discontinued his study of penicillin because of the perceived instability of the substance also probably helped to temporarily quell interest in this new compound and thereby further delayed the introduction of penicillin into the pharmacological armamentarium. In addition, progress in this field was hindered by the fact that the concept of chemotherapy was still considered to be an anathema by many microbiologists at the time. The introduction of sulfonamides by Domagk in 1935 certainly helped to nullify this bias and paved the way for more expansive views of microbial antagonism to emerge.

4. Ernst Chain and Sir Howard Florey. By the end of the 1930s, major pharmaceutical firms in the United States began to become aware of penicillin as a potential therapeutic agent. But despite these sporadic signs of

interest, penicillin remained a laboratory curiosity until 1940, when it was revitalized by Ernst Chain (Fig. 16) and Sir Howard Florey (Fig. 17) by a series of remarkable circumstances. Ironically, the fascist regime of Germany was indirectly responsible for the development of penicillin. During the early 1930s after Adolf Hitler came to power, Chain was a young Jew with left-wing views living in Germany. It did not take him long to realize that Hitler's Germany was not the safest place for him to call home, and so he fled his native country in 1933. After spending 2 years at Cambridge University working on phospholipids, Chain was hired by Florey, Head of the School of Pathology at Oxford, to organize a biochemical group for his department.

Chain was a first-rate biochemist and scientist who also happened to be a gifted musician. But he was endowed with a rather difficult personality and instigated many personal confrontations, including those among his colleagues and associates. Part of his difficult personality may have stemmed from his belief that he did not receive a fair share of the credit from his colleagues for his scientific achievements. Florey, an Australian by birth, was not trained as a microbiologist and therefore did not possess the expertise needed to isolate and study the penicillin-producing mold. Nevertheless, his extraordinary ability to lead, identify a person to perform a given task, and organize a research team would eventually bring about the epoch-making advance in chemotherapy.

Chain was recruited by Florey because of his knowledge of the biochemistry of fungi. During one of their discussions about research projects to consider, Florey suggested that Chain examine the mode of action of the bacteriolytic enzyme lysozyme, previously discovered by Alexander Fleming in 1922. Chain agreed, and without difficulty isolated lysozyme in pure form and demonstrated that its enzymatic action involved the destruction of a polysaccharide localized to the bacterial cell wall. After expeditiously dispensing with the lysozyme problem in 1937, Chain turned to investigating other antimicrobial products produced in nature. During this



FIG. 17. Howard Florey (1898–1968). Florey's ability to commandeer talented people for appropriate tasks led to the development of penicillin as a therapeutic agent. One of Florey's team members was Norman Heatley (1911–2004; not shown), whose experimental prowess was crucial to the purification of penicillin.

interval, Chain began searching the scientific literature for older, related studies and came across Fleming's 1929 publication. In reading Fleming's article, Chain was impressed by the lytic effect of the unidentified compound in the mold and concluded that it might be related to lysozyme. Florey also expressed interest in the subject because of his professional interactions with Fleming as well as his discussions with Cecil Paine, both of which had made him aware of the potentially curative power of penicillin.

Before Chain could repeat Fleming's experiments, he had to obtain a culture of the original strain of *P. notatum*. By an extraordinary coincidence, Chain was able to acquire a subculture from Florey's own department at Oxford. For some unfathomable reason, the reading of Fleming's article prompted Chain to recall a laboratory technician walking through the corridor carrying bottles of culture fluid; on the surface of the fluid he observed a growth of mold. Chain later discovered that it was the same mold that Fleming had described in his 1929 article. Further investigation divulged that the late Professor Dreyer, Florey's predecessor, had requested the mold from Fleming, because he erroneously surmised that it might serve some useful purpose in his experiments with bacteriophage. So Fleming's contaminant mold had been sent to Dreyer in 1930 and was subsequently subcultured and sequestered in the culture collection of the Department of Pathology for several years.

After learning of its availability, Chain immediately approached Florey about conducting experiments with



FIG. 16. Ernst Chain (1906–1979). Courtesy of the National Library of Medicine.

the mold. Confident of his skills as a chemist, Chain was convinced that despite its instability, he could at least devise a method for partially purifying the substance. After Florey agreed to the project, Chain began the isolation of what he thought was an enzyme that might hydrolyze a cell surface substrate shared by many bacterial pathogens. The original objective of this study was mechanistic in nature, since Fleming's article failed to provide Chain with any assurance that penicillin would possess utility as a therapeutic agent. In fact, when Chain and Florey initially undertook the work, they were unable to reproduce the bacteriolytic effect that had taken place in Fleming's laboratory. It took them several years to uncover the fact that penicillin would attack bacteria only when the microorganisms were able to undergo at least one division. It was then that Chain and Florey realized in retrospect that Fleming's somewhat untidy manner of running his laboratory had accidentally produced disease-like conditions, thereby enabling the developing microorganisms to generate colonies and at the same time produce an adequate amount of penicillin to lyse the bacteria. This serendipitous sequence of events prompted Chain to whimsically conclude that the discovery of penicillin might never have been made if Fleming's laboratory had been maintained in a more orderly and sanitary state (Chain, 1979).

Chain and his colleagues developed a simple and precise assay for measuring penicillin, whereas Florey and his group were charged with carrying out the toxicity tests. However, it was Norman Heatley, rather than Chain, who devised the crucial step that would expedite the purification of penicillin by developing the simple and reliable "cylinder plate technique." At about this time, a rift began to develop between Chain and Florey. Chain, in Florey's temporary absence, had decided to carry out toxicity tests on his own by injecting partially purified penicillin into two rats, which showed no evidence of toxicity. This infringement on Florey's responsibility caused an estrangement that was to widen over the next few years. The strained relations, in part, would eventually convince Chain to move to the Institute of Public Health in Rome by the late 1940s, where he achieved financial success by developing and producing modified penicillins.

Despite their personal differences, Chain and Florey led their respective teams in successfully isolating penicillin and testing it on culture plates of various bacteria with varied success, depending upon the type of microorganism. To obtain evidence as to its effectiveness in animals, Chain and Florey found that mice pretreated with penicillin and then challenged with lethal doses of streptococci survived 2 days longer than infected mice not pretreated with penicillin. Mice had been used for these experiments simply because of their small size, thereby curtailing depletion of the limited stores of antibiotic available at the time. But the use of mice

rather than guinea pigs afforded Chain and Florey an unsuspected conduit to the ultimate success of this project, since humans and mice responded favorably to penicillin, whereas the guinea pig would exhibit toxic effects from the drug. Therefore, if guinea pigs had been used for the toxicity studies, the clinical trials might have been delayed or even discontinued. The utilization of drug-treated mice also demonstrated significant antibacterial activity in urine. This important finding revealed that the unknown substance was not rapidly metabolized but was excreted and therefore had the potential to be used as a therapeutic agent.

After a series of animal (mice) experiments yielded spectacular results, an article was published by Chain and Florey in *The Lancet* in August 1940 (Chain et al., 1940). It described the production and purification of penicillin and documented its antibacterial action in animals pretreated with *Streptococcus pyogenes* and *Staphylococcus aureus*. In 1941, a sufficient amount of the agent was made available to carry out clinical trials on patients suffering from infections caused by *S. pyogenes* and *S. aureus*. In addition, a policeman suffering from a severe bacterial infection dramatically improved when given penicillin. However, he subsequently died when the supply of drug ran out. Although Florey always argued that the basis of his interest in penicillin was heuristic, he was greatly disturbed by the demise of the policeman due to an inadequate supply of the drug. He then vowed to accomplish whatever was needed to ensure that penicillin was made available to the general public. Despite the scant supply of penicillin, a second article that reported the dramatically favorable clinical results on so-called hopeless cases was published the following year.

At about this time, Alexander Fleming paid a visit to Florey's laboratory and was graciously provided with a sample of purified penicillin. Upon returning to St. Mary's Hospital, Fleming resourcefully injected the drug directly into the cerebrospinal fluid of a patient suffering from *Streptococcal meningitis*. After a rapid cure was reported in the London-based newspaper *The Times*, Fleming was catapulted into the limelight, despite his earlier reluctance to pursue studies on animals and humans.

After Florey recruited Edward Abraham to aid in developing large-scale purification methods and to determine its structure as a prelude to its synthesis, the structural formula of penicillin was determined by the Oxford group in October 1943. Although during World War II, British companies, including Burroughs Wellcome, Imperial Chemical Industries, and Glaxo, provided sufficient drugs to fulfill the needs of their military forces, the amounts produced were only comparable to those generated at the academic laboratories at Oxford. So in the spring of 1941, because the Americans employed better methods for isolating the substance and were not hampered by wartime bombing, Florey turned

to the United States to inaugurate large-scale production of penicillin. To promote such collaborations, Florey and Heatley made several transatlantic trips, first to enlist and then to establish and fortify the cooperation of the American scientists and pharmaceutical firms. After surface culture methods became obsolete and deep fermentation methods were adopted, a rapid characterization of the chemical and physical properties of penicillin was achieved by an army of scientists both in the United States and in the UK. Its low toxicity, broad antibacterial spectrum, and potency in animals were also verified.

The cooperative efforts expended on both sides of the Atlantic, involving both academia and the pharmaceutical industry, eventually enabled penicillin production to be put on a fast track, so that the drug could become more readily available and effectively used by the Allied forces during World War II. As a result, from D-Day onward (June 1944), the rate of Allied soldiers dying from infected war wounds inexorably declined. By contrast, the Germans never succeeded in producing penicillin on a large scale and had to rely on the much less effective sulfonamides. The superiority that the Allies experienced with regard to chemotherapeutic agents may have at least in part contributed to an earlier end to the war in Europe.

By 1944, the programs cultivated mainly by Howard Florey had promoted a prodigious growth in the production of penicillin. But despite an abundant supply, the exorbitant cost of the drug was responsible for the continued promiscuous sale of the crude extract through the black market. For Florey, the black market was a constant source of consternation, because of the dangers that the use of the crude extract portended. After the war, the cooperation of workers from both the United States and the UK in crystallizing the drug led to its successful testing in such diseases as septicemia, cerebral meningitis, gas gangrene, pneumonia, syphilis, and gonorrhea. Needless to say, the impact of the combined efforts of Fleming, Florey, and Chain was profound, because in many cases patients who were terminally ill with an infectious disease could now be completely cured by the new "miracle drug." However, the successful treatment of tuberculosis and typhoid fever still had to await the discovery of streptomycin, as well as other antibiotics with wider spectra of action.

Although it is clear that serendipity played a key role in the discovery of penicillin, it should be emphasized that the events surrounding the development of penicillin represent a striking example of how basic research can be used to address a clinical problem. After bacterial lysis was accidentally discovered, Fleming pursued the finding because of his long-term interest in this field. Chain's incentive in studying penicillin stemmed from reading Fleming's article and realizing that it contained findings of major scientific significance. Thus, according to Chain, the discovery of penicillin represented a prime

example of fundamental progress in pharmacotherapy being made simply because of the ability to follow up on potentially interesting biological findings and not because of aspirations to cure infectious diseases.

For the discovery of penicillin, Fleming, Florey, and Chain were awarded the Nobel Prize in 1945. The rapidity by which their discovery was acknowledged by the scientific community is ample testimony to the enormity of their discovery. I still remember as a young boy shortly after World War II when penicillin became available to the general public. It was very expensive, highly valued, and treated like pure gold, unlike today when it is readily available in many different forms. Even though the hand of fate was in part responsible for a favorable outcome, the story of penicillin represents a prime example of the commitment and effort expended by a group of international investigators to produce a therapeutic agent that effectively combats infectious disease and thereby promotes the preservation of life.

5. *Jack Strominger.* During the late 1950s and early 60s, as the production of newer antibiotics expanded, interest in their mode of action heightened. Key evidence bearing on this problem was adduced in the late 1950s by Jack Strominger (Fig. 18) and James Park. At the time, Strominger held a position in the Department of Pharmacology at Washington University in St. Louis, while Park worked nearby at St. Louis University. The Chair of Pharmacology at Washington University was then occupied by Oliver Lowry, who earned acclaim for his expertise in developing a variety of important bio-



FIG. 18. Jack Strominger (1925–). Courtesy of the Strominger laboratory, Harvard University. Reprinted with permission from the *Annual Review of Immunology*, volume 24. © 2006 by Annual Reviews.

chemical techniques. His protein assay is one of the most quoted references in the scientific literature. Lowry's department was also a stimulating one for research, and he recruited outstanding young scientists, including Jack Strominger and Robert Furchgott. During his years of medical training, Floyd Bloom also served as an instructor in the Department of Pharmacology. Bloom asserts that being able to interact with Oliver Lowry and the rest of the pharmacology faculty during brown-bag luncheon sessions was a key factor in making his decision to pursue a career in pharmacology.

Jack Strominger's collaboration with James Park led to the demonstration that several strains of *staphylococci* inhibited by penicillin accumulated an intermediate that was identified as UDP-acetylmuramyl-pentapeptide (Park and Strominger, 1957). This acetylmuramic intermediate was given the name the "Park nucleotide." The striking similarity between the chemical composition of the acetylmuramic peptide and the bacterial cell wall suggested to Park and Strominger that the peptide was a precursor of a major component of the cell wall, which turned out to be peptidoglycan. Strominger later demonstrated that the mode of action and selective toxicity of penicillin were related to an inhibition of a step in peptidoglycan biosynthesis, thereby causing the accumulation of the acetylmuramic peptide (Park nucleotide). As a result, the low toxicity of penicillin in eukaryotic cells could now be ascribed to a lack of either a structure analogous to the bacterial cell wall or the chemical equivalent of the acetylmuramic acid-peptide fragment. Although this groundbreaking work has not been recognized by the Nobel Committee, it did eventually earn Strominger a faculty position in the Department of Pharmacology at the University of Wisconsin and subsequently the Higgins Professorship at Harvard.

By forging a novel strategy for investigating the mode of action of antibiotics, the work of Jack Strominger prompted the scientific literature to take on an added dimension in this area of research. These mechanistic approaches, when linked together together with the more practical contributions made by Fleming, Florey, and Chain, not only helped to promote the search for new and more useful antibiotics but also enabled physicians to devise more effective treatment regimens and to cope more proficiently with drug resistance. To provide some idea of the impact that the availability of sulfa drugs, penicillin, and other available antibiotics exerted on the world community, the National Office of Vital Statistics estimated that 1.5 million human lives were saved during the 15 years that preceded 1958 (Wainwright, 1990).

J. Selman Waksman: Streptomycin: The First Antibiotic Effective against Tuberculosis

Although Robert Koch will long be remembered for his discovery of the tubercle bacillus in 1882, it was not until some 60 years later that conceptual and technical ad-

vances regarding chemotherapy had developed to the point that an effective treatment for tuberculosis was possible. In contrast to the discovery of penicillin, which was largely based upon a chance observation made by Alexander Fleming, the discovery of streptomycin by Selman Waksman (Fig. 19) and his colleagues was the result of prolonged, systematic, and arduous research. Moreover, this story was a turbulent drama of pathos, leading not only to euphoria and exhilaration associated with an important discovery but also to anguish and despair engendered by unresolved controversies.

Selman Waksman, like Ernst Chain, was a refugee from antisemitism. During the early 1900s, it was extremely difficult for Jews in Russia to obtain a university education, so Waksman immigrated to the United States in 1910. After receiving his doctorate in biochemistry at the University of California, Berkeley, he assumed a faculty position at his undergraduate alma mater Rutgers. Because of his basic interest in understanding how microorganisms interact with one another in the soil, Waksman nurtured a passion for the subject of microbial antagonism. After becoming convinced that microbes could produce substances that prevented the growth of other microorganisms, he began a comprehensive study to find an effective *in vivo* antagonist to the tubercle bacillus. This important undertaking was urged on by Waksman's son Byron, also a prominent scientist, after he exuberantly praised his father for the simplicity of the method that he had developed in isolating antibiotic substances that produced fungi.

So, by 1944, Waksman and his colleagues initiated a screening program that they hoped might lead to the discovery of new antibiotics that would complement the actions of penicillin and sulfa drugs. Since he had studied *actinomycetes* for many years and recognized their broad distribution and activity against other microorganisms, Waksman directed his attention to these particular microbes. From a large number of chemical substances isolated from *actinomycetes* from 1940 through

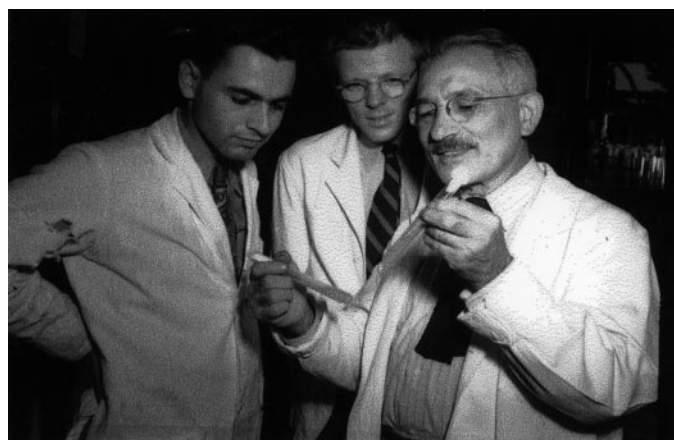


FIG. 19. Selman Waksman (1888–1973) and Albert Schatz (1920–2005); in the center is Donald Reynolds (1918–2001). Courtesy of Rutgers University Archives and Special Collections.

1952, Waksman's team eventually characterized 10 antibiotics, 3 of which had practical applications: actinomycin in 1940, streptomycin in 1944, and neomycin in 1949 (Waksman and Woodruff, 1940; Schatz et al., 1944; Waksman and Lechevalier, 1949). One of Waksman's graduate assistants, Albert Schatz, isolated one of two streptomycin-producing strains of actinomycetes and then extracted and tested the new antibiotic. Schatz wrote up this work for his thesis, which was accepted by Rutgers in 1945 for the Ph.D. degree. The first report of the discovery of streptomycin was made in 1944 (Schatz et al., 1944).

As the next step, expertise was required to test the effectiveness of the new drug on animals. For this work, William Feldman and H. C. Hinshaw of the Mayo Clinic were enlisted to conduct trials on four guinea pigs that had been infected with the tubercle bacillus (Feldman and Hinshaw, 1944). This study was so successful that clinical tests were quickly undertaken using a supply of the purified drug provided by Merck. When streptomycin was tested on patients with tuberculosis by Feldman and Hinshaw, its curative action was established on two of the more serious forms of the disease: miliary tuberculosis and tubercular meningitis (Feldman et al., 1945; Hinshaw et al., 1946). Streptomycin also proved to be particularly effective in treating pneumonic plague, the most deadly form of bubonic plague (The Black Death), as well as brucellosis, typhoid fever, and tularemia. Thus, the discovery of streptomycin now made it possible to treat effectively a number of gram-negative bacteria, including several that were insensitive to penicillin.

Because streptomycin proved effective against several types of microorganisms, Merck decided to establish a plant in New Jersey to manufacture the drug for clinical trials. Waksman greatly benefited from his staunch alliance with Merck, who helped to purify the antibiotic and bring it to the marketplace. Merck's involvement was mandated in part by the fact that Waksman's request for government funds to develop streptomycin had been denied, because the work was considered too "theoretical" for funding during wartime. Financial support for developing the drug was also provided by a private foundation, The Commonwealth Fund. Meanwhile, Schatz and Bugie produced larger quantities of the drug and provided additional information about its properties. By 1946, the results of the first large-scale clinical trial were published, proclaiming that streptomycin was effective in the treatment of tuberculosis. The biomedical community hailed the discovery as monumental, because during the 1930s tuberculosis had been the principal cause of death in the United States.

Waksman devised the term *antibiotic* to describe the actions of streptomycin because he envisioned the drug to be a substance produced by one microorganism that antagonized the effect of another microorganism. On the other hand, he also identified situations in which streptomycin was ineffective. He noted that anaerobic

bacteria, viruses, and fungi were resistant to streptomycin, as they were to penicillin. In addition, he described other limitations that streptomycin posed as a therapeutic agent. Thus, the activity of streptomycin was bacteriostatic, in contrast to penicillin, which was bacteriocidal. Furthermore, unlike penicillin, streptomycin had to be administered parenterally, and a major side effect that resulted from prolonged treatment with the drug was vestibular (inner ear) dysfunction, including tinnitus and vertigo (Waksman, 1975). Although Waksman seemed unaware that drug resistance might portend a long-term problem, his discovery of streptomycin did spur other researchers and clinicians to mount searches for newer and more effective antibiotics to combat drug resistance. Later on, it became general knowledge that the inclusion in the treatment regimen of other antitubercular compounds, such as pyrazinamide, isoniazid, and rifampicin, tended to slow the development of drug resistance. Still, the use of streptomycin has now been markedly curtailed, being surpassed by newer, more effective, and less toxic agents for the treatment of tuberculosis and certain other infectious diseases.

When Waksman was awarded the Nobel Prize in 1952, the Nobel Committee acclaimed him as "one of the greatest benefactors to mankind" because he had dramatically altered the prognosis for tuberculosis (http://nobelprize.org/nobel_prizes/medicine/laureates/1952/press.html). His discovery had transformed it from a chronic, debilitating disease that frequently culminated in a negative outcome to one that could be effectively treated and even cured. It is not hyperbole to state that the advent of the streptomycin era revolutionized the treatment of tuberculosis. Prior to the entry of the antibiotic into pharmacotherapy, this chronic malady was treated with rest, fresh air, and supportive care, with minimal therapeutic intervention. Edward Livingston Trudeau (Fig. 20), a New York physician, who himself suffered from the dreaded disease, was a major proponent of this approach. Emphasizing the importance of the interaction between disease and the environment, Trudeau raised money to build the Adirondack Cottage Sanitarium in the pristine environs of tiny Saranac Lake in upstate New York. Between the late 1880s and the mid-1950s, this facility achieved international acclaim as a treatment, research, and teaching center engaged in combating tuberculosis.

The notoriety of this institution was such that Robert Louis Stevenson, the famous author, spent 1887–1888 at Trudeau's sanitarium to enhance his recovery from the disease. My uncle, while in his late 30s, was diagnosed with tuberculosis around 1940 and sent to the Trudeau Sanitarium with the hope of arresting the disease by focusing on relaxation and recuperation. Unfortunately, like many other cases, the diagnosis was made too late to save his life. When the effective mode of drug treatment for tuberculosis became available in 1946, the Trudeau Sani-



FIG. 20. Edward Livingston Trudeau (1848–1915). Christmas seal of 1935 to recognize and support sanatoriums to treat tuberculosis patients. Courtesy of the National Library of Medicine.

torium and similar facilities around the world were rendered obsolete, and their doors gradually closed. But the spirit of Edward Trudeau's work continued to flourish when in 1964 his grandson, Francis Jr., aided by many appreciative benefactors who had been patients at the Trudeau Sanitarium, transformed it into a world-class center for basic research in infectious disease and immunology. Today, the Trudeau Institute continues to attract outstanding scientists from all over the world.

1. Albert Schatz. In concluding the story of Selman Waksman and streptomycin, I would be negligent in not considering the rather celebrated and unresolved controversy that stemmed from the development of the drug. During World War I, Albert Schatz (Fig. 19) served in the U.S. military by working in a medical laboratory. In this venue, he developed expertise in the handling of pathogenic bacteria. So when Schatz came to work with Selman Waksman as a predoctoral student, he had already gained a good deal of experience as a researcher and probably expected to have some independence in conducting his experiments. In addition, because Waksman had a phobic fear of the tubercle bacillus, Schatz was assigned a basement laboratory several floors below Waksman's office. This physical separation contributed

further to the independence of Schatz in carrying out his research activities.

Schatz's thesis work involved using the screening program developed by Waksman to isolate streptomycin-producing strains of actinomycetes (Schatz, 1945). After Schatz isolated, extracted, and tested the new antibiotic on various microorganisms, including the tubercle bacillus, several articles were published on the subject, with Schatz as first author (e.g., Schatz and Waksman, 1944; Schatz et al., 1944). Prior to 1942, the relationship between Waksman and Schatz was quite amicable, and before taking up a position at the Hopkins Marine Station in California, Schatz entered into an agreement with Waksman that neither would profit from the discovery of the drug. However, shortly thereafter, Waksman began receiving a significant sum in royalties from the Rutgers Research and Endowment Foundation. While in the process of addressing some taxation questions by mail, Schatz became aware of Waksman's apparent duplicity, which prompted a contentious letter from Schatz (letter from Schatz to Waksman, January 22, 1949: Selman Waksman Papers, Rutgers Archives and Special Collections). Waksman's indignant reply reflected his belief that Schatz's contribution represented "only a very small part of the picture in the development of streptomycin as a whole" (letter from Waksman to Schatz, February 8, 1949: Selman Waksman Papers, Rutgers Archives and Special Collections; Lechevalier, 1980).

Waksman considered himself chiefly responsible for the discovery of streptomycin, since it was only one of many positive outcomes that stemmed from this particular research project that had begun some 8 years earlier. Waksman also felt that his influence as an eminent microbiologist expedited the production of streptomycin on a large scale, which ultimately led to its development as a clinically useful antibiotic. However, Waksman's perspective was compromised by the fact that Schatz was listed as the first author on several of the early articles on the subject, and his name appeared on the patent application for streptomycin. In the interim, Elizabeth Bugie had signed an affidavit certifying that she was not involved in the discovery. After objectively surveying the situation, it seems fair to conclude that Waksman initiated and supervised a research program that directly led to the discovery of streptomycin, although Schatz actually made the discovery.

The dispute culminated in a lawsuit filed by Schatz in March 1950 against Waksman and the Rutgers Research Foundation. The basis of the lawsuit was to obtain what Schatz believed was his share of the credit. At the conclusion of the protracted court proceedings, Schatz was named a codiscoverer of the antibiotic in an out-of-court settlement and was awarded 3% of the royalties. The settlement of the case mandated that Waksman would receive 10% of the royalties, but he magnanimously reduced his share to 5% to help sponsor another

foundation. Royalties received by Rutgers were also used to create and maintain the Waksman Institute of Microbiology. It is to Waksman's credit that he became responsible for developing the institute into a facility where microbiologists could interact with colleagues from other disciplines and pursue scientific problems of varied interest. As a result, the study of antibiotics constitutes only a minor fraction of the overall research program, and drugs are studied without necessarily considering their practical applications (Lechevalier, 1980).

The Schatz case was not the only regrettable incident that cast a shadow over Waksman's scientific career. In the early 1920s, Waksman became involved in an acrimonious dispute that was predicated on a claim by a colleague named J. S. Joffe. He asserted that Waksman had assumed an inordinate amount of credit for the discovery of *Thiobacillus thiooxidans*, the first sulfur-oxidizing bacterium. Waksman was also involved in another lawsuit filed by a Mary Marcus in 1954, which added to his legal woes. Marcus's complaint was based upon her assertion that she had given Waksman cultures of actinomycetes *psoriaticus* and had instructed him as to their therapeutic value in treating psoriasis. Although this suit was eventually dismissed, these legal wrangles did not constitute pleasant days for Waksman, despite his important discovery (Lechevalier, 1980). Conflicting views of Waksman as a person were also prevalent. On the one hand, certain colleagues were effusive in their praise of him and considered him to be avuncular and gracious; others viewed him as self-serving and arrogant. Whatever his personal flaws, however, there is no doubt that Waksman was a gifted scientist who provided major contributions to several important areas of microbiology and chemotherapy.

Despite the court judgment in favor of Schatz, Waksman, as laboratory head, was the sole recipient of the Nobel Prize awarded in 1952. Schatz, then a Professor of Microbiology at the National Agricultural College in Pennsylvania, was quite disgruntled and wrote letters to the most distinguished scientists of the time, elaborating in detail his point of view on the subject. However, not surprisingly, he was rebuffed by the scientific establishment and even rebuked for his disloyalty. In addition, Schatz's petition to the Nobel Prize Committee for a share of the award was rejected. In considering Waksman's perspective in the dispute, he was considered a member of the old school of European scientists who regarded graduate students as privileged individuals by virtue of being able to work with an expert in his/her chosen field. So Waksman began to view Schatz as a malcontent who professed gross disloyalty. Nevertheless, much of the notoriety attributed to Waksman for the discovery would probably not have been compromised had he been gracious enough to provide Schatz with a more equitable share of the credit and patent royalties.

Still, Albert Schatz did flourish in his own way during an eclectic career in science and education. He received many honorary degrees, medals, and titles for his work on streptomycin, as well as for nystatin, an antibiotic that is still used against fungal and yeast infections. He was also named an honorary member of several scientific societies in the United States, Latin America, and Europe. He even taught science to students at the collegiate level who were education majors and developed a science unit for teaching microbiology to elementary school children. Nevertheless, Schatz was shuffled back to the ranks of scientists who have been lost to history despite their invaluable contributions, whereas Waksman came to be regarded as one of the fathers of antibiotics.

Schatz passed away in January 2005, still tormented by the controversy that surrounded the discovery of streptomycin and unyielding in the advocacy of his case. In an attempt to document Waksman's initial disinterest in the project, Schatz noted in his memoirs written in 2003 and reprinted in his obituary in 2005 that "Dr. Waksman was so afraid of contracting tuberculosis that he banished me to a basement laboratory to conduct the experiments involving the tubercle bacillus, and he ordered me never to bring a culture of the tubercle bacillus to the third floor" (http://oralhistory.rutgers.edu/Docs/memoirs/schatz_albert/schatz_albert_memoir.html). Schatz's viewpoint in the dispute was finally redeemed in 1994 when he was given Rutgers' highest honor, the University Medal. So, as time passes, perhaps the primary focus of this discovery should be mainly devoted to the life-saving benefits enjoyed by those who have been successfully treated with streptomycin and other antibiotics, whereas the controversy that surrounds the credit for its discovery should remain at best only a secondary aspect of the overall picture (Waksman, 1954; Sneider, 1985).

K. Sir Frederick Banting, Charles Best, John Macleod, and James Collip

Prior to the turn of the 20th century, endocrinology was an uncultivated discipline. The functions of most endocrine glands were unknown, and advances made were generally a result of clinical observations or by observing the functional effects of selectively removing tissues from animals. However, a milestone was reached in 1895 when Oliver and Schaefer described the physiological effects of an intravenous injection of an aqueous extract of the adrenal gland in animals (Oliver and Schaefer, 1895). Within a few more years, the active principle was isolated from the extract, identified, and synthesized. This body of work was of major significance since, as we have seen, it led to the first expression of the theory of chemical transmission by Thomas Elliott (see section II.A.). It would also provide the basis for comparable studies that would identify physiologically active substances produced and secreted by other endocrine

glands. However, these advances would not be forthcoming until the early 1920s.

The endocrine pancreas had long interested researchers and physicians, particularly when the association between the endocrine pancreas and diabetes mellitus was made. Children suffering from diabetes generally died at an early age, whereas older individuals endured multiple complications, frequently lapsed into comas, and too often succumbed. Because diabetics were often placed on starvation diets and expressed an emaciated phenotype, physicians who observed pictures of the survivors of Nazi concentration camps during the 1940s were reminded of the diabetic patients prior to the advent of insulin.

Diabetes mellitus had been known to afflict mankind for many centuries. *Mellitus*, the Latin word for honey, was attached to diabetes because of its link with sweet urine. Important insight into its pathophysiology was finally attained in the latter part of the 19th century when Paul Langerhans, a German medical student, identified islet cells in the pancreas. However, he was unable to explain their function. In 1889, the association between the endocrine pancreas and diabetes was established when von Mehring and Minkowski reported that diabetes developed when the pancreas was removed from dogs (von Mehring and Minkowski, 1889).

By the end of World War I, scientists had become aware of a substance localized in the pancreas that lowered blood sugar. But no one had been able to either isolate it or use pancreatic extract to successfully treat diabetic patients. After further studies reaffirmed the link between the pancreas and diabetes, research pinpointed pancreatic extracts to treat the disease. Attempts to supply the missing hormone by oral administration failed because the hormone was destroyed in the gastrointestinal tract. The key to success, therefore, was to extract insulin from the pancreas before proteolytic enzymes destroyed it. Sir Frederick Banting and Charles Best (Fig. 21) were destined to accomplish this daunting task.

During the early 1920s, Banting was a young orthopedic surgeon, struggling to make a success of his medical practice in the Canadian town of London, ON. With an insufficient number of patients to earn a living wage, Banting took a part-time job teaching physiology to medical students for \$2 per hour. After preparing a lecture on carbohydrate metabolism, which he knew very little about, Banting went to bed. However, he was unable to sleep. In preparing the lecture, he had become intrigued by an article authored by a pathologist named Moses Barron at the University of Minnesota. During a routine autopsy, Barron had observed the rare case of a pancreatic stone that obstructed the main pancreatic duct. The occlusion had produced a degeneration of the acinar cells, whereas the islet cells remained virtually intact (Barron, 1920). After reading this article, Banting theorized that previous attempts to isolate the active princi-

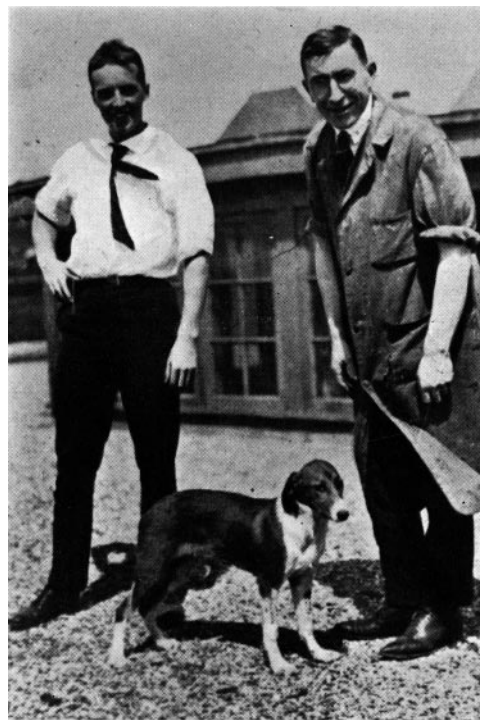


FIG. 21. Frederick Banting (1891–1941) and Charles Best (1899–1978) pose with a dog that was rescued through administration of insulin. Collaborators not shown in the photo include John Macleod (1876–1935) and James Collip (1892–1965).

ple during extraction of the pancreas failed because the unknown substance was degraded by digestive enzymes in the acinar tissue. At 2 AM, Banting wrote down 25 words that would highlight a momentous discovery: “Diabetes Ligate pancreatic duct of dogs. Keep dogs alive till acini degenerate leaving Islets. Try to isolate the internal secretion of these to relieve glycosurea” (http://diabetes.ca/Section_About/BantingIdea.asp).

Banting’s rudimentary knowledge of this subject was exemplified by the fact that he was unable to correctly spell *diabetes* and *glycosuria*. Moreover, he had never treated a diabetic patient, even with basic dietary restrictions. Nevertheless, he became obsessed by his idea and its ultimate success. Since Banting’s clinical practice was floundering (it is said that he took in a total of \$4 in his first month of practice), he made up his mind to travel to Toronto to discuss his idea with an expert in the field. His objective was John J. R. Macleod, who had left Western Reserve University a few years earlier to become Head of the Department of Physiology at the University of Toronto. Banting had been advised by a senior colleague to contact Macleod because he was a leading authority on carbohydrate metabolism and diabetes, having published almost 50 articles on those subjects alone.

Unlike many scientific novices, Banting presented a hypothesis that was entirely feasible. Nevertheless, Banting was viewed by Macleod as ill-cast to experimentally test his idea, since he possessed no credentials such as an advanced research degree or any publications or

experience in research. So Macleod naturally inquired as to why Banting thought he could succeed when others with far more research experience had faltered. Nicolas Paulesco, a Romanian scientist, had already published articles (in French) that described successful experiments with pancreatic extracts in 1921; however, Paulesco had been delayed in the clinical testing of his extracts (Paulesco, 1921). Several others joined the list of those making unsuccessful pancreatic extracts, including Georg Zuelzer, a German researcher who had produced a pancreatic extract called *acomato* (Zuelzer, 1908). However, no drug company was willing to undertake the production of his extract. Two Americans named E. L. Scott and Israel Kleiner also flirted with success. But neither was able to eliminate the toxic properties of the extract and convince the scientific world that the hormone had actually been obtained (Scott, 1912).

In addition to Banting's complete lack of experience and training, Macleod had other reservations about a successful outcome. The perceptive Macleod realized that Banting, who lacked the necessary background knowledge of the field and the chemical testing procedures involved, would require a great deal of assistance and direction. In fact, Banting himself once admitted that if he had been thoroughly acquainted with the literature and the travails that beset others, he might not have undertaken the project. Nevertheless, Macleod gave some consideration to Banting's idea, because he surmised that even negative results might be of some theoretical value. Although initially Banting had reservations about moving to Toronto, he finally opted to meet again with Macleod.

Banting's persistence, plus the likely possibility of more reliable results with the recent advances in the methodology for measuring glucose in blood and urine, may have contributed to the more positive stance eventually taken by Macleod. So in the spring of 1921, after finally persuading Macleod to provide him with some laboratory space, two graduate assistants, and several dogs, Banting moved to Toronto. Although Banting possessed the necessary surgical skills, experience in physiology and biochemistry was also needed to tackle this project. So Macleod selected Charles Best and Clark Noble, who were 4th-year students in the honors physiology and biochemistry course, to assist Banting. The prime motivation for these students was to earn some extra money during the summer hiatus. A coin toss decided that Best would work with Banting first. The month was May, the 17th day to be exact, in the year 1921. It was 1 day after Best had completed his examinations for his undergraduate degree when Banting and Best, along with Macleod, began their experiments that would soon bequeath scientific immortality to all of them.

The general design of the research plan was formulated by Macleod, who gave his younger associates sug-

gestions about surgical techniques and the preparation of extracts and then helped them get started on their first dog. A couple of weeks later, Macleod departed for his native Scotland on summer holiday. The widely held belief that Macleod immediately departed after putting Banting and Best to work is not true. Prior to Macleod's departure, Banting and Best had been experimenting for 1 month and consulting with him during this time. Moreover, Macleod apparently reviewed the status of the project before leaving, provided his summer address, and gave fairly explicit parting instructions to his younger colleagues.

Banting was tasked with performing surgery to tie up the pancreatic ducts in dogs and allowing ample time for the glands to atrophy. At that point, the pancreata were removed, and an extract was prepared. Although Banting and Best had expected to spend only 8 weeks on the project, it was late in July before they were able to prepare and inject an extract. At first, the most effective extracts were capable of producing only a modest prolongation of life in the diabetic dogs. Nevertheless, the Banting and Best team seemed to be working well, with Banting doing the surgery and Best analyzing the blood and urine samples. Because of the progress that Banting and Best were achieving together, Clark Noble graciously declined his turn with Banting. So with the goal of keeping their diabetic dogs alive for extended periods of time, the two young researchers continued to toil feverishly over the next few months to improve the quality of the extract, which they called "isletin." By this time, Banting became so confident of the ultimate success of the project that he completely divested himself of his clinical commitments in London, even before Macleod returned and critiqued the work that had been done.

After returning in September, Macleod at first cast a critical eye on the results and requested that the experiments be confirmed before proceeding to the purification and assay phases of the project. Banting did not take Macleod's criticisms lightly; in fact, he even threatened to move the project to another institution outside of Canada. To mollify Banting, Macleod rewarded him with additional personnel and resources to continue with the experiments. Macleod's willingness to provide more assistance for Banting may have, at least in part, been prompted by Professor of Pharmacology Velyien Henderson's involvement in Banting's affairs. Just 4 months after arriving in Toronto, Banting had a chance meeting with Henderson in his office. They naturally began to discuss Banting's work and his future prospects. After learning about Banting's precarious financial situation (he was working without salary) and his efforts to achieve success under spartan working conditions, Henderson volunteered to provide laboratory space for Banting. When in mid-September the one junior member of the pharmacology department left for another assignment, Henderson pulled strings to re-

place him with Banting. So, because of Henderson, Banting had adequate laboratory space and was on the payroll of the university as a special lecturer in pharmacology. He would now be able to concentrate fully on his work.

Banting and Best published their first article "The Internal Secretion of the Pancreas" in the February issue of the *Journal of Laboratory and Clinical Medicine* (Banting and Best, 1922). Macleod edited the first draft but declined to be listed as a coauthor because he deemed it to be primarily the work of his young colleagues. Before their article was published, Banting and Best publically presented their work at the American Physiological Society Meeting in New Haven, CT in late 1921. As president of the Society, Macleod introduced his colleagues (<http://www.the-aps.org/about/pres/introjjm.htm>). However, as the designated speaker, Banting spoke haltingly and without confidence. As a result, Macleod felt compelled to adequately address the questions posed by members of the distinguished audience. Banting deeply resented Macleod's interference and felt that the senior member of the team was trying to dominate the limelight. This episode aggravated Banting's concerns about Macleod's motives, which had first surfaced after Macleod returned from his holiday, and would endure throughout Banting's life.

In retrospect, some colleagues claimed that Banting's early experiments were quite crude and did not document the validity of his idea. Yet Macleod still understood the enormous potential value of this study and eventually turned over the entire resources of his laboratory to accomplish the intensive work that still had to be done. In addition, to further expedite progress, it was decided to add another individual to the group. Apparently, it was Banting who first suggested to Macleod that James B. Collip should join the team to accelerate the production of quality insulin. Due to a positive twist of fate, Collip, a biochemist on sabbatical leave from the University of Alberta, had been working in Macleod's laboratory as a Rockefeller Fellow. But fortuitously, Collip possessed significant knowledge of preparing tissue extracts. So he provided invaluable assistance with the purification process and soon achieved a less toxic and more effective product by gradually increasing the concentration of alcohol in the extracts.

In addition to his key role in preparing effective tissue extracts, Collip was also responsible for developing a more rapid and less cumbersome method for assaying blood sugar in rabbits. This method proved to be a key factor in facilitating the acquisition of positive results. Further progress was achieved in August 1921 when the supply of duct-ligated dogs was exhausted and a new approach was developed using normal pancreas to isolate an effective extract (Collip, 1923). Other advances in the preparation of extracts were later established. Banting, who grew up on a farm and was familiar with stock breeding, identified another source of extracts in pancreata of unborn calves. Eventually, extracts taken

from glands of fully grown cows and pigs were successfully employed therapeutically. This was a providential finding at the time, because only much later was it determined that the genetic sequence of human insulin differs only by three amino acids from the bovine form and by just one amino acid from the porcine form. As a result, the various animal extracts employed early on fortuitously proved to be effective modes of treatment in humans.

However, success began to fuel animosity from another source. Collip was housed in the pathology laboratory several blocks away from the dog labs, so Banting began to perceive that a new phase of the research was underway that seemed to exclude him. To allay his suspicions, Banting insisted that the first clinical applications be carried out with an extract that he had made, despite the fact that Banting had no qualifications for experimenting on patients. Still, Macleod interceded with the head of the clinic to allow Banting to use his preparation on the first patient, who happened to be a moribund teenager. Banting's extract produced minimal clinical benefits. However, treatment of the boy resumed a few weeks later, this time with a purified extract prepared by Collip. Daily injections produced marked and immediate improvement in his clinical status. In February 1922, the treatment of six more patients also produced very favorable results. Word of the spectacular success of this new agent spread rapidly. The advent of insulin now gave hope to diabetics throughout the world of improving the quality and duration of their lives.

One of Banting's private patients of note was Elizabeth Evans Hughes, the teenage daughter of Charles Evans Hughes, the unsuccessful Republican candidate for U.S. President in 1916, the former U.S. Secretary of State, and later Chief Justice of the Supreme Court. Her recovery, after weighing only approximately 50 pounds and almost unable to walk, was publicized throughout the world. As a result, Banting's standing in the medical community rapidly reached heroic status. This adulation was not misguided since Elizabeth lived on to the age of 60. The successful treatment of Elizabeth Hughes underscored the significance of this discovery, because a year earlier the diagnosis of diabetes was a virtual death sentence for this young lady; with insulin treatment, she would now live a full and productive life.

The chemistry of insulin eventually progressed from the preparation of the first crystalline form by John Jacob Abel in 1926 to the establishment of its amino acid sequence by Frederick Sanger, who received the Nobel Prize in Chemistry for this work, and finally to the complete synthesis of the hormone in 1966 by Katsoyannis. Sanger's work was responsible for defining the first complete structure of a protein (Abel, 1926; Sanger and Tuppy, 1951a,b; Sanger and Thompson, 1953a,b; Katsoyannis, 1966). In 1967, after decades of effort, Dorothy Crowfoot Hodgkin determined the spatial conformation of the molecule by X-ray diffraction, and she too

was awarded the Nobel Prize (Blundell et al., 1971). The emergence of DNA technology in the 1970s led to the synthesis of a human type of insulin, which obviated the need to maintain stockpiles of animal pancreata. Production of the first recombinant DNA insulin was announced in 1978 (Crea et al., 1978). Although recombinant insulin may not have constituted a major breakthrough in pharmacotherapy, this discovery did set the stage for the development of genetically engineered drugs for human use and for the establishment of close relations between biotech firms and the pharmaceutical industry.

Despite his possessive posture regarding the project, Banting did not claim a patent for insulin. He wanted to make it available to the world, so he magnanimously sold the patent to the University of Toronto for \$1. To manufacture larger quantities of the material, the local Connaught Antitoxin Laboratories were enlisted to finance and administer production. Collip was charged with overseeing its manufacture. However, George Clowes, Research Director at Eli Lilly, had been present at the New Haven meeting several months earlier. Realizing the significance and the potential commercial value of the findings, Clowes inquired whether the Toronto group would collaborate with Eli Lilly to produce the extract commercially. Macleod declined the offer at the time, because he felt that further progress had to be made in isolating and purifying the hormone before considering commercial preparation. Still, Macleod was impressed by the enlightened attitude expressed by Clowes and Eli Lilly toward research and by their willingness to develop close ties with the scientific community.

So when the Toronto team began to experience problems consistently isolating and purifying the valuable substance, a collaboration was established with Eli Lilly in May 1922. This agreement led to the development of methods for the large-scale production of insulin. Best and Collip even traveled to Indianapolis to advise the chemists at Eli Lilly with regard to the details of the process. As a result, by that summer the first commercial supply of the hormone became available. After Eli Lilly sold more than a million dollars worth of insulin during its first year of production, the company was transformed into a pharmaceutical giant. The collaboration of the Toronto team and Eli Lilly, which obviously turned out extraordinarily well for everyone concerned, was another testament to the successes that can be achieved by the cooperative interaction of academia and the pharmaceutical industry.

As with several other Nobel Prize-winning discoveries, controversy and animosity engulfed the discovery of insulin almost from the beginning. Banting was known as a person who, although dynamic and forceful, was also impatient and insolent. The seeds for the estrangement of Banting and Macleod were sown when Macleod returned from his summer holiday and strongly criticized the work that Banting and Best had done in his

absence. Banting's growing dislike of Macleod seemed to escalate even further after the meeting in New Haven, when Macleod took over the discussion of their article after Banting had faltered. Although the project continued to proceed forward, Banting began to gradually withdraw from interacting with members of his team. Having begun the project, Banting now perceived it to be taken over by others only when obvious success was apparent and the most positive results were forthcoming. Indeed, many of Banting's concerns were bolstered by the fact that Macleod took charge of the efforts to produce larger quantities of insulin, whereas Collip continued to work alone to improve the quality of the extract.

To complicate matters further, Collip also became embroiled in controversy. After the successful treatment of patients with insulin, Collip apparently agreed to share all information with Banting and Best about the extraction method. However, over time, Collip began to view the disagreements that involved Banting and Macleod as onerous and unprofessional. He was particularly annoyed by Banting's vexing attitude. As a result, Collip threatened to withdraw from the group and take out a patent in his own name. This led to a physical confrontation between Banting and Collip. Eventually, relative sanity prevailed and Banting, Best, and Collip each agreed not to seek his own patent.

Some of these issues were resolved in April 1922 when the team prepared an article that summarized all of their work to date. The authors were listed in the following order: Banting, Best, Collip, Campbell, Fletcher, Macleod, and Noble. W. R. Campbell and A. A. Fletcher were clinicians who had dealt with problems that arose from the new treatment, whereas Clark Noble, the second student assistant originally assigned to the project, had returned to assist in the rabbit studies and the glycogen experiments. In this article, the group gave its discovery the name insulin (Banting et al., 1922).

To provide a public report of their momentous findings, all of the authors agreed that because he was a Society member, Macleod would speak at the meeting of Association of American Physicians in Washington in May. There is little doubt that Macleod was the appropriate person to serve as the voice of the group. He was widely known as an honest, dedicated scientist who was respected for his high standards of research, and most importantly he was skilled in conveying ideas and information. Just 2 weeks short of a year after Banting and Best began their work, Macleod announced to the world that the Toronto group had discovered insulin and described its therapeutic efficacy. The audience, which was composed of many distinguished scientists and experts in diabetes, received the presentation with a standing ovation. So what had begun as a summer project turned into one of the greatest medical discoveries in the history of science.

As a consequence of his inspiring presentation, Macleod was credited with the discovery by some lay reports. As expected, the accolades directed toward Macleod did little to allay Banting's suspicions about his motives and in fact fostered deep resentment in Banting. Although tensions between the two men did not affect the progress of the work, they were persistent. Whatever Macleod seemed to do relative to the project tended to evoke some response from Banting, and the response was often intemperate. Although initially Macleod had been unaware of Banting's negative feelings toward him, his animosity toward Banting in turn slowly grew. Macleod now considered Banting an ungrateful young doctor who did not appreciate what he, as the senior professor, was contributing to the project. At every step, Macleod believed that he had given Banting and Best requisite assistance, adequate support, and credit for discovering the hormone. In addition to the actual discovery, Macleod envisioned the project to consist of two other components, Collip's isolation of the active principle and the investigation of its physiological effects by clinical investigators. So, although Macleod has too frequently been profiled as a dark figure in this drama, and perhaps not deserving of the tributes that were afforded him, one may just as well argue that he played a vital role by keeping the feuding team together until success was achieved.

Despite the high praise attributed to Macleod's talk in Washington, after Banting gave his side of the story to *The Toronto Daily Star*, his name now became closely associated with the discovery. As a consequence, innumerable honors were bestowed upon him, including an appointment as Professor of Medical Research and the establishment of the Banting Institute at the University of Toronto. Banting also became an honorary member of many of the major scientific and medical societies throughout the world and was eventually knighted. In June, 1923 the Canadian House of Commons granted Banting a lifetime annuity of \$7500, a generous award in those days. However, members of the House of Commons could not have had the foresight to envision that of the four principal players in this story, only Banting would fail to make another important discovery during his lifetime. Still, in 2004, Banting was selected 4th in the top 10 of the "Greatest Canadians" as determined by the Canadian Broadcasting Corporation.

Even though the names Fred Banting and Charles Best are closely identified with this celebrated discovery, in 1923, the Nobel Prize was awarded to Banting and Macleod. Although the Nobel Committee was well aware of Best's contribution, he was only a student at the time and did not present the findings at any of the meetings. Therefore, the Committee did not view his role in the discovery as a key one. However, Banting vigorously objected to the recognition given to Macleod by the Nobel Committee and had to be dissuaded from turning down the award. Banting did what he could to correct the

perceived injustice by sharing half of his monetary prize with Best. Collip was also overlooked by the Nobel Committee, even though he succeeded in purifying insulin far beyond what Banting and Best had achieved. His reward was a sharing of the prize money with Macleod. But despite all of the personal turmoil experienced by this quartet, the selfless act of this team to eschew the opportunity to obtain a patent for their therapeutic agent cost them a fortune in royalties. But more importantly, the success of the insulin work succeeded in enhancing the life expectancy of diabetics some 25-fold. Extraordinary success had been achieved despite the fact that the Toronto team lacked any knowledge about how insulin regulated glucose utilization in the body.

One year after the discovery of insulin's therapeutic potential, another future Nobel Laureate, Bernardo Houssay, took the study of insulin secretion to another level by focusing on the interactions of the pituitary gland and endocrine pancreas (Houssay, 1936). Later in 1936, Cyril Norman Hugh Long and Francis Lukens also provided convincing evidence that the adrenal cortex, as well as the adenohypophysis, exerted hormonal effects that were antagonistic to insulin. These studies sparked a new paradigm by revealing that diabetes could be caused by an excess of certain pituitary and adrenal hormones, as well as by a deficiency of insulin (Long and Lukens, 1936).

Despite his personal frailties, Banting was always cognizant of the fact that the failure of his medical practice provided the entrée to his future successes, and he wistfully proclaimed that "had I not failed in my one year at London [Ontario], I might never have started my research work" (http://diabetes.ca/Section_About/BantingIdea.asp). Although Banting's intemperate behavior toward his colleagues seemed to reflect an unusually strong emotional attachment to the discovery, like most scientists who are associated with important discoveries, he did exhibit qualities of perseverance, intuition, and courage in the face of what might have been insurmountable obstacles to others. During the ensuing years, Banting spent much of his time working with younger colleagues and carrying out various unsuccessful research projects. Perhaps emboldened by his earlier success, Banting undertook the monumental task of trying to cure cancer. However, he achieved greater success and fulfillment by participating in the creation of the G-suit, which was used by pilots of the Royal Air Force during World War II to cope with high-speed flight.

Banting was not at odds with everyone he interacted with professionally. As noted above, soon after his arrival in Toronto, Banting developed an association with Velyien Henderson, Professor of Pharmacology, who remained Banting's closest confidant throughout his life. When problems surfaced with Banting's coworkers, Henderson's advice and counsel prevented him from leaving Toronto, along with his discovery. In addition, sometime after the discovery, Henderson mounted a campaign to enhance public recognition of Banting's ac-

complishments, which culminated in a special research chair being awarded to Banting. To further express his loyalty, Henderson even served as Banting's spokesperson on several of his ill-fated sojourns into other areas of research. So it was not surprising that Banting believed strongly that Velyien Henderson was, in large measure, responsible for the success engendered by the insulin work.

Banting's life and career came to a tragic end in 1941, when a plane taking him to the UK on a secret mission for the Canadian Army Medical Corps crashed in Newfoundland. As a testament to Banting's strong will and heroic nature, he was said to have treated the surviving pilot's wounds before succumbing to his own injuries. The announcement of his death reverberated throughout Canada and the rest of the world. The Canadian House of Commons interrupted its work to mourn his passing. The funeral service was carried out amid pomp and fanfare, the likes of which Canada had rarely seen. His body rested in state in the University of Toronto's Convocation Hall. Following the memorial service, the flag-draped casket was placed on a gun carriage, and the cortege, which included a 200-man military escort, wound its way through downtown Toronto. Dressed in the uniform of a major in the Canadian Army, and wearing medals earned during his World War I exploits, Fred Banting was lowered into his grave amid the firing of three volleys and four trumpeters playing reveille—a true hero indeed.

Macleod, mentally fatigued by all of the controversy that surrounded the great discovery, returned to Scotland in 1927 to become Professor of Physiology at his alma mater, the University of Aberdeen. He later became Dean of the Medical School, although he was tormented in his later years by debilitating arthritis.

After his work with insulin was completed, Charles Best completed his education by graduating from medical school. He then sailed to England to study with Sir Henry Dale, now Director of the Biochemistry and Pharmacology Department at the National Institute for Medical Research. Dale had maintained a strong interest in the insulin project since its inception. Prior to Best's arrival, Dale volunteered to travel to Toronto in September 1922 along with the biochemist Harold Dudley to evaluate the credibility and possible utility of the alleged discovery for the British government. Although British scientists were skeptical that a major discovery could be made in "outback country," Dale quickly realized that a major scientific breakthrough was occurring. He later wrote that "whatever might or might not finally be decided about Banting and Best experiments, nobody could deny that a first-rate discovery has been made" (Bliss, 1982). Dale's prestige as a scientist, taken together with his persuasive arguments, ultimately led to the commercialization of insulin in Great Britain.

In addition to obtaining invaluable experience in Dale's laboratory, Best was advised by Dale to avoid the

limelight and get additional training to complement his work with insulin. Dale felt that this strategy would prepare Best for his future scientific endeavors. Best's many successes throughout his distinguished career attest to the fact that he followed this sage advice. When he returned to Toronto at the age of 29, Best assumed Macleod's position, and by doing so became Banting's superior as Head of Physiology. His discovery of histaminase, mechanistic studies that led to the treatment of thrombosis with heparin, and basic studies on the dietary factor choline are only a few examples of his additional accomplishments in research (Best and McHenry, 1930; Best, 1938; Best and Rideout, 1939). He also used his prodigious wealth of knowledge to coauthor with Norman Taylor the classic textbook *The Physiological Basis of Medical Practice*. Best also received many honors related to his insulin work throughout his lifetime and, like Banting, Macleod, and Collip, was elected a Fellow of the British Royal Society. All of the honors notwithstanding, Best probably derived the most satisfaction from the statement put forward by authors of the official history of the Nobel Prize. In 1950, this prestigious group acknowledged that an error had been made in 1923 and it "would have been right to include Best among the prize-winners . . ." (<http://discoveryofinsulin.com/Best.htm>). So, despite not receiving the ultimate award, Charles Best came to be fully recognized by history.

Best's relationship with Fred Banting was a proverbial roller coaster ride. As Macleod's student and Banting's coworker, Best seemed to be caught in the middle of a difficult situation involving the two feuding members of the team. Later on, their relationship began to deteriorate when Best found that he was unable to respect Banting as a scientist and physician. When Banting died prematurely in 1941, Best took charge of the Banting and Best Department, and in 1953 the Best Institute was erected next door to the Banting Institute. Despite the ill will harbored by both men, after Banting's death, Best maintained that he and Banting were solely responsible for the discovery of insulin. Best outlived everyone else in this drama but passed away in 1978 shortly after learning of the death of his eldest son.

Collip always manifested a low profile in discussing the discovery of insulin and modestly felt that his role was "very trivial by comparison with Banting's contribution" (Collip, 1941). Not long after the discovery and establishment of a program for producing insulin on a large scale, Collip returned to Alberta. He eventually became a leading endocrinologist and achieved international fame for his research on the parathyroid gland and hormones of the adenohypophysis. His modification of the method to measure serum calcium that he developed in 1925 was widely employed in clinical chemistry laboratories over the next 40 years (Clark and Collip, 1925).

Despite the conflicts in personality, the relationships among the protagonists were not always hostile. In fact, during the 1930s, Collip and Banting became friends, and the night before Banting took his ill-fated flight, he paid a visit to Collip. Both had mellowed from the earlier days, and they agreed that the discovery of insulin was the result of a well coordinated interaction of all concerned parties. When word came about the accident, Collip took the tragic news with deep sorrow. In his obituary tribute, Collip graciously endowed Banting with the major share of the credit for the discovery. Ironically, Collip succeeded Banting as senior administrator of medical research in Canada. He eventually assumed a faculty position at McGill University and later served as Dean and then President of the University of Western Ontario.

Deferring any judgments about the validity of each one's point of view, all of the major participants in this drama seemed unaware that scholars and introspective individuals, who comprehend and learn from the important lessons of history, would ultimately bestow rightful credit for the discovery to each of them (Bliss, 1982). At the same time, the former members of the team may also have been dismissive or unconcerned about the impact that the discovery portended from another, more global perspective. The unchallenged success of this project and the remarkable effect that it had on the human condition served to transform endocrinology into an important area of research and heightened interest in using tissues as therapeutic tools. The discovery of adrenal corticosteroids and their therapeutic applications, which followed the discovery of insulin some 15 years later, would also be facilitated in great measure by the combined efforts of academia and the pharmaceutical industry. These efforts would culminate in even greater opportunities for future drug development.

The Banting House National Historical site now stands as a monument to the turbulent career of Sir Frederick Banting by portraying the history of insulin's development. One important component of the Banting Museum is the Flame of Hope, which was lit by the Queen Mother in 1989 and serves as a reminder that a cure for diabetes must still be found. When the cure is finally revealed, the doctor or team responsible for the discovery will be invited to the museum site to extinguish the flame. Only then can the final chapter in the story of insulin be written (http://diabetes.ca/Section_About/BantingSquare.asp).

L. Philip Hench, Edward Kendall, and Tadeus Reichstein: Hormones of the Adrenal Cortex, Their Structure, and Biological Effects

1. Philip Hench. The adrenal gland, like the endocrine pancreas, occupies a pivotal role in the evolution of endocrine pharmacology. Insight into the physiological significance of the adrenal gland began in the 1850s when Thomas Addison described the clinical syndrome associ-

ated with adrenal insufficiency (Addison, 1855). Some 45 years later, Oliver and Schaefer reported the striking pressor effects of adrenal extracts. The suggestion made by Oliver that adrenal extracts might be used to treat Addison's disease spawned a chemical analysis of the extract, resulting in the isolation and purification of the active principle as epinephrine (Oliver and Schafer, 1895). Although by the 3rd decade of the 20th century it was generally agreed that the cortex and not the medulla was obligatory for maintaining life, arguments continued to rage about whether the deficiency state involved a basic defect in carbohydrate metabolism or electrolyte imbalance. The answer to this question, and to related ones, could not be addressed until the hormones of the cortex were isolated and identified and the factors that regulated their activity were defined. However, because of limitations in methodology, the identification of the adrenal hormones proved to be a formidable task.

It was against this background that Philip Hench (Fig. 22), Head of the Rheumatic Disease Service at the Mayo Clinic, began a protracted study in 1929 to define the etiological basis of rheumatoid arthritis and develop a treatment for this disorder. At the time, the pathophysiology of rheumatoid arthritis, like many other diseases, was still an enigma. Hench had long been affected by the painful and crippling nature of the disease and aspired to relieve his patients of its debilitating symptoms. Toward this end, he observed that arthritic patients who became pregnant exhibited a rapidly developing amelioration of their symptoms, which even disappeared for varying periods of time. These observations, taken together with the fact that Hench had observed that the adrenal glands were enlarged during pregnancy, led him to postulate that a "metabolite" (unimaginatively called substance X) elaborated by the adrenal glands was responsible for the remission of arthritic symptoms. Another aspect of this story emerged



FIG. 22. Edward Kendall (1886–1972) and Philip Hench (1896–1965). Copyright 2007 by the Mayo Foundation for Medical Education and Research.

in 1929, when a 65-year-old patient reported to Hench that after suffering from the debilitating disease for 4 years, jaundice suddenly developed, and within a week most of his symptoms had subsided (Hench, 1938).

These clinical findings were considered to be of major significance, because they provided Hench with the first clue that rheumatoid arthritis might be a reversible process rather than a relentlessly progressive one. The striking reversibility of symptoms sometimes observed with the onset of jaundice or pregnancy also bolstered his view that symptomatic relief was brought about by some normal constituent of the body. Knowing that during jaundice bile acids were retained, Hench initially theorized that a temporary excess of a normal biliary constituent, such as bile acids or bilirubin, might be responsible for the reversibility of the symptoms. His clinical observation that a reversal of the symptoms was more striking following the appearance of jaundice than the onset of pregnancy probably bolstered his belief of a possible relationship between biliary secretion and the symptoms of arthritis. However, after conducting further studies, Hench excluded the liver as a possible source of the unidentified substance and thereby eliminated jaundice as a key element in this pathophysiological process.

So Hench turned his attention to a possible hormonal source of substance X. Cognizant of the greater incidence of rheumatoid arthritis among women, Hench observed that one of the major physiological changes observed during pregnancy was a marked increase in the concentrations of certain hormones in the body. He determined that substance X was not an estrogen/progesterone-type hormone when the administration of female sex hormones to both male and female patients failed to bring about an abatement of symptoms. On the basis of these and other clinical findings, Hench proposed that substance X was a hormone that was elaborated by both normal males and females and was specific in nature and function. At this juncture, Hench held the belief that the hormone quelled the symptoms of rheumatoid arthritis by either eliminating a chemical deficiency or exerting an antibacterial effect (Hench, 1938).

2. *Edward Kendall and Tadeus Reichstein.* Aware that the symptom of fatigue observed in arthritics exhibited similar characteristics to those observed in adrenal insufficiency, Hench pinpointed the adrenal glands as the possible source of substance X. In doing so, he was most fortunate in developing an extended and fruitful collaboration with Edward Kendall (Fig. 22), who was Professor of Physiological Chemistry at the Mayo Clinic. Kendall was known as a very dedicated researcher who was described by a colleague as "a man who celebrated Christmas Day in his laboratory (Lloyd, 2002)." In 1914, at the age of 28, he became the first chemist to isolate thyroxine (thyroid hormone) in crystalline form (Kendall, 1914). In addition, his entrepreneurial skills were exhibited at this

early stage in his career when he wasted no time in patenting his thyroid hormone preparation. Kendall assigned the intellectual property of his preparation to the University of Minnesota, which had an affiliation with the Mayo Clinic. In 1919, the university granted Squibb Pharmaceuticals an exclusive license to market the drug. The hormone enjoyed broad appeal in the treatment of hypothyroidism and as a general metabolic stimulant. Because of Kendall's administrative skills, the successful marketing of thyroxine provided another example as to how basic research could foster the development of effective therapeutic agents.

During the 1930s, Kendall's interest had turned to adrenal research. By this time, progress in this field had been fueled by advances in technology. Most significantly, adsorption chromatography now made it possible to purify adrenal extracts that were completely free of medullary catecholamine. This accomplishment propelled Kendall to the forefront in the quest to bring a cortical hormone to the marketplace. The active principle was later found to be soluble in organic solvent (lipophilic) and yielded a product that could prolong the life of adrenalectomized animals for an extended period of time (Mason et al., 1936). These findings set the stage for the production of the active principle, called cortin, in pure form and the elucidation of its chemical nature.

Kendall then engineered a coordinated interaction between research scientists and the pharmaceutical industry to accelerate the process for making steroid therapy available to the general public. Kendall initiated the process by brokering an agreement with Parke-Davis Pharmaceuticals so that they would provide a continuous supply of adrenal glands in exchange for epinephrine. Kendall was able to forge this agreement because his laboratory possessed the technological expertise to differentially recover cortical hormones and epinephrine from extracts. In addition, Kendall also negotiated an agreement with Wilson Laboratories, a subsidiary of a large meat-packing company, to standardize the potency of the firm's cortical extracts in exchange for additional supplies of frozen adrenal glands. In this way, Kendall would become the chief source of purified cortical hormones in North America, and Mayo would emerge as a leading center for the treatment of adrenal insufficiency.

Chemists working in Kendall's laboratory not only isolated, identified, and synthesized compound E [17-hydroxy-11-dehydrocorticosterone (cortisone)] from adrenal extracts, they also succeeded in identifying more than 20 chemically related compounds (Mason et al., 1937, 1938). Six of these substances proved to be effective in maintaining adrenalectomized animals. The industrial scale of his efforts to purify hormone aided Kendall in competing with other laboratories both in the United States and in Europe. In Switzerland, Tadeus Reichstein (Fig. 23) proved to be Kendall's most competitive rival, since collaborations with the pharma-



FIG. 23. Tadeus Reichstein (1897–1996). Copyright Nobelstiftelsen.

ceutical firm Organon supplied Reichstein with large batches of adrenal extract. In return, Organon received Reichstein's patents for improved methods to prepare extracts.

The laboratory directed by Reichstein, which was located in the Department of Pharmacy at the University of Basel, also isolated and identified 25 adrenal steroids, all but 6 of which were biologically active (Reichstein, 1936). Like Kendall, who had developed a reputation as a chemist by synthesizing thyroxine, Reichstein's credentials as an expert chemist were established in 1933 when he synthesized vitamin C (ascorbic acid) (Reichstein et al., 1933). For Reichstein, the isolation and identification of the various adrenocortical steroids were tedious and laborious processes, as illustrated by the fact that more than one *ton* of animal adrenal gland was required to produce 1 g of corticosteroid. Nevertheless, the production of desoxycorticosterone by Reichstein in 1937 not only firmly established the steroidal nature of these molecules but was also responsible for the first clinical application of a corticosteroid (von Steiger and Reichstein, 1937). The success of Reichstein's laboratory in synthesizing desoxycorticosterone, which had a relatively higher mineralocorticoid activity than did corticosterone, also confirmed that distinct hormones were responsible for regulating mineral metabolism and carbohydrate metabolism. Despite his prodigious achievements, Reichstein admitted that when he undertook the investigation, he firmly believed that the solubility properties of adrenocortical hormones argued against the possibility that they would be identified as steroids.

By 1940, 28 adrenal steroids had been isolated by Kendall and Reichstein, including cortisone, hydrocortisone (cortisol), corticosterone, and 11-deoxycortico-

sterone. Since compound E (cortisone) was found to be particularly effective in preserving the lives of adrenalectomized animals, Hench concluded that compound E might be substance X. So Hench and Kendall now focused their attention on endocrine-like factors being involved in rheumatic diseases. However, due to the onset of World War II, it would be almost 8 years before a sufficient amount of hormone became available for clinical use and more comprehensive studies could be conducted to define the pathophysiological processes associated with rheumatoid arthritis. Meanwhile, the years of World War II passed, with Hench steadfastly adhering to his personal pledge to employ cortisone as a therapeutic agent.

Another fortunate turn of events in this saga occurred during the war, when the development of adrenal steroids became a program of high priority for the United States government. Intelligence reports claimed that the Germans were injecting Luftwaffe pilots with adrenal extracts, enabling them to fly their planes at unprecedented altitudes. To add to the intrigue, these reports disclosed that this project would be implemented after Germany obtained large quantities of bovine adrenal glands from Argentina. At the time, this South American country was also controlled by a fascist government and would later become the safe haven for a number of Nazi war criminals.

In October 1941, as a consequence of these intelligence reports, the federal government's Committee on Medical Research convened a committee composed of the most eminent scientists available. Their responsibility was to assess the current status of adrenal cortical hormones, as well as their future prospects. Included among these luminaries was Edward Kendall. Following intense debate, this group decided that a top priority should be given to the production of cortisone so that it could be made available on a commercial scale. Kendall's entrepreneurial skills and initiative were instrumental in reaching the decision that several different laboratories would be given the same responsibility, i.e., to repeat Kendall's work by synthesizing cortisone and then testing its military capabilities. All of the distinguished academic researchers and the industrial and pharmaceutical companies that participated in this project accepted no remuneration from the government.

By mid-1943, the potential of cortisone was assessed from a military perspective, and the results were rather unimpressive. The entire data derived from the participating laboratories suggested that cortisone would not improve pilot performance, and although hormone treatment might have limited ability to prevent death at very high altitudes, an appropriate training regimen would be at least as effective. As a result, research to assess the functional value of corticosteroids in military combat was summarily terminated (Rasmussen, 2002).

Although the rumors and intelligence reports about the military use of corticosteroids by the Germans were

eventually proven to be false, a surprising mandate by the United States government to continue supporting the chemical aspects of the investigations helped immeasurably to promote the ultimate development of corticosteroids as therapeutic agents. The mandate was predicated upon the possibility that some military significance could still emerge from this project. In 1943, Reichstein was the first to successfully synthesize compound A (11-dehydrocorticosterone), although the chemists at Merck began using Kendall's novel method for synthesizing compound A as a precursor to produce modest amounts of cortisone (Lardon and Reichstein, 1943). This strategy employed at Merck increased the likelihood that sufficient quantities of the hormone would soon become available for treating patients.

At the end of World War II, to increase the production of cortisone, Merck considered enlarging its facilities, despite the fact that a possible therapeutic use for this compound had not been identified. Although there were several sound financial and commercial reasons not to augment the production of cortisone, Merck fortunately decided to take on this assignment. So, by 1947, with the help of Kendall and his team, Merck chemists developed an economical method for synthesizing cortisone (Sarett, 1948). Hench, who had been waiting 2 decades for corticosteroids to become available for clinical use, seized the opportunity to formally request the compound. After learning that the entire allotment had already been committed, Hench, as one might expect, persisted. His persistence, taken together with the help of Kendall, enabled Hench to obtain a small supply of the drug from Merck. In this way, researchers once again became partners in the commercial endeavors of a pharmaceutical firm.

Despite now having a sufficient supply of the precious agent, Hench and Kendall approached this project with great trepidation. They realized that the ultimate success of their study was problematic at best; however, much more was at stake than the simple success or failure of a given experiment. They were aware that failure would result in a major setback in their quest for an effective treatment for rheumatoid arthritis. But, perhaps more significantly, it might dissuade Merck from continuing to sponsor this project.

Such a series of events would undermine all that they had strived for those many years. So, for his first patient, Hench, with great care, selected a 29-year-old woman who had suffered from severe rheumatoid arthritis for 5 years and was virtually sedentary. Cortisone was administered at a dose of 100 mg for 4 days. The dose administered was considered rather high, but Hench and Kendall did not want to risk failure by treating the patient with an inadequate dose. After 4 days of this regimen, the patient walked out of the hospital without assistance. Since the dramatic clinical results obtained with cortisone were later duplicated by adrenocorticotrophin (ACTH), it was concluded that ACTH

exerted its effects by promoting the liberation of corticosteroids from the adrenal gland.

This very encouraging result understandably prompted a plea for Merck to supply Hench with an additional supply of cortisone. As a result, the company repeated a 36-stage chemical process to produce 1 kg of the hormone within a few weeks. An additional 15 arthritic patients were then treated over the next few months with consistently positive outcomes. After Hench presented his findings at a meeting of the staff at the Mayo Clinic on April 13, 1948, he received a standing ovation (Hench et al., 1949). Daily newspapers helped to disseminate word of this extraordinary discovery by hailing compound E as the "modern miracle drug." The hormone was later renamed *cortisone*, because of the confusion that arose from newspaper reports that erroneously identified the new therapeutic agent as vitamin E.

The first published report of the efficacy of cortisone in the treatment of rheumatoid arthritis, which appeared in 1949, had such an enormous impact on the scientific community that the Nobel Prize in Medicine was jointly awarded to Philip Hench, Edward Kendall, and Tadeus Reichstein just 1 year later. Although physicians were soon harassed by patients seeking treatment with this new miracle drug, Hench and Kendall persisted in arguing that the "use of these hormones should be considered an investigative procedure" (Hench et al., 1950). Nevertheless, in November 1950, Merck made cortisone available to physicians in the United States at a price of \$200 per gram. G.D. Searle & Company and Upjohn also developed novel methods for producing corticosteroids, most notably *hydrocortisone*, thereby making corticosteroids available to the general public at a more reasonable cost.

Hench quickly realized that he would have to employ much higher doses of corticosteroid to treat arthritis than those needed in replacement therapy for adrenal insufficiency. So it was not long before he became aware of the serious side effects that cortisone could produce when used in high concentrations for extended periods of time. Because of such untoward side effects as edema, osteoporosis, diabetogenic activity, hirsutism, and psychic disorders, Hench denied cortisone treatment to patients suffering from hypertension, diabetes, osteoporosis, and psychoses. Hench also observed that the administration of suprphysiological doses of cortisone often produced a reduction in activity of the hypophyseal-pituitary-adrenal axis (negative feedback). But despite the known side effects produced by high doses of adrenal steroids, by the time Hench retired in 1957, a biological method of production (using *Rhizopus nigricans*) had been found, and glucocorticoids became a standard treatment for rheumatoid arthritis.

Hench later became cognizant of the antiallergenic effects of glucocorticoids, which he employed in the treatment of a variety of diseases with an allergenic

basis, including bronchial asthma, lupus erythematosus, acute rheumatic fever, ulcerative colitis, and psoriasis. Although Hench knew very little about structure-activity relationships and the cellular and biochemical mechanisms involved in steroid action, he did correctly predict that the manipulation of the steroid nucleus would ultimately produce agents with glucocorticoid activity that possessed fewer side effects, while maintaining their potent pharmacological actions. So, despite the limitations inherent in corticosteroid usage, the unquestioned significance of steroids as therapeutic and research tools has advanced the accomplishments of Hench, Kendall, and Reichstein far beyond their own individual contributions. By fueling the interest of others to become more invested in this line of research, their work has led to the further amelioration of the pain, discomfort, and disability caused by inflammatory disorders (Lloyd, 2002).

M. Sune Bergström, Bengt Samuelsson, and John Vane: Prostaglandins and Related Biologically Active Substances

Nature has contrived complex regulatory systems to curtail the possibility that cellular homeostasis is perturbed by internal and external factors. Eicosanoids and related substances constitute one such regulatory system. The eicosanoids, which include the prostaglandins, thromboxanes, leukotrienes, lipoxins, and prostacyclin, are a family of lipid-derived compounds that are formed from unsaturated fatty acid precursors, such as arachidonic acid. The widespread involvement of the prostaglandin system in both normal and pathological processes is exemplified by the fact that almost every cell in the body produces one or another of this group of autacoids (local hormones), and they elicit a wide spectrum of biological actions.

1. Ulf von Euler and Sune Bergström. This story had its genesis in the 1930s, when at the age of 25, Ulf von Euler was working in the laboratory of Sir Henry Dale with Sir John Henry Gaddum, another future luminary in the annals of pharmacology. They discovered an atropine-resistant factor that lowered blood pressure and contracted isolated intestinal smooth muscle. This factor was later named substance P (von Euler and Gaddum, 1931). Intense discipline and determination were hallmarks of von Euler's approach to experimental research, so he followed up his discovery of substance P by describing its peptide nature, its general distribution in the body, and methods for its purification and assay. The commitment of von Euler to scientific pursuits stemmed in part from his heritage. His father, Hans von Euler, was a renowned chemist who was awarded the Nobel Prize in 1929. The influence of father on son is illustrated by the fact that the elder von Euler was coauthor on the younger von Euler's first article, which was published when he was just 17 years old.

The discovery of substance P fueled von Euler's interest in hypotensive factors. This commitment culminated in the identification some 3 years later of a lipid-soluble organic acid with hypotensive- and smooth muscle-stimulating activity in accessory genital glands and human semen. He called this factor of unknown biological significance *prostaglandin* (von Euler, 1935). Although von Euler examined various biological effects of this factor, he realized that more comprehensive knowledge concerning the chemistry and biology of prostaglandin was needed. After progress in this field was interrupted for about 10 years, in part by World War II, interest was revived in 1945 at a meeting at the Karolinska Institute in Stockholm. At this meeting, von Euler seized the opportunity to persuade Sune Bergström (Fig. 24) to extend the chemical analysis of lipid extracts of sheep vesicular glands that von Euler had conscientiously safeguarded since the outbreak of the war. After purifying the crude extract about 500 times, Bergström found that it was composed of unsaturated hydroxyl acids that lacked a nitrogen moiety (Bergström, 1949).

This work, which was initiated at the behest of von Euler, was directly responsible for spawning Bergström's long-term involvement in prostaglandin research. However, further exploration of this field was again stalled for several years by Bergström's move to the University of Lund. When work on this project resumed in 1957, Bergström and his colleagues accumulated a largesse of sheep vesicular glands from all over the world, and by using counter current fractionation and partition chromatography, they isolated the prostaglandins PGE₁ and PGF_{1α}. However, due to the necessity of having to measure pico- and nanogram quantities of the prostaglandins, a more



FIG. 24. Sune Bergström (1916–2004). Copyright Nobelstiftelsen.

sensitive method of detection was needed. Fortunately, when Bergström's group moved to the Karolinska Institute in the late 1950s, mass spectrometry became available for analysis. So, by 1962, Bergström and his colleagues were able to identify six prostaglandins in a number of different tissues and then determine their respective chemical structures (Bergström et al., 1962a,b).

Bergström and coworkers were also able to demonstrate that arachidonic acid provided a substrate precursor for this system by conducting short-term incubations with the radioactive form of the unsaturated fatty acid. Using this approach, endoperoxides were isolated and characterized, as well as a hydroperoxide, 12-hydroxyicosatetraenoic acid, and thromboxane B_2 . The thromboxanes were found to exert powerful actions on platelet aggregation and vascular smooth muscle contractility. These results suggested to Bergström that thromboxanes played a key role in the regulation of hemostasis and in the etiology of certain pathophysiological conditions, including thrombosis. By 1964, although Bergström and his collaborators had isolated several different prostaglandins, the biochemical pathways involved in prostaglandin synthesis and metabolism were still virtually unknown. The mapping of these pathways had to await the key experiments to be conducted by Bergström's student Bengt Samuelsson.

In his writings, Bergström laments the difficulties that he encountered in persuading colleagues to investigate the pharmacological properties of prostaglandins in various *in vitro* systems. However, this knowledge gap was markedly reduced when Martha Vaughan, Daniel Steinberg, and Jack Orloff at the NIH, in collaboration with Bergström, tackled this question. For example, it was found that PGE_1 and PGE_2 inhibited the stimulatory effects of epinephrine, glucagon, and corticotrophin on lipolysis in rat adipose tissue (Steinberg et al., 1963, 1964). These inhibitory effects were mediated by a reduction in cAMP levels (Orloff et al., 1965). Subsequent experiments, inspired by the independent work of both Rodbell and Gilman (see section II.P.), demonstrated that prostanoid receptors were coupled to effector mechanisms through G proteins, frequently involving adenylyl cyclase or phospholipase C. The fact that G proteins functionally couple the activation of receptors to the regulation of a myriad of effector systems helped to explain why various prostaglandins possess such diverse and widespread effects. Major differences in functional responses observed between the effects of various prostaglandins and their analogs, and between the same prostaglandin and different animal species, were also found to contribute to the diversity in the actions of prostaglandins.

2. *Bengt Samuelsson.* At this juncture, Bengt Samuelsson (Fig. 25) took over the leadership of the project, and during the latter part of the 1960s undertook the staggering task of mapping the major biosynthetic pathways of prostaglandin metabolism through cyclooxygen-



FIG. 25. Bengt Samuelsson (1934–). Copyright Nobelstiftelsen.

ase- and lipoxygenase-catalyzed reactions. Samuelsson found that the cyclooxygenases possessed endoperoxide activity that converted PGG to PGH. These labile intermediates were metabolized enzymatically to a number of different products, including PGE and thromboxane. Samuelsson also demonstrated that the lipoxygenases were a family of cytosolic enzymes that catalyzed the oxygenation of polyenoic fatty acids to corresponding lipid peroxides and the leukotrienes (Borgeat et al., 1976). In addition to identifying a metabolite of arachidonic acid that was called leukotriene A_4 , Samuelsson also discovered cysteine-containing leukotrienes in a variety of biological systems. These proinflammatory and immunoregulatory mediators (LTB_4 , LTC_4 , LTD_4 , and LTE_4) were named leukotrienes because they were first identified in leukocytes (neutrophils), and a conjugated triene was a common structural feature. The biological effects of leukotrienes included potent bronchoconstrictor, vasoconstrictor, and chemoattractant activities, as well as negative inotropic effects on the heart (Borgeat and Samuelsson, 1979a,b). Because leukotrienes possess these diverse actions, they have been implicated in the pathophysiological processes associated with airway anaphylaxis.

Samuelsson's contributions to this field merit the highest of accolades because it became clear from his work that prostaglandin metabolism was infinitely more complex than anyone had ever imagined. In addition to the large number of components, their short-lived nature in many cases made the elucidation of these pathways a formidable undertaking. Nevertheless, Samuelsson and his colleagues succeeded in identifying the

many substances involved in prostaglandin metabolism and positioning them in their correct sequence. This was an accomplishment of monumental proportions.

3. *John Vane*. The key experiments performed by Bergström and Samuelsson provided a fitting prelude to the complementary contributions made by John Vane (Fig. 26). In the late 1960s, Vane began an examination of the role of prostaglandins in the inflammatory process at Wellcome Research Laboratories in the UK. Although the development of sophisticated chemical methods was in large measure responsible for advancing the field of prostaglandin research, the bioassay also contributed significantly to its initial development, primarily as a result of the major contributions made by John Vane and his team. In fact, the bioassay represents a key component of pharmacological lore and is a laboratory technique that is inherently the domain of the pharmacologist.

Whereas the eminent pharmacologist J. H. Burn deserves singular recognition for pioneering the development of the biological assay, John Vane recognized that unstable products of arachidonic acid metabolism might be more easily identified using bioassay techniques rather than biochemical methodology. Vane also understood that the bioassay could distinguish between physiologically relevant compounds and biologically unimportant metabolites. To provide assay specificity, Vane employed cascade superfusion, which used a combination of tissues such as the stomach strip and rat colon together with the chick rectum (Vane, 1964). In addition, strips of bovine coronary artery were particularly useful for identifying and quantitating specific eicosanoids, because this prepa-

ration contracted in the presence of PGE₂ and relaxed in response to PGI₂ (prostacyclin).

Vane also established that the selectivity of the bioassay could be augmented by judiciously employing specific antagonists. For example, contractions of the rat stomach strip elicited by serotonin were abolished by a serotonin antagonist (methysergide), thereby producing a preparation more sensitive to prostaglandins. In addition to prostaglandins, catecholamines, angiotensin, histamine, and bradykinin could be quantitated by this technique. Although Vane and many other pharmacologists/physiologists used the bioassay to make important discoveries, Sir John Henry Gaddum (Fig. 27) was prophetic in predicting that the bioassay would eventually be replaced by chemical methods that possessed greater sensitivity and specificity. However, he also noted that bioassay could always serve a useful purpose by validating results attained by chemical methods (Gaddum, 1959).

After deciding that much more could be learned about prostaglandins from a physiological and pharmacological perspective, Vane theorized that any tissue that was perturbed or distorted in any way would liberate prostaglandins. He further speculated that pulmonary blood flow was regulated by a distention of the lung to discharge prostaglandins. In carrying out experiments to test this hypothesis, Vane was distracted by the unexpected finding that the infusion of aspirin into a hyper-ventilated dog resulted in an attenuation of the hypotensive response. His interest was further aroused when he found that the abatement of the hypotensive response was accompanied by a reduction in prostaglandin re-



FIG. 26. John Vane (1927–2004). Copyright Nobelstiftelsen.



FIG. 27. Sir John Henry Gaddum (1900–1965). Courtesy of the Department of Pharmacology at University College London.

lease. On the basis of these findings, Vane was not only drawn to conclude that the release of prostaglandins from the lung was a key factor in regulating regional blood flow, he also postulated that aspirin interfered with the synthesis of prostaglandins (Piper and Vane, 1969). The idea that drugs could relieve pain by inhibiting the synthesis of prostaglandins was supported by evidence that certain prostaglandins played a key role in pain perception.

About this time, reports appeared in the scientific literature that supported the idea that prostaglandins also participated in the pathogenesis of inflammation and fever. Such findings favored the idea that the inhibition of prostaglandin synthesis could also account for the anti-inflammatory and antipyretic actions of aspirin and related drugs. To investigate this matter further, Vane incubated homogenates of guinea pig lung with arachidonic acid and measured by bioassay the levels of PGE_2 and $\text{PGF}_{2\alpha}$ formed in the presence and absence of aspirin and indomethacin. The latter drug is another nonsteroidal anti-inflammatory drug (NSAID) with properties similar to those of aspirin. The results of this experiment reaffirmed that aspirin and indomethacin inhibited prostaglandin formation (Vane, 1971).

This important discovery not only offered profound insight into the mechanism of action of NSAIDs, it also provided an important pharmacological tool for probing the role of these lipid-derived eicosanoids in physiological and biochemical processes. It is now a pharmacological maxim that eicosanoids serve as a prime target for many anti-inflammatory drugs and that the side effects of these drugs are frequently attributed to impaired eicosanoid production. In elucidating the mechanism of action of NSAIDs, Vane was well aware of the fact that the mechanism of action of adrenal steroids as anti-inflammatory agents was different from that of aspirin. Steroids depressed prostaglandin synthesis by blocking the release of arachidonic acid from phospholipids, whereas NSAIDs interfere with the cyclooxygenase-mediated conversion of arachidonic acid into eicosanoids.

Vane expanded his accomplishments in this field by being the first to identify prostacyclin (PGI_2), another putative regulator of the cardiovascular system. Following the isolation of the endoperoxides and the discovery of the thromboxanes by Bergström and Samuelsson, Vane found that incubating microsomal fractions of pig aorta together with endoperoxide rapidly generated an unknown substance produced by endothelial cells, which he first called PGX (and later named prostacyclin or PGI_2). The structure of PGI_2 was eventually established by a joint research team of scientists at Upjohn and the Wellcome Foundation where Vane conducted his experiments.

PGI_2 proved to be the major product of arachidonic acid metabolism in vascular tissues. It also exhibited some rather contrasting properties relative to thrombox-

ane A_2 , with regard to its instability, potent antiaggregatory activity, and relaxation of isolated vascular smooth muscle. In fact, PGI_2 was found to be an effective hypotensive agent *in vivo* and was one of the most potent endogenous inhibitors of platelet aggregation (Moncada et al., 1976, 1977). On the basis of the mounting evidence, Vane postulated that the combination of PGI_2 and TXA_2 formed by blood vessel walls served to control thrombus formation by regulating blood vessel tone and platelet aggregation *in vivo* (Dyerberg et al., 1978).

In pointing out the outstanding contributions made by Bergström and Samuelsson in Sweden and by Vane in the UK, one should also recognize the valued participation of Upjohn with regard to its research and development work in this field. The generous policy of Upjohn toward the academic community in supplying prostaglandins for experimentation also deserves much praise. Although pharmaceutical firms have also been very active in developing prostaglandins as therapeutic agents, the clinical use of these substances has been limited somewhat by their relatively short duration of action and the diversity of their effects. Still, because of the importance of their physiological actions, there are still a number of disorders in which eicosanoids have proven to be therapeutically useful, including primary pulmonary hypertension, the maintenance of the patent ductus arteriosus in infants with congenital heart disease, impotence, and bronchial asthma. The putative role of prostaglandins in regulating fertility mechanisms has also had a strong impact on their therapeutic utility, where they have been employed for labor induction, postpartum bleeding, and uterine dilation for diagnostic surgery.

The ability of certain prostaglandin analogs to curtail gastric ulceration has led to the use of the prostaglandin analog misoprostol in preventing ulcers that frequently occur during long-term treatment with NSAIDs. In addition, the discovery of constitutive (COX-1) and inducible (COX-2) forms of the cyclooxygenase enzyme resulted in the development of COX-2 inhibitors. These more selective inhibitors of prostaglandin synthesis exhibit fewer adverse effects on the gastrointestinal tract because they possess a preferential inhibitory action on COX-2 activity induced by inflammatory mediators.

Today, the modulation of prostaglandin metabolism still represents a primary focus of studies related to anti-inflammatory therapies. However, the disturbing reports of thrombotic disorders being associated with prolonged use of COX-2 inhibitors such as Vioxx may redirect interest in this field toward developing anti-inflammatory agents that act by inhibiting the biosynthetic pathway downstream to the cyclooxygenases. In any case, the extensive knowledge about the identity, metabolism, and biological effects of arachidonic acid metabolites provided by the work of Bergström, Samuelsson, and Vane should facilitate the development of more selective therapeutic agents for the treatment of

gastrointestinal, cardiovascular, and respiratory diseases. For their discoveries concerned with prostaglandins, which are paving the way for therapeutic advances in this field, Sune Bergström, Bengt Samuelsson, and Sir John Vane were awarded the Nobel Prize in 1982.

Finally, at this juncture, particular recognition must be afforded the Burroughs Wellcome Foundation for providing the positive environment in which a number of Nobel Laureates were able to carry out their experiments both in the United States and in the UK. In 1936, Sir Henry Dale, an early Director of Research at Wellcome Laboratories, won the Nobel Prize for his studies on chemical transmission of nerve impulses. In 1982, the Nobel Prize was awarded to Sir John Vane, who, like Dale, worked at the Wellcome Research Laboratories in the UK and discovered prostacyclin, as well as the mode of action of aspirin. Sir James Black served for 6 years as Director of Therapeutic Research at Wellcome Laboratories in the UK, where he discovered β -adrenoceptor antagonists, whereas Gertrude Elion and George Hitchings carried out extended studies at Burroughs Wellcome in the United States, where they made invaluable contributions to drug development, mainly in the field of cancer. The extraordinary work carried out under the auspices of the Wellcome Research Laboratories has contributed immeasurably to drug discovery and enabled humankind to remain competitive in its constant battle with disease (Garfield, 1989).

N. Earl W. Sutherland: Cyclic AMP

Although the first hormone (epinephrine) was discovered by Oliver and Schaefer during the latter part of the 19th century (Oliver and Schaefer, 1895), the mechanism of hormone action was not productively examined until Earl Sutherland (Fig. 28) initiated his investigations on the effects of epinephrine and glucagon a half century later. As a student, Sutherland had been fascinated by the diverse effects of hormones. He was also intrigued by the complex responses that followed the administration of minute amounts of these substances or by the various functional defects produced by the extirpation of specific endocrine tissues. Thus, he was an excellent candidate to address these issues.

Sutherland began his scientific career in the early 1940s in the laboratory of Carl and Gerty Cori at Washington University in St. Louis. The work of the Cori team on glucose metabolism was carried out in the Department of Pharmacology from 1931 through 1946, before Carl Cori was appointed Chair of the newly formed Department of Biochemistry. As a member of the Cori laboratory, Sutherland had successfully investigated the enzymatic conversion of glucose to glycogen with Sidney Colowick during the early 1940s (Sutherland et al., 1941). Although much of Sutherland's initial efforts involved adding various hormones to different enzyme preparations and examining the resultant effects, as late as 1955, he expressed doubts about the prospect of



FIG. 28. Earl Sutherland (1915–1974). Copyright Nobelstiftelsen.

analyzing hormone action in broken cell preparations. These early views on the subject were shared by most researchers. However, Ted Rall, one of Sutherland's first colleagues, seemed to be at least partly responsible for eventually convincing Sutherland to embark on the types of *in vitro* experiments that would ultimately determine whether the regulation of certain cellular constituents might be coupled to specific components on the cell surface (Bourne and Rall, 1990).

Despite his initial reservations about using broken cell preparations, Sutherland found studies devoted to the glycogenolytic action of epinephrine and glucagon in liver particularly attractive for several reasons. He had an excellent grasp of the basic biochemistry and enzymology of glycogen breakdown, which had been established by the Cori laboratory. Moreover, the effects of epinephrine and glucagon on glycogen breakdown were rapid, robust, and reproducible, and a large number of slices could be prepared from a single liver. So Sutherland and his associates, working first at Washington University and then after 1953 in the Department of Pharmacology at Case Western Reserve University, began by focusing on phosphorylase, the rate-limiting enzyme that catalyzes the conversion of glycogen to glucose. Sutherland predicted that to be successful in this endeavor it would be necessary to establish reproducible hormonal effects in a soluble system and then extrapolate *in vitro* findings to the physiological action of the hormone *in vivo*. However, at the time, epinephrine- or glucagon-induced activation of phosphorylase, although readily demonstrable in intact liver preparations, was not reproducible if the cells were first broken prior to hormone stimulation.

Earl Sutherland and Ted Rall, then a young assistant professor, began their groundbreaking work on the mechanisms of hormone action by demonstrating that the regulation of phosphorylase activity involved a balance between the donation of a phosphate group to the enzyme (phosphorylation) and the inactivation of this process by a phosphatase (dephosphorylation) (Rall et al., 1956; Sutherland and Wosilait, 1956; Wosilait and Sutherland, 1956). About the same time, two other future Nobel Laureates, Edwin Krebs and Edmond Fischer, who had been studying phosphorylase activation in rabbit skeletal muscle extracts, demonstrated its requirement for ATP and Mg^{2+} (Fischer and Krebs, 1955). Armed with this information, Sutherland and Rall began adding hormones to cell extracts in the presence of ATP and Mg^{2+} . Using this modified approach, they were able to elicit stimulatory effects of epinephrine and glucagon on phosphorylase activity in cell-free systems that seemed physiologically relevant (Rall et al., 1957). Their success in demonstrating effects of hormones in broken cell systems represented a landmark finding and emboldened the two investigators to continue their studies on hormone action.

Sutherland and Rall went on to observe that the hormonal response was lost when the liver homogenate was centrifuged to remove cellular debris but could be restored by recombining the particulate fraction with the supernatant. By differential centrifugation studies, Sutherland and Rall also found that the addition of hormone to the particulate fraction resulted in the production of a heat-stable factor, which in turn activated phosphorylase when the factor was added to the supernatant fraction. The heat-stable factor was determined to be an adenine nucleotide, which was produced by the liver, heart, skeletal muscle, and brain. The factor was later identified as cAMP (Rall and Sutherland, 1958; Sutherland and Rall, 1958).

The discovery by Sutherland and Rall that epinephrine enhances glucose production by elevating cAMP levels leading to the conversion of inactive phosphorylase to active enzyme explained the cellular mechanism whereby receptor recognition of a hormone triggered the response of an effector. Sutherland's unerring instinct in identifying phosphorylase as the target enzyme for his experiments represented a crucial element in successfully defining the steps leading to the discovery of cAMP. These studies provided the basis for understanding how cell signaling operates.

Rall uses a self-deprecatory posture to recall his experiences in ultimately achieving success by reflecting on "how many wrong ideas got us to do the right experiments" (Bourne and Rall, 1990). For example, Rall initially used rats rather than dogs to make liver homogenates. He later discovered that he was using a completely inappropriate animal model, because in rats the actions of epinephrine are mainly expressed by a calcium-mediated α -adrenergic pathway and not

by a cAMP-mediated β -adrenergic receptor pathway. Rall also initially employed an inadequate protocol for centrifugation. The original protocol was modified for him by a Belgian postdoctoral fellow named Jacques Berthet, who fortunately for Rall and Sutherland, happened to be a disciple of another Nobel Laureate, Christian DeDuve. The modification of the protocol then enabled Rall to successfully perform the key experiment in which a supernatant fraction responded to hormones after a small aliquot of the particulate fraction was added back to the supernatant (Rall et al., 1957).

During this time, Edwin Krebs and Edmond Fischer not only found that phosphorylase activation involves reversible protein phosphorylation, but Krebs also went on to complement Sutherland's work by discovering that cAMP-dependent protein kinase mediates many of the diverse actions of cAMP elicited by hormones and pharmacological agents (Walsh et al., 1968). After it became apparent that reversible protein phosphorylation was widespread in nature and affected a multitude of cellular processes, Krebs and Fischer were awarded the Nobel Prize in 1992. Krebs served as Chair of the Department of Pharmacology at the University of Washington from 1977 through 1984, where he led a major expansion of research in molecular pharmacology. An Edwin G. Krebs Lectureship in Molecular Pharmacology at the University of Washington is an enduring tribute to his achievements in the field of molecular pharmacology.

As with many discoveries that provide new insights into basic biological processes, the concept of an intracellular mediator of hormone action was met with some skepticism by the scientific establishment. The proposition that the compound in question was a nucleotide that was resistant to boiling in hydrochloric acid was particularly difficult to fathom, since at the time only acid-labile phosphates had been identified. Moreover, many in the scientific community believed that it was highly unlikely that a single substance could elicit a variety of cell-specific actions that were triggered by diverse hormones. To deflect such criticisms, studies on the effects of hormones on another tissue was examined. Robert Haynes, working in a laboratory in proximity to that of Sutherland, was able to show that ACTH stimulated the formation of the "heat-stable factor" in the adrenal cortex (Haynes, 1958). This finding suggested that the concept of an intracellular messenger was a more generalized process, involving diverse tissues. The skepticism expressed for this new concept began to fade in 1957 when David Lipkin and his colleagues, who were members of the Chemistry Department at Washington University, isolated cAMP from a barium hydroxide digestate of adenosine triphosphate, established its structure, and described its chemical properties (Lipkin et al., 1959).

By 1960, Sutherland felt that the evidence was sufficiently decisive to suggest that cAMP might serve as a

second messenger for a variety of hormones and neurotransmitters. In Sutherland's view, since different cells contain a distinct set of enzymes, the alteration in cAMP levels would produce diverse effects from one cell type to another. For example, cAMP would produce phosphorylase activation in liver cells and activate steroidogenesis in adrenocortical cells. Sutherland and coworkers also made the important discovery that cAMP was synthesized within the cell membrane, implying that primary hormones acted on the surface of the cell (Davoren and Sutherland, 1963). The idea that a second messenger, rather than the primary messenger, triggers a biochemical response that subserves a specialized function of a given cell represented a novel biological concept that explained the functional specificity of a variety of hormones and neurotransmitters.

The ever-increasing recognition that hormones could stimulate cAMP synthesis naturally prompted scientific inquiry into how hormones accomplished this process. So before long Sutherland and his coworkers provided a natural sequel to their experiments by identifying adenylyl cyclase, the enzyme that catalyzed the synthesis of cAMP (Sutherland et al., 1962). Although cAMP was unaffected by known phosphatases, Sutherland and colleagues also discovered phosphodiesterase, an enzyme system present in diverse tissues that degraded cAMP to adenosine 5'-phosphate (Butcher and Sutherland, 1962). These results provided support for the view that tissue levels of cAMP at any given time represented a balance between factors that synthesized cAMP (the adenylyl cyclase system) and those that degraded it (phosphodiesterases). Both of these enzyme systems were found to be widely distributed, not only in mammalian tissues but also in other phyla of the animal kingdom. The importance of the contributions made by Sutherland, Rall, and their colleagues cannot be overemphasized since the discovery of cAMP provided the template for formulating the fundamental concepts of hormone-sensitive production of cellular messengers and transmembrane signaling.

To firmly establish cAMP as a cell messenger, Sutherland mandated that the molecule satisfy several rigorous criteria. First, adenylyl cyclase should be stimulated by hormones that elevate cAMP levels, and hormones unable to produce a cAMP response should not stimulate effectors. Second, a correlation should exist in terms of dose-response and temporal relationships between cAMP levels and the cellular response. Third, drugs that inhibit phosphodiesterase, such as theophylline and isobutylmethylxanthine, should enhance cAMP levels and effector responses elicited by hormones. Finally, the effects of exogenous administration of cAMP (or a derivative) should mimic those of the primary hormone. Scientific inquiry still employs these criteria today in identifying putative second messenger systems.

After moving to Vanderbilt University in the early 1960s, Sutherland was joined by a number of talented

colleagues, including Reginald Butcher, Joel Hardman, and G. Alan Robison, who provided new lines of approach that continued and expanded this work. Experiments using rat heart were of particular interest and significance since they formed the initial basis for drawing inferences regarding the relationship between a biochemical event (changes in cAMP levels) and functional activity (cardiac contraction). Evidence bearing on the hypothesis that the positive inotropic response to epinephrine, as well as other β -adrenergic stimulants, is mediated by cAMP, had enormous ramifications in the research laboratory (Robison et al., 1965). This key finding also directly led to the development of markedly improved therapy for such cardiovascular and respiratory disorders as hypertension, bradycardia, heart block, congestive heart failure, and bronchial asthma.

Sutherland eventually broadened his analysis by envisioning the generation of multiple "second messengers" to explain the actions of hormones. Toward this end, together with Joel Hardman, Sutherland diverted some of his attention away from cAMP and focused on cGMP. This cyclic nucleotide was detected as a major organic phosphate component of rat urine by T. D. Price et al. at Columbia University in 1959 (Ashman et al., 1963). Cyclic GMP was subsequently identified in diverse mammalian tissues and in a number of lower phyla by Sutherland, Hardman, and Ferid Murad, among others.

Because the concentration of cGMP in most tissues was found to be generally 1 to 2 orders of magnitude less than the concentration of cAMP, the problems associated with the quantitation of low tissue levels of cGMP hampered progress in the functional aspects of this field for several years. Nevertheless, Sutherland and Hardman did adduce evidence that cGMP synthesis was regulated by guanylyl cyclase, an enzyme that differed from adenylyl cyclase with regard to cellular distribution, solubility, and ion activation (Hardman and Sutherland, 1969). Although an initial analysis by Sutherland and coworkers failed to detect an effect of any hormone on guanylyl cyclase activity, subsequent studies demonstrated that the enzyme could be stimulated by cholinergic agonists, nitroso compounds, and certain vasoactive peptides. Later work showed that cGMP was a key regulator of phototransduction in retinal cells, certain ion channels, and smooth muscle relaxation (see essays on Furchgott, Ignarro, and Murad in section II.Q.).

In 1973, Sutherland left Vanderbilt to become Distinguished Professor of Biochemistry at the University of Miami. It was about this time that experimental findings from several laboratories began to surface that were compatible with the idea that cAMP played a key role in cellular activation elicited by a wide variety of hormones, including ACTH, luteinizing hormone, thyroid hormone, thyroid stimulating hormone, and parathyroid hormone. However, Sutherland was not carried away by the cAMP frenzy and acknowledged that it was

simplistic to conclude that all hormones act by raising intracellular cAMP levels. Indeed, we now know that the biochemical mechanisms involved in hormone action are associated with an ever-growing number of second messengers that includes calcium, arachidonic acid and its metabolites (eicosanoids), nitric oxide (NO), inositol trisphosphate, and diacylglycerol.

In establishing a basic understanding as to how physiological signals mediated by second messenger pathways are integrated within tissues, Earl Sutherland and his associates also provided the foundation of knowledge for important subsequent work. For example, the studies carried out by Paul Greengard that demonstrated that the activation of cAMP-dependent protein phosphorylation in brain is a key factor in controlling neuronal excitability was based upon Sutherland's legacy (see following paragraph). This work not only earned Greengard the Nobel Prize, it helped to develop more effective therapeutic agents for treating certain neurologic and psychic disorders. Sutherland's scientific contributions also laid the groundwork for other Nobel Prize-winning discoveries made by Furchgott, Ignarro, and Murad on the role of nitric oxide-cGMP in regulating smooth muscle contraction, as well as Gilman and Rodbell for their elucidation of the role of G proteins in the regulation of adenylyl cyclase. In recognition of his accomplishments in providing the foundation for delineating the molecular mechanisms involved in hormone action, Earl Sutherland was awarded the Nobel Prize in 1971. Moreover, he was the first sole recipient in 11 years.

Looking back on Sutherland's scientific career, his legacy was further defined by the fact that he was responsible for mentoring a number of young investigators who then went on to distinguish themselves in their own right. In addition to Ted Rall, who was an indispensable collaborator for 7 years, these gifted individuals included the Nobel Laureate Ferid Murad, as well as Joseph Beavo, Reginald Butcher, Joel Hardman, Roger Johnson, G. Alan Robison, and Günter Schultz. The individual accomplishments of these first-class scientists perpetuate the remarkable profile of Earl Sutherland as a mentor and collaborator, as well as an elite scientific investigator. Earl Sutherland accomplished so very much in his professional career in spite of the fact that he passed away at a relatively early age, still battling his own personal demons.

There is one personal anecdote that is only peripherally related to the momentous contributions made by Earl Sutherland that should be recounted because I believe it exemplifies a prevailing view that existed among scientists at the time. As a pharmacology graduate student during the early 1960s, I frequently attended classes in the Biochemistry Seminar Room at the Albert Einstein College of Medicine. My foray into biochemistry was prompted by the suggestion of our Chair, the senior Alfred Gilman, that I take an advanced course in enzymology. On one such day, as I entered the semi-

nar room prepared to deal with a subject that I found quite bewildering, I overheard a remark made by the venerable Abe White, the Chair of the Department of Biochemistry, to one of his colleagues. Dr. White, a most affable and gregarious gentleman, was describing in glowing terms the groundbreaking work of Earl Sutherland, and apparently in an attempt to enhance its significance, specifically noted that it was being carried out in a pharmacology department and not in a biochemistry department (at Case Western Reserve University).

Because Dr. White introduced this qualifier, I was puzzled as to whether his intent was to imply that Sutherland's work involved the melding of two scientific disciplines or whether he wished to convey the notion that he was surprised that Sutherland's prodigious feat could actually be accomplished in a pharmacology department. If the latter interpretation was, indeed, the correct one, then this brief reflection should not be construed as a criticism of Dr. White but only an illustration of a prevailing view of an earlier time.

To further highlight this experience, I recall another occasion related by Nobel Laureate Alfred G. Gilman in an interview in the ASPET journal *Molecular Interventions* in 2001. Gilman reflects on the fact that in exploring his options for postgraduate education, he had "tried to avoid pharmacology" and was "more attracted to biochemistry." When attempting to recruit him to Case Western Reserve University as an M.D./Ph.D. student, Sutherland tempered Gilman's reluctance to join a pharmacology department by telling him that "It's OK. The kind of pharmacology that we do around here is really biochemistry with a purpose" (Gilman, 2001). Although this remark made by Sutherland may be interpreted in several ways, it could reflect a type of condescension with which many biochemists and even physiologists viewed pharmacology at the time. Hopefully, the fact that pharmacologists such as Robert Furchgott, Julius Axelrod, Louis Ignarro, Ferid Murad, Sir James Black, and John Vane have earned the Nobel Prize in recent times would justify the belief that the discipline of pharmacology now shares at least equal status with the other basic sciences from which it was spawned (Beavo and Brunton, 2002)

O. Paul Greengard: Signal Transduction in the Nervous System

The independent work of Earl Sutherland and Edwin Krebs shaped scientific thought concerning the cellular and biochemical mechanism(s) involved in hormone action. The demonstration that the effects of a number of hormones were mediated by an increase in cellular cAMP levels leading to enhanced protein phosphorylation now made it possible to carry out parallel studies on nonendocrine systems.

Inspired by the work of Sutherland and Krebs, Paul Greengard (Fig. 29) and his associates embarked on a mission, first at Yale and then at The Rockefeller Uni-



FIG. 29. Paul Greengard (1925–). Copyright Nobelstiftelsen.

versity, to prove that neuronal communication was governed by at least some of the same general principles associated with hormone action. Although the concept of chemical transmission across synapses both in the peripheral and central nervous systems had been accepted for some time and a link between biogenic amines and brain function had been established by Arvid Carlsson, gaining deeper knowledge about the processes that regulated neuronal activity in brain was still considered to be a formidable undertaking. The human brain, with its infinite number of interactive pathways, was arguably viewed as the most complicated biological organ known. The complex architecture of the central nervous system precluded the possibility of analyzing drug effects on individual isolated units, a strategy that had been successfully used by studies involving peripheral neurons.

Undaunted, Greengard and his coworkers set out to obtain knowledge about the biological processes that regulate synaptic transmission in brain. In searching for brain enzymes, they soon identified a family of dopamine-sensitive adenylyl cyclases, analogous to those enzymes found by Sutherland in liver and other tissues. This enzyme family not only catalyzed cAMP synthesis, but the evidence that Greengard's group adduced led to the conclusion that the adenylyl cyclases played a key role in synaptic transmission (Kebabian and Greengard, 1971). Coincident with these results, the fact that cAMP-dependent protein kinase activity (PKA) was detected in brain in concentrations much higher than those found in liver implied significant physiological relevance to the actions of PKA in nerve function (Miyamoto et al., 1969). The additional finding that the enzyme was concentrated in the synaptic region of nerve

cells was also consistent with a possible role for cAMP-dependent protein kinase in synaptic transmission (De Robertis et al., 1967).

Again, borrowing from Sutherland's concept of hormone action, Greengard and his colleagues formulated the hypothesis that a neurotransmitter in the nervous system functions in an analogous manner to that of a hormone by activating adenylyl cyclase to elevate cAMP levels. The cyclic nucleotide then activates PKA, which catalyzes the phosphorylation of a substrate protein. The phosphorylated substrate, by means of one or more additional reactions, elicits the physiological response characteristic of the neurotransmitter in question. Collaborative studies subsequently performed by Greengard's team provided evidence for a causal relationship between protein phosphorylation and the physiological response in neurons and neurosecretory cells. The impact of this work was profound, since it provided insight into the biological processes that regulate synaptic transmission and therefore presented a more detailed understanding of neuronal function (Castellucci et al., 1980; Kaczmarek et al., 1980).

During the 1970s, Greengard and his associates built on their original observations by conducting a systematic search for other endogenous substrates for protein kinases in brain. Their approach was rather unconventional, yet innovative, in that its goal was to identify substrates by demonstrating the ability of endogenous protein kinases to phosphorylate them. Employing this strategy encompassed the difficult task of determining the function of these substrate proteins, but only after they were detected, purified, and characterized. Despite these potential difficulties, Greengard and colleagues identified cGMP- and Ca^{2+} -calmodulin-dependent kinases, which established that second messengers other than cAMP were also involved in brain signaling mechanisms (Greengard and Kuo, 1970; Kuo and Greengard, 1970; Schulman and Greengard, 1978a,b).

During the 1990s, the Greengard laboratory expanded its findings by demonstrating that the magnitude of neurotransmitter release was governed by the phosphorylation state of certain proteins localized to the presynaptic nerve endings. Included among these proteins were the synapsins, so named because they were detected in synaptic vesicles localized to nerve endings (De Camilli et al., 1983a,b). In collaboration with Eric Kandel of Columbia University, Greengard and colleagues showed conclusively that the magnitude of neurotransmitter release in response to a nerve impulse was regulated by phosphorylation/dephosphorylation reactions. As a consequence, a basic foundation was laid for elucidating the biological processes associated with synaptic transmission.

However, this work, although of immeasurable value, did not portend an end to the pursuit of knowledge in this important area, because the study of synaptic transmission involves areas of research that

are unlimited in scope. Moreover, the concept of a biochemical basis for nerve function proposed by Greengard was originally greeted with some skepticism. The most substantive criticism involved explaining how the relatively slow enzymatic reactions involving phosphorylation/dephosphorylation could be implicated in fast synaptic transmission, which occurs in milliseconds. Greengard's laboratory addressed this caveat by proposing that, in contrast to fast synaptic transmission, slow synaptic transmission involves complex interactions among various interactive signaling pathways, with dopamine playing a pivotal role. By activating a subtype of receptors, dopamine was thought to elevate cAMP levels, enhance PKA activity, and promote the phosphorylation of DARPP-32, a molecule highly concentrated in the neostriatum (caudate and putamen) (Walaas et al., 1983; Greengard et al., 1999). This reaction sequence was postulated to play a key role in mediating the interactions of dopamine with other neurotransmitters, therapeutic agents, and drugs of abuse.

In generating a deeper understanding about the functions performed by dopamine and other neurotransmitters in processes associated with synaptic transmission in the brain, these advances resulted in the development of drugs that more selectively activate or depress the various neuronal pathways involved in cell signaling. As a result, neurological and psychiatric disorders associated with aberrations in dopamine signaling could now be more effectively addressed.

Finally, it should be noted that the individual contributions attributed to Paul Greengard and Arvid Carlsson—whose work was previously chronicled—were complementary (and even synergistic). Whereas Carlsson first discovered that dopamine played a key role in brain function, Greengard identified and characterized the process by which dopamine and other neurotransmitters elicit their effects. So, in recognition of their accomplishments and their confluent efforts to advance our understanding of cell signaling mechanisms in the brain, Paul Greengard and Arvid Carlsson were jointly awarded the Nobel Prize in 2000.

I was fortunate enough to first interact with Paul Greengard during the early 1960s, when I was a graduate student in the Department of Pharmacology at the Albert Einstein College of Medicine. At the time, Greengard held an adjunct faculty position at Albert Einstein College of Medicine while working at Ciba Pharmaceuticals, which was located in Westchester County north of New York City. Greengard had developed a very sensitive fluorometric method for detecting adenine nucleotide levels, even to the purported level of a single molecule. He was asked by his friend and colleague (and my mentor) Bill Douglas to help him provide evidence for the theory of exocytotic catecholamine secretion by measuring very low levels of adenine nucleotides in the effluent of perfused adrenal glands. I was tasked with perfusing the glands and providing Greengard with

samples of perfusate to carry out the enzymatic assay. To optimize the conditions for success, the assay required that the room be kept as dark as possible. So, while Greengard feverishly grappled with our newly acquired Aminco-Bowman spectrophotofluorometer, Alan Poisner, a postdoctoral fellow, and I sat in complete darkness for several days, waiting and wondering what, if anything, was going to transpire. As a dutiful graduate student, I always prided myself in trying to comprehend the various lessons that my mentors were attempting to teach me. But in this particular case, the object lesson gleaned by sitting in total darkness for an extended period of time remains moot even to this day.

Although the success that Greengard and Douglas had anticipated never materialized, this story has a happy ending. Soon after, Poisner succeeded in developing a method for assaying nucleotides in the adrenal perfusate, which was found to be coincident with the release of catecholamines. These findings provided convincing support for exocytosis as the mode of secretion. Meanwhile, Greengard went on to make an indelible mark in neurobiology, first in the Department of Pharmacology at Yale, and then at Rockefeller University, by defining the biochemical events that take place during synaptic transmission.

P. Martin Rodbell and Alfred G. Gilman: G Proteins and Their Role in Signal Transduction in Cells

The concept of "receptors" has been linked to drug-tissue interactions since John Newport Langley and Paul Ehrlich independently put forward their theories in the late 19th and early 20th centuries. In 1905, Langley proposed the existence of a "receptive substance" with which curare and nicotine both interacted. About the same time, Ehrlich envisioned the importance of surface receptors on cells, and postulated a "lock and key" hypothesis to explain drug-receptor interactions. Ehrlich understood that to achieve the greatest degree of selectivity with regard to drug action the eventual goal in pharmacological research should be to identify each receptor and exploit its distinctive characteristics. However, for many years no one could provide convincing evidence that receptors actually existed. They were studied as theoretical entities by such eminent pharmacologists as Gaddum, Schild, Paton, Ariens, and Furchgott until well into the second half of the 20th century, when biochemical procedures for isolating receptors were finally developed.

A major breakthrough in the understanding of agonist-receptor interactions was reached in the 1950s and 60s when Earl Sutherland and his many talented colleagues established that cAMP mediates the actions of a primary signal triggered at the surface of the cell. They also showed that the effects of epinephrine and its analogs on adenylyl cyclase conformed to Raymond Ahlquist's concept of a β -adrenergic receptor (Ahlquist, 1948). These key advances fostered a need to generate new paradigms about

how receptor activation by hormones or other agonists culminated in the response of an effector. During the 1960s, the question relating to the biochemical mechanisms involved in agonist-receptor interactions was initially addressed by Martin Rodbell, when he postulated that an intermediary was interposed between receptor and enzyme (effector).

1. Martin Rodbell. Martin Rodbell (Fig. 30), who began his groundbreaking work at the NIH, first in the National Heart Institute, then in the National Institutes of Arthritis and Metabolic Diseases, had harbored a burning interest in biological communication and cell signaling for a long time. However, he realized that his long-term goal to elucidate the biochemical and molecular basis for hormone action could not be achieved by existing methodology. So, with experience in cell culturing gained during a fellowship in The Netherlands, Rodbell devised a method of enzymatically digesting the matrix of adipose tissue to generate isolated fat cells. The ability to effectively purify fat cells from mainly vascular cells allowed Rodbell to design a protocol to investigate whether lipoprotein lipase was synthesized and released from fat cells.

At the outset, Rodbell's innovative work was inspired by Bernardo Houssay, the renowned endocrinologist from Argentina who happened to be visiting Rodbell's laboratory in 1963. Although obviously impressed by Rodbell's technique for isolating fat cells, Houssay questioned whether this preparation was metabolically viable. After Rodbell demonstrated the stimulatory action of insulin on glucose utilization using this preparation, Houssay was ecstatic and used highly laudatory language to proclaim that Rodbell's discovery represented a

landmark event in endocrinology. Encouraged by Houssay's ardent support, Rodbell further demonstrated that the effects of insulin were mimicked by exogenous phospholipases. This suggested to Rodbell that insulin acted on the surface of the adipose cell to stimulate phospholipase activity.

In 1964 and 1966, Rodbell published a series of three articles in the *Journal of Biological Chemistry* under the title "The Metabolism of Isolated Fat Cells," in which he described how insulin bound directly to the receptors of individual fat cells to stimulate glucose metabolism (Rodbell, 1964, 1966). These publications became very highly cited in the scientific literature and convinced Rodbell to devote his life's work to studying the nature of the molecular processes associated with the interaction of hormones with cell surface receptors.

In 1965, Rodbell, like other resourceful researchers, took advantage of an accident of timing to develop another line of research that would reap major dividends. After hearing a seminar presented at the NIH by Earl Sutherland, Rodbell became preoccupied with addressing the question as to how cell surface receptors interact with adenylyl cyclase. Rodbell was already aware that adenylyl cyclase was an allosterically regulated enzyme system that consisted of two distinct sites, a regulatory (receptor) and a catalytic site. Judiciously using this information, along with the fat cell preparation as the experimental model, Rodbell and Lutz Birnbaumer demonstrated in a series of studies that took place between 1969 and 1971 that the action of insulin was mediated by a GTP-dependent process (Birnbaumer and Rodbell, 1969; Birnbaumer et al., 1971; Pohl et al., 1971; Rodbell et al., 1971a,b,c).

Although at the time it was not clear what function GTP performed, Rodbell, encouraged by these preliminary findings, then set out to determine whether all hormones interacted with the same enzyme or each receptor was coupled to a distinct enzyme (in this case cyclase). The finding that additivity was not observed using a combination of hormones at maximal concentrations was of great importance because it revealed that adipocyte cyclase was composed of multiple receptors that interacted with a common catalytic unit. This idea encompassed a rather complex system, in which each receptor contained specific binding regions plus a common element that interacted with a catalytic component to promote the conversion of ATP to cAMP.

To define how cells receive signals and disseminate them throughout the cell, Rodbell coined the term *signal transduction* in 1969. The term, which was to revolutionize the study of cellular and molecular biology, was initially framed by Rodbell after informal conversations with Oscar Hechter, a noted endocrinologist and steroid biochemist. Rodbell profited greatly from his discussions with Hechter, who was a pioneer in challenging the prevailing concept that hormones acted directly on adenylyl cyclase (Hechter and Halkerston, 1964).

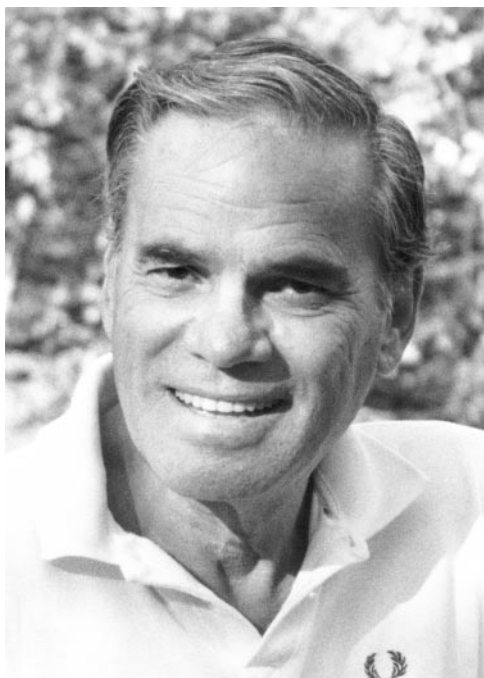


FIG. 30. Martin Rodbell (1925–1998). Copyright Nobelstiftelsen.

The one-on-one talks between Rodbell and Hechter took place at a hotel bar in Washington, DC prior to a meeting organized by Rodbell to honor Earl Sutherland. It was at this meeting that Rodbell proposed a three-step mechanistic model to describe the steps involved in what we now call "cell signaling." These steps involved a discriminator, transducer, and amplifier. Extrapolating from his knowledge of transfer theory, Rodbell coined the term *discriminator* to define the cell surface receptor that recognized the source of the external signal. The *amplifier* represented the role played by adenylyl cyclase to ensure that the effector produced a physiologically relevant response. But most importantly, Rodbell postulated the existence of a switch (or coupling process) interposed between the discriminator (receptor) and amplifier (enzyme), which he called the *transducer*. He also proposed that the transducer be called G proteins, because they bound GTP and mediated the process of transmitting signals across the cell membrane.

Rodbell also theorized that G proteins were composed of three subunits, an α -subunit capable of binding and degrading GTP, plus a complex of β and γ subunits. Implicit in this concept was the postulate that GTP turnover was essential for the rapid and sustained effects of a variety of diverse hormones. As a corollary to proving that cellular communication was composed of a biological transducer, Rodbell optimistically predicted that experimental validation of the transducer concept would lead to a better understanding of the mechanisms that linked receptor to enzyme (adenylyl cyclase) (Rodbell, 1985).

Cognizant of the complexities involved in investigating the adenylyl cyclase system in adipocytes that expressed multiple receptors, Rodbell turned his attention to glucagon-sensitive adenylyl cyclase in liver, in part because Sutherland had used this tissue for his early experiments. Using a recently published plasma membrane preparation of rat liver, Rodbell found that the responses to a combination of maximally effective concentrations of hormones were not additive. This key finding provided compelling evidence that receptors and adenylyl cyclase were distinct cellular components, and in accordance with Hechter's idea, that hormones did not exert a direct action on the effector (Birnbauer and Rodbell, 1969). Rodbell's finding was confirmed in the 1970s, when the introduction of ligand-binding assays made it possible to dissociate the β -adrenoceptor from adenylyl cyclase.

The ability of Rodbell's laboratory to develop expertise in synthesizing ^{125}I -glucagon made it possible to elucidate the general properties of the glucagon receptor, as well as the relationship between hormone binding and the activation of adenylyl cyclase. So, in late 1969 and early 1970, Rodbell began working with a team that set out to characterize ^{125}I -glucagon binding to a rat liver membrane receptor. Based upon the known ability of hormones to activate adenylyl cyclase, it was expected

that ^{125}I -glucagon binding would proceed rapidly and be readily reversed by washing the membranes. However, binding failed to occur rapidly and was not readily reversed (Rodbell et al., 1971a).

In confronting this problem, Rodbell, who knew from earlier experiments that commercial ATP contained GTP as an impurity, conjectured that this impurity might be responsible for the confounding results. Indeed, not only did GTP reverse glucagon binding to its receptor, the magnitude of its effect at equal concentrations was almost 4-fold greater than that of ATP. As a result, Rodbell correctly deduced that GTP was the physiologically relevant factor in dissociating glucagon from its receptor. The G protein, then activated by GTP, would serve as the principal component of the transducer. Although the discovery of the role of GTP in hormonal activation of adenylyl cyclase is attributed to Rodbell and his coworkers, they were not able to explain how GTP stimulated the G protein or how GTP permitted signal transduction to proceed (Rodbell et al., 1971b). The biochemical characterization of this process would await further examination by Alfred G. Gilman and his coworkers.

In 1973, Rodbell and several colleagues arranged the manufacture of a synthetic analog of GTP, which, although poorly hydrolyzed and therefore resistant to degradation, could stimulate G proteins and adenylyl cyclase. The use of the synthetic guanine nucleotide analogs made it possible to provide convincing evidence that favored an action of GTP at the transduction site. These experimental results also supported the idea that the GTP regulatory site might be the locus of a GTPase, which would serve as the controlling element of enzyme activity. As a result of these findings, the potential significance of the transducer to mediate transfer of information between receptor and enzyme was established (Harwood et al., 1973).

The additional finding that fat cells also contained adenosine receptors that expressed their effects by *inhibiting* adenylyl cyclase via a GTP-dependent process provided decisive evidence that guanine nucleotides could subservise a negative role in signal transduction. These studies, which were performed in Rodbell's laboratory as well as several other laboratories, spawned a novel paradigm of hormone action. This paradigm included the idea that transduction involved both stimulatory and inhibitory processes that were mediated by distinct GTP-binding proteins. Although these nucleotide regulatory proteins were initially called N_s and N_i to identify the stimulatory and inhibitory G proteins, respectively, they were ultimately designated G_s and G_i .

By 1980, additional studies from Rodbell's laboratory conclusively demonstrated that the actions of guanine nucleotide in mediating hormonal effects extended far beyond the realm of adenylyl cyclase to reflect a more global role for G proteins. The biological importance of GTP-binding proteins was elevated to

even greater heights when researchers found that the activity or inactivity of specific G proteins might be implicated in the pathophysiology of diseases such as cholera, pseudohypoparathyroidism, acromegaly, and certain types of cancer.

In providing an encapsulated view of Martin Rodbell's fundamental contributions to signal transduction, I can recall a Gordon Conference during one summer in the late 1960s, which I attended as a fledgling investigator. Despite the large number of scientific luminaries who were in attendance, including George Palade, Marilyn Farquhar, and Isidore Edelman, I noted a buzz emanating from the group concerning the anticipated arrival of one Martin Rodbell. At the time, my research knowledge was limited to perfusing adrenal glands. But because of the stir created by the attendees, I too began to anticipate Rodbell's arrival. Suffice to say that after hearing Rodbell's talk I came to the realization that this esteemed group of scientists, unlike myself, had already been well aware of the profound significance his discoveries engendered.

After leaving the National Institutes of Arthritis and Metabolic Diseases in 1985, Rodbell was appointed Scientific Director of the National Institute of Environmental Health Sciences at Research Triangle Park, NC, a position he held until 1989. He then served as Chief of the Section on Signal Transduction until his retirement in 1994. Particularly in these later years, because he believed that the information processing systems of cells and computers were similar, Rodbell continued to describe G proteins in terms of computer science. In addition, he used the language of cellular regulation to describe his perceptions of modern society and seemed to view his scientific career as inseparable from his experiences with people and world events.

The fact that Rodbell was also gifted in composing poetry and verse enabled him to express his strong philosophical and introspective persona. As an example, by composing and reading a poem entitled "To my Friends: Thoughts from 'On High,'" he communicated his sincere gratitude to colleagues who had contributed to the concept of signal transduction. He read this poem while standing next to the King of Sweden when accepting the Nobel Prize. The poem seems to represent Rodbell's perspective of science and the profound respect and affection he felt toward his colleagues who were not in attendance: ". . . So, I extol the intuitions encapsulated in the folds of my mind/from whence occasionally they hurtle to the forebrain and in a twinkling of a/proton's discharge bring to fruition a thought, an idea borne on the feathery/appendages of teeming neurons wedded in a seamless synergy. Those fleeting/moments are cherished as are those precious impulses imparted by the/innumerable individuals who nurtured and instilled unknowingly their/encrypted thoughts with mine" (<http://nobelprize.org/medicine/laureates/1994/rodbell-lecture.pdf>). Much more could be written about this multifaceted individ-

ual, but it would not begin to accurately reflect the essence of Martin Rodbell as a scientist and a person. If the reader wishes to learn more about Rodbell's noteworthy scientific contributions, a review of his work on G proteins was published in *Nature* in 1980 (Rodbell, 1980). Building on the groundwork laid by Rodbell and his colleagues, Alfred G. Gilman and his coworkers would prove the validity of Rodbell's theories by using a combination of biochemical and genetic techniques to show conclusively that G proteins were required for hormone action.

2. *Alfred G. Gilman.* Alfred Goodman Gilman (Fig. 31) was gifted with an excellent heritage as the son of Alfred Gilman, who with Louis Goodman first coauthored and then edited the classical textbook *The Pharmacological Basis of Therapeutics*, now in its 11th edition. My first encounter with the younger Gilman came in the early 1960s at a New Year's Day party given by his parents at their home in White Plains, NY. The elder Dr. Gilman was then Chairman of the Department of Pharmacology at the newly established Albert Einstein College of Medicine in the Bronx. Invitations were handed out to all of the faculty members, as well as the handful of graduate students (like me) who found themselves in a position to socialize with a faculty whose main function—we students felt—was to intimidate. It was a very festive gathering to say the least, with an abundance of food and especially drink. Only peripherally relevant to the subject at hand, the younger Gilman, then at the end of his college career, was given the task of serving the food and beverages. From his facial expressions I perceived how much he disdained this activity. Nevertheless, the party was a huge success, and the guests (at least those who were able) all departed with a fond remembrance of the event. The students took away



FIG. 31. Alfred G. Gilman (1941–). Copyright Nobelstiftelsen.

something else: a more balanced perspective of the pharmacology faculty.

Alfred G. Gilman then went on to obtain his M.D./Ph.D. degree at Case Western Reserve University in the Department of Pharmacology. Although originally recruited to Cleveland by Earl Sutherland, Gilman became a student in Ted Rall's laboratory after Sutherland departed for Vanderbilt University in 1963. Not surprisingly, his thesis work involved the role of cAMP in the thyroid gland. After completing his doctoral dissertation in 1969, Gilman began a 3-year postdoctoral fellowship funded by the National Institute of General Medical Sciences (a branch of the NIH) in the laboratory of Marshall Nirenberg. Although Gilman has stated that during his scientific training he was "forced" to work on cAMP, at the NIH he succeeded in developing a relatively simple, yet sensitive assay for cAMP (Gilman, 1970). This method afforded the general scientific community the means to investigate this newly discovered second messenger on a broad scale.

My next encounter with the younger Gilman came sometime after 1974 when I was a faculty member at the Medical College of Virginia and he had assumed a faculty position in the Department of Pharmacology at the University of Virginia in Charlottesville, some 60 miles away. This department was headed by Joseph Lerner, a very distinguished investigator in his own right, and included among others Ted Rall, who had collaborated with Earl Sutherland on his classic experiments, the future Nobel Laureates Ferid Murad and Alfred G. Gilman, and Tom Westfall and Robert Haynes. After being invited to Charlottesville to present a seminar, I was profoundly impressed by the members of the department; it was clear to me at the time that a bright, productive future awaited these talented investigators.

During the 1970s, Alfred G. Gilman and his coworkers began a venture that would revolutionize the concepts of hormone and drug action in terms of cell signaling. It was around this time that attempts were being made to solubilize and purify components of hormone-sensitive adenylyl cyclase systems. In most cases, such experiments proved unsuccessful because of the extreme lability of the enzyme and because hormonal responses were invariably lost following solubilization of the preparation with detergents. As a result of the obstacles that had to be overcome, Gilman and his colleagues understood that a novel strategy was needed to penetrate many of the mysteries that still existed regarding the biochemical and molecular mechanisms involved in hormone action.

The approach that Gilman and Elliott Ross took to explore the events associated with catecholamine-induced stimulation of adenylyl cyclase was to reconstitute hormone-sensitive enzyme activity in intact membranes depleted of key constituents. Toward this end, they became aware of a variant of a clonal S49 lymphoma cell line that Henry Bourne and his associates

had recently isolated (Bourne et al., 1975). This clone expressed β -adrenergic receptors but seemed to lack adenylyl cyclase activity as a result of genetic manipulation. Gilman's team also selected for another clone that failed to generate cAMP in response to hormonal stimulation. Gilman and Ross then judiciously used these cell variants to reconstitute hormone-sensitive adenylyl cyclase activity.

Gilman and Ross initially surmised that the success of the reconstitution experiments would be predicated upon a mixing of receptor and enzyme extracted from cells that expressed complementary phenotypes. So they mixed a detergent extract of plasma membranes of murine cells, which contained adenylyl cyclase activity, with plasma membranes from the variant clone of S49 cells (*cyc*-), which seemingly lacked cyclase activity, but had retained the β -receptor. However, after conducting control experiments that revealed that the reconstituted system resembled a wild-type S49 cell, they concluded that the *cyc*- cells were not devoid of cyclase but rather lacked a third, more heat-stable protein that was also required for the expression of enzyme activity. On the basis of these experiments, Gilman and Ross proposed that the function of the heat-stable protein was to allow adenylyl cyclase to catalyze the synthesis of cAMP in response to hormone and that the hormone receptor served to regulate the interaction between the heat-stable protein and enzyme (Ross and Gilman, 1977).

Another facet of the interaction between receptor and effector emerged from further studies by Gilman and coworkers. They directly demonstrated that the regulatory protein identified in the detergent extract was a guanine nucleotide-binding protein capable of activating adenylyl cyclase. The regulatory protein was originally designated G/F but was later named G_s as the locus of action of GTP (Ross et al., 1978; Howlett et al., 1979; Sternweis and Gilman, 1979; Northup et al., 1980). Follow-up experiments in Gilman's laboratory also resulted in the isolation of the α - and β -subunits of the G protein (Northup et al., 1983a,b). The third component, the γ -subunit, was identified later. Eventually, it was determined that hormonal activation of an appropriate receptor triggers the exchange of GTP for bound GDP, causing a conformational change in the G protein complex. The change in conformation leads to the dissociation of the α -subunit from the β - γ subunit, causing the activation of adenylyl cyclase by $G\alpha$ -GTP. General acceptance of the regulatory effects (both inhibitory and excitatory) exerted by the β - γ complex was established later, as was the ability of the β - and γ -subunits to dissociate to exert their differential cellular effects. The reassociation of the subunits was theorized to occur when the GTP bound to the α -subunit was hydrolyzed to GDP by GTPase.

By the early 1980s, Gilman moved to the University of Texas Southwestern Medical Center in 1981 to head the Department of Pharmacology, ironically after Martin Rodbell had turned down the job. Gilman's laboratory

continued its groundbreaking work by showing that hormonal stimulation of adenylyl cyclase was demonstrable *in vitro* after purified β -adrenergic receptor, G_s and G_i , and adenylyl cyclase were all reconstituted into phospholipid vesicles (May et al., 1985). Further insight into the mechanism of action of GTP was obtained when they found that GTP selectively reduced the affinity of the β_2 -adrenoceptor for agonists but not for antagonists. These experiments not only provided further support for the idea that G proteins regulate cell signaling, they also helped to spawn a wave of scientific articles that described the role of G proteins in the regulation of cellular responses in diverse tissues.

After demonstrating that G proteins play an essential role in transducing the signal expressed by agonist-receptor interactions at the plasma membrane, Gilman and coworkers went on to show that G proteins permit the amplification of the signaling process. In addition to single G proteins interacting with multiple effectors, different G proteins were found to converge on a single effector to modify its activity. These findings enabled Gilman and his associates to define the complexity of diverging and converging transducing systems, which allow each cell to formulate its own mechanisms of regulation. Gilman's team later found that G proteins not only exerted their effects on a number of diverse enzyme systems but could also directly modify the activity of functional responses, such as the activation of ligand-gated ion channels. The effects of G proteins on the regulation of ion channels are particularly interesting from the point of view that they were found to be associated with purified μ - and δ -opioid receptors. However, the diverse second messenger pathways expressed by opioid receptors suggest that the cellular mechanisms involved in the adaptive changes produced by chronic opioid administration extend far beyond the scope of G proteins in their complexity (Grudt and Williams, 1995).

Today, Rodbell's three-step model, which postulates that G proteins serve as a primary switch to mediate agonist-receptor interactions, remains a fundamental biological principle. As a result, we now have detailed knowledge of how a given cell responds to a myriad of external signals that impinge upon it. The model also helps to explain how changes in the duration of the signal are transmitted and how effector mechanisms are activated or depressed. The full significance of the contributions made by Rodbell and Gilman in proving that G proteins are an essential component of cell signaling can also be gleaned from the fact that the G protein-coupled receptor superfamily also represents a vital drug target. Drugs targeted to adrenergic receptors and their subtypes, for example, have emerged as more effective and safer therapy for such disorders as hypertension and atherosclerosis. Further knowledge concerned with the nuances of G protein-mediated transduction pathways should ultimately lead to even more sophisticated drug designs and thus to the continued advancement of pharmacotherapy. For their fundamental contributions to

our basic understanding of signal transduction and how cells respond in an integrated manner to cellular messengers, Martin Rodbell and Alfred G. Gilman were jointly awarded the Nobel Prize in 1994.

Q. Robert Furchgott, Ferid Murad, and Louis Ignarro: Nitric Oxide as a Signaling Molecule in the Cardiovascular System

1. *Robert Furchgott.* Inquiry into a novel signaling mechanism involving NO was begun in the late 1970s, when Robert Furchgott (Fig. 32), the Chair of the Department of Pharmacology at The State University of New York Health Science Center in Brooklyn, NY, first described the putative role of endothelial cells in regulating vascular tone. The preparation that he mainly used for these studies was the helical strip of rabbit aorta. In a review published in 1955, Furchgott had documented the utility of this preparation as an experimental model for studying drug-receptor interactions (Furchgott, 1955). In his experimental analysis, Furchgott observed one paradoxical finding. Aware that ACh was a potent dilator *in vivo* and in isolated perfused organs, he surprisingly observed that ACh elicited a contractile response of the aortic strip (Furchgott and Bhadrakom, 1953). This anomalous effect of ACh remained an enigma until 1978, when due to an inadvertent error made by his technician, Furchgott found that muscarinic agonists would elicit relaxation if the preparation was pretreated with a contractile agent such as norepinephrine (Furchgott, 1996).

After several weeks, Furchgott seemed to resolve the apparent paradox when he observed that general rubbing of the intimal surface of the vasculature abolished



FIG. 32. Robert Furchgott (1916–). Copyright Nobelstiftelsen.

the relaxation response induced by ACh following pre-treatment with norepinephrine. He further determined by microscopic analysis that the rubbing of the intimal surface caused an annulment of the relaxation response by the loss of endothelial cells from the aortic strip. Using the so-called “sandwich procedure,” Furchgott bolstered his conclusions by demonstrating that a transverse strip of aorta devoid of endothelial cells would relax after exposure to ACh if it were mounted with its endothelium-free surface placed against an intimal surface of a second strip possessing endothelial cells (Furchgott and Zawadzki, 1980).

On the basis of these experiments, Furchgott proposed in 1982 that ACh interacted with muscarinic receptors on the surface of endothelial cells to bring about the release of an unknown substance from the endothelium. This substance would then diffuse to nearby smooth muscle cells to induce relaxation. He named this smooth muscle relaxant endothelium-relaxing factor (EDRF). Furchgott extended these findings by determining that histamine, serotonin, and bradykinin could also serve as endothelium-dependent relaxing agents on vascular smooth muscle (Cherry et al., 1982).

The early studies on EDRF were carried out without any preconceived idea about its identity or its chemical structure, and several erroneous hypotheses about the nature of EDRF were contrived during the late 1970s. Sometime during the early 1980s, Dr. Furchgott invited me back to The State University of New York Health Sciences Center (then called the Downstate Medical Center), the institution that provided me with my initial experience as an independent researcher. Although he had already determined that cyclooxygenase inhibitors did not alter EDRF-dependent relaxation, Furchgott questioned me about any knowledge I might have concerning arachidonic acid and its metabolites as possible mediators of muscle relaxation. Not really comprehending the significance of his questions, I failed to furnish him with any substantive information that he had been seeking. But based upon the knowledge that in certain smooth muscle preparations there was a positive relationship between cGMP levels and relaxation, Furchgott eventually proposed a pathway in which ACh-induced EDRF release stimulated guanylyl cyclase of vascular smooth muscle, causing an increase in cGMP. This sequence of events then somehow provided the signal for bringing about the relaxation of smooth muscle. This theory was to gain in importance as time went on, and evidence in its favor gradually grew stronger. The link between cGMP and smooth muscle relaxation also derived support from studies carried out by several other groups, including those of Ferid Murad and Louis Ignarro. They employed bovine coronary and pulmonary artery, as well as rabbit aorta, to provide evidence that favored a causal role for cGMP in the relaxation of vascular smooth muscle. However, the identity of EDRF still remained elusive.

In attempting to ferret out the critical factor responsible for activating guanylyl cyclase, Furchgott was also aware that Ferid Murad had demonstrated that NO was a potent activator of guanylyl cyclase. He also knew that Louis Ignarro had shown that the relaxation of bovine coronary artery by NO was accompanied by a rise in cGMP. However, as noted by Furchgott in his Nobel Laureate presentation, these experiments failed to directly link EDRF to NO. So, displaying his characteristic caution and thoroughness, Furchgott followed up on these studies by carrying out a rigorous comparison of the properties of NO-induced muscle relaxation with those of EDRF released by ACh. After finding that the functional characteristics of NO and EDRF were remarkably similar, Furchgott proposed at a symposium at the Mayo Clinic in July 1986 that EDRF was NO (Furchgott, 1988). At the same meeting, Louis Ignarro of UCLA (see section II.Q.3.) independently came to the same general conclusion by reporting that NO caused the relaxation of bovine pulmonary artery. Before long, decisive evidence became available that endothelium-dependent relaxation correlated with an increase in cGMP levels, and the conclusion that EDRF and NO were the same substance became inescapable.

Now 91 years old, Robert Furchgott is the essence of dignity, honesty, and fair-mindedness. His self-effacing demeanor has always engendered the highest respect among his peers. And knowing him as I do, I am sure that he would like to be remembered as a colleague who successfully accomplished his goal, because he possessed the innovative ideas and the resolute approach required to doggedly pursue a certain path for more than 20 years.

2. Ferid Murad. Ferid Murad's (Fig. 33) involvement in this story stemmed from his long-term interest in the study of cGMP. Like Alfred G. Gilman, Ferid Murad received his M.D./Ph.D. degree in the Department of Pharmacology at Case Western Reserve University and was mentored by Earl Sutherland and Ted Rall. Suth-



FIG. 33. Ferid Murad (1936–). Photo by David Reischl; courtesy of the Medical University of Graz, Graz, Austria.

erland and Rall had discovered cAMP a year prior to Murad's arrival in Cleveland, and Murad was tasked with demonstrating that the effects of catecholamines on cAMP formation were mediated through the β -adrenergic receptor. Murad expeditiously completed this work and went on to show that ACh inhibits adenylyl cyclase. This latter study represented the first report of hormones or neurotransmitters *blocking* enhanced cAMP formation (Murad et al., 1962). However, Murad's findings that agonists could both activate and inhibit cAMP accumulation raised some doubts about the validity of Sutherland's concept that receptors and adenylyl cyclase were either a single entity or a tightly associated complex. The subsequent discovery of G proteins and the elucidation of their mechanism of action by Martin Rodbell and Alfred G. Gilman eventually filled this knowledge gap.

After completing his clinical training in Cleveland and then gaining additional research experience at the NIH in Martha Vaughan's laboratory, Murad embarked on his career as an independent investigator in 1970 in the Department of Pharmacology at the University of Virginia. During this period, cGMP had begun to emerge as another putative second messenger, and so, for his first project, Murad probed possible biological functions that might be mediated by cGMP. This cyclic nucleotide, which was first identified in human urine by T. D. Price and coworkers (Ashman et al., 1963), had already been comprehensively investigated by Earl Sutherland and Joel Hardman. So Murad spent a few nonproductive years adding various stimuli to preparations and measuring cGMP levels. After the laboratories of Sutherland and Gerald Aurbach at the NIH had independently identified guanylyl cyclase as the enzyme that catalyzed cGMP synthesis from GTP, Murad concluded that it would probably be more productive if he focused his attention on studying guanylyl cyclase in cell-free systems. The strategy of employing cell-free systems was reminiscent of the general approach first taken by Sutherland in examining cAMP. Murad and his coworkers began their exploration into this new area of research by detecting guanylyl cyclase activity in both cytosolic and particulate fractions. The finding that these cell fractions expressed different isoforms of the enzyme with different properties emboldened Murad to delve further into this problem (Kimura and Murad, 1974, 1975).

In his attempt to gain insight into the mechanism involved in guanylyl cyclase activation, Murad showed that azide, hydroxylamine, and nitrite activated several *in vitro* preparations of the enzyme (Kimura et al., 1975a,b). He and his coworkers then decided to focus on azide because they reasoned that if the mechanism of azide activation was elucidated in cell-free preparations, then some insight into the possible mechanism of enzyme activation by more physiologically relevant hormones might be forged. However, azide was a well known metabolic poison, and its use to delineate phys-

iologically relevant mechanisms was criticized, and even ridiculed, by many of Murad's colleagues. Nevertheless, Murad persisted in this endeavor and was able to temporally link azide-enhanced cGMP levels and the relaxation of various smooth muscle preparations that had previously been contracted. Murad's team obtained additional evidence bearing on this problem by demonstrating that other smooth muscle relaxants such as nitroglycerin and nitroprusside also augmented tissue cGMP levels (Katsuki et al., 1977a,b).

These studies by Murad and his coworkers not only provided insight into the physiological mechanisms regulating smooth muscle contractility, they also finally made available to clinicians important information about the mechanism governing the action of nitroglycerin, despite a century of clinical use. Ironically, Alfred Nobel, the Swedish industrialist who became a wealthy man in the 1860s by inventing a process that manufactured dynamite from nitroglycerin, was prescribed nitroglycerin for angina pectoris by his physician later in life. But Nobel refused medication because he was aware of the vascular headaches experienced by his factory workers with cardiovascular problems because of the vasodilatory properties of the drug.

Subsequent experiments carried out in Murad's laboratory revealed that certain factors present in liver and heart extracts were required for azide-induced activation and inhibition, respectively. The stimulatory factor isolated from liver extracts proved to be the heme protein catalase, whereas the inhibitory factors present in heart extracts were identified as hemoglobin and myoglobin (Murad et al., 1978). Murad's discovery of the stimulatory and inhibitory effects of different heme-containing proteins on the action of azide represented key findings because they complemented earlier work carried out in other laboratories that had described the interactions between azide and catalase to generate NO. In addition, these studies, which were conducted several years prior to Furchgott's discovery of EDRF, would represent the first report of a biological effect of NO and ultimately provide important clues toward the identification of EDRF as NO.

The suggestion by Murad that EDRF might be NO was predicated upon the growing list of agents with nitro- or nitroso-functional groups that activated guanylyl cyclase, as well as the effects of the heme-containing macromolecules that inhibited or activated the enzyme. In 1976, Shuji Katsuki and William Arnold, two postdoctoral fellows working in Murad's laboratory, performed a key experiment in which they generated NO from various nitroso compounds to activate guanylyl cyclase preparations from rat lung and bovine tracheal muscle. This experiment was followed by studies that revealed that NO could activate guanylyl cyclase and augment cGMP levels in almost every tissue examined (Arnold et al., 1977). The additional finding that the stimulatory effects of NO and the nitroso compounds

were not additive suggested that they shared a common mechanism of action. This was a pivotal finding because it enabled Murad to propose as early as 1978 that NO, formed from some endogenous precursor, could be the trigger for augmenting cGMP synthesis in intact tissues. However, at the time, the assays for measuring NO and its oxidation products (nitrite and nitrate) were not sufficiently sensitive. So the hypothesis would not be validated until more sensitive methods were developed several years later.

The paths of Murad and Furchgott crossed in 1980, when Furchgott presented a seminar at the University of Virginia. Furchgott spoke about his discovery of EDRF and his efforts to disclose its identity. Upon hearing the presentation, Murad became aware of the fact that EDRF shared many of the characteristic features of nitroso compounds, and he suggested to Furchgott that they establish a collaboration to investigate this issue. However, soon after this meeting, Murad moved to Stanford, and so the anticipated collaboration never materialized. Nevertheless, in 1982, Murad's laboratory demonstrated that EDRF did indeed augment cGMP levels in rat aortic smooth muscle. The effects of EDRF on cGMP formation were found to be similar to those induced by ACh and other endothelial-dependent vasodilators, thereby establishing a link between the primary action of ACh and cGMP-induced smooth muscle relaxation (Rapoport and Murad, 1983).

The similarities between the effects of EDRF-producing agents and nitrovasodilators made the case for viewing EDRF as an "endogenous nitrovasodilator." Shortly thereafter (in 1986), Furchgott and Ignarro independently proposed that EDRF was NO, although Murad continued to argue for several years that EDRF was actually comprised of a *family* of NO adducts or complexes, as well as NO. Nevertheless, the key role that Ferid Murad played in the discovery of NO was predicated upon his important finding that nitroso compounds activate guanylyl cyclase, which increases tissue levels of cGMP leading to muscle relaxation. This finding, taken together with the demonstration that NO and the nitro compounds shared a common mechanism of action, was in large measure responsible for developing the conceptual framework that eventually led to the identification of EDRF as NO.

At first, the belief that NO could serve as a cellular messenger was deemed so contentious that certain prestigious journals were reluctant to accept articles that reported these controversial results and conclusions. In particular, the scientific establishment was dubious about contemplating a concept involving a colorless gas and a free radical that served as a second messenger for activating an enzyme. In personifying a risk-taker who was not afraid of failure, Murad recalls, "For years, colleagues said I was crazy to invest so much time and effort in NO. . . . But I was certain I was right from the beginning" (<http://www.albanian.ca/murad.htm>). In car-

rying out his work with strong conviction and commitment, Murad played a fundamental role in helping to open a new avenue of research.

3. *Louis Ignarro*. In 1976, Louis Ignarro (Fig. 34) and his associates at UCLA provided a major contribution to the putative role that NO played in smooth muscle relaxation. They demonstrated that nitroglycerin liberated NO as part of its action and that NO-induced relaxation of an isolated bovine coronary artery preparation was associated with an increase in cGMP levels. When in 1979 Ignarro and his team confirmed the observation made by Murad and coworkers that nitro compounds brought about cGMP-elicited smooth muscle relaxation by liberating NO, they concluded that the relaxant effect of ACh was pharmacologically similar to that of NO (Gruetter et al., 1979). A further series of experiments was then conducted to test the as-yet unpublished hypothesis that EDRF might be NO. Employing a bioassay cascade preparation similar to the ones used by Gaddum, Burn, and Vane, Ignarro and colleagues compared the chemical and pharmacological properties of EDRF and NO. These experiments disclosed striking similarities between the two substances, prompting Ignarro in July 1986 to propose independently from Furchgott that EDRF was NO (Ignarro et al., 1986).

An additional experiment performed by Ignarro's group provided final proof of EDRF's identity. They found that EDRF produced a spectral shift in reduced hemoglobin that was identical to the shift produced by authentic NO (Ignarro et al., 1987). Despite his indelible influence on the discoveries associated with NO as a cellular mediator, Ignarro admitted that he and his

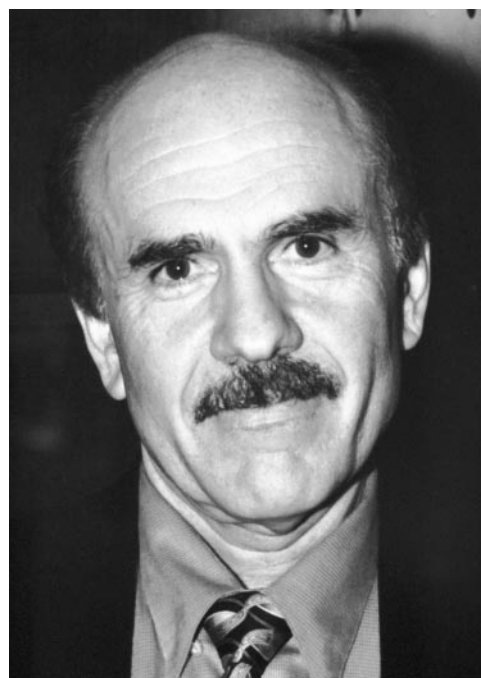


FIG. 34. Louis Ignarro (1941–). Copyright Nobelstiftelsen.

group initiated their investigation of endothelium-dependent relaxation in 1983, not because they thought that EDRF might be NO, but because of the assumption that cGMP played a role in the process of muscle relaxation. So, once again, serendipity played a key role in a discovery of major significance.

The work of Furchgott, Murad, and Ignarro has had far reaching ramifications both from a heuristic and therapeutic perspective. The discovery of NO as a vasodilator represented the emergence of a novel biological process. This process involves an endogenously produced gas that serves as a signaling molecule. The generation of NO leads to the activation or inhibition of multiple effectors, including muscle relaxation, neurotransmission, renal function, host defense reactions, and brain function. In addition, the utilization of NO as a therapeutic agent has afforded physicians the opportunity to make significant advances in the diagnosis, treatment, and prevention of cardiopulmonary disorders in the neonate and pediatric patients. In adults, altered NO production is associated with a variety of chronic cardiovascular disorders, as well as metabolic, inflammatory, and neuronal diseases. As a result, research devoted to targeting drug delivery to the endothelium is presently enjoying a wave of interest. In this connection, Furchgott's prophetic comment in the early 1980s warrants recounting: "... once the source, chemical identity, and mechanism of action of the endothelial-derived relaxing substance (or substances) have been elucidated, we may, hopefully, have a new basis for the development of drugs that are useful in treating certain circulatory disorders" (Furchgott, 1981).

In addition, the studies of Ignarro and colleagues have shed light on a signal transduction pathway that is modulated by drugs to control impotence. A very successful approach to treating this disorder with Sildenafil (Viagra) is based upon enhancing the effects of NO by selectively inhibiting phosphodiesterase-5 in the corpus cavernosum (smooth muscle) of the penis. As a result, cGMP is allowed to accumulate, causing vasodilation of the corpus cavernosum. So, in recognition of their invaluable scientific achievements, which provided major conceptual advances in our understanding of basic cardiovascular mechanisms and at the same time enhanced the opportunities for drug development, Robert Furchgott, Ferid Murad, and Louis Ignarro were jointly awarded the Nobel Prize in 1998.

III. Epilogue

The essays contained herein hopefully attest to how far our scientific knowledge has advanced and how far it has yet to go before we approach a more complete understanding of many of nature's well kept secrets. We have documented how a gifted experimentalist can have the foresight not shared by his/her scientific colleagues, and despite logistical problems, is capable of generating

and testing novel hypotheses that constitute the fundamental basis of new knowledge. By taking lessons from the past and putting them in the context of present and future goals, these unique individuals were able to forge new frontiers of knowledge and at the same time annul previous dogma. The impact that a discovery may have on the public mind was also chronicled to document how the fruits of science have contributed to the well being of recent generations. It is hoped that the stories presented in these essays support the assumption that every age is an age of important information, each in its own way. Even though much about science has changed over the years, the constancy of excellence has seemingly always prevailed. As a result, the outstanding work recounted in this series of essays, as well as that contributed by many others, will endure as an inspiration for advancing knowledge and respecting the truth, which, after all, are the true goals of the scientific method.

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