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The Discovery of Chemical Neurotransmitters

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Neurotransmitters have become such an intrinsic part of our theories about brain function that many today are unaware of how difficult it was to prove their existence or the protracted dispute over the nature of synaptic transmission. The story is important not only because it is fascinating science history, but also because it exemplifies much of what is best in science and deserving to be emulated. The friendships formed among such major figures in this history as Henry Dale, Otto Loewi, Wilhelm Feldberg, Walter Cannon, and others extended over two world wars, enriching their lives and facilitating their research. Even the dispute—the ''war of the sparks and the soups''—between neurophysiologists and pharmacologists over whether synaptic transmission is electrical or chemical played a positive role in stimulating the research needed to provide convincing proof.

Neurotransmitters have become such an intrinsic part of our theories about brain function that many today are unaware of how difficult it was to prove their existence or the protracted dispute over whether transmission across synapses is chemical or electrical. The dispute, which primarily pitted neurophysiologists against pharmacologists, has been called the ''war of the sparks and soups'' (Cook, 1986). The story is important not only as history but also because it exemplifies much of what is best in science. It illustrates, for example, how controversy can facilitate progress and how friendships formed among scientists facilitate research and, when circumstances arise, can reach across national borders to support colleagues in need of help.

The history of the discovery of neurotransmitters is too long and complex to describe adequately in one article. It involves the 35-year accumulation of evidence that eventually demonstrated that neurons secrete chemical substances at all synapses in the peripheral nervous system. Although there were still leading neurophysiologists who contested the point, it was generally conceded to have been proven when the Nobel Prize was awarded to Otto Loewi and Henry Dale in 1936. This was followed by another approximately 30-year period, disrupted by World War II, before it would be accepted that the same principles apply to the brain. In this article, only the first half of this history is told. To make the history manageable and to give it a human face, this account emphasizes the research and lives of Dale and Loewi, but this is not meant to diminish the significance of the hundreds of other researchers who also contributed to the solution of this problem.

The discovery of neurotransmitters in the peripheral nervous system arose directly out of studies of the way drugs affect visceral organs. It was only natural that drug

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research should have begun there because the visceral organs, together with the skeletal muscles, were the most obvious and technically feasible end points for studying what drugs do and how they do it. The eventual proof that nerves secrete chemical substances emerged from the realization that certain classes of drugs mimic the effects of stimulating the autonomic nerves that innervate visceral organs. The story is intimately tied, therefore, to studies of how visceral organs are innervated by the autonomic nervous system.

CONTRIBUTIONS OF GASKELL AND LANGLEY AND THEIR STUDENTS AND COLLEAGUES

It was Walter H. Gaskell (1847–1914) and John Newport Langley (1852–1925), colleagues in the Department of Physiology at Cambridge University, who were indisputably most responsible for establishing the foundation for what is now known about the autonomic nervous system. A contemporary physiologist wrote that to read an account of the autonomic nervous system before Gaskell ''is like reading a description of the circulation before Harvey,'' and he added that this should in no way subtract from Langley, ''whose beautiful and accurate research on this subject [is] of paramount importance'' (Brown, 1923, p. 1). Of the two, Gaskell was perhaps the more interested in the anatomy, whereas Langley's research emphasized the physiology and pharmacology of the system. Between them, they traced the preganglionic nerves from where they emerge from the spinal cord and brain to where they synapse in ganglia on the postganglionic nerves that terminate on visceral organs. They also described the differences in anatomy and function of the sympathetic and parasympathetic divisions of this system and began exploring how they differ in response to drugs (Gaskell, 1914, published posthumously in 1916; Langley, 1921).

Around 1900, Langley, who introduced the term ''autonomic nervous system'' in place of Gaskell's ''involuntary'' or ''visceral'' nervous system, initiated a series of studies demonstrating similarities between the effects of adrenaline and sympathetic nerve stimulation.[1] At the time, it was thought that adrenaline and other drugs affected visceral organs by either paralyzing or activating the nerves that innervate them. Langley, however, disagreed. He demonstrated that adrenaline had the same effect on organs even after their nerves had been cut and had degenerated. Moreover, because adrenaline caused some organs to contract and others to relax, Langley proposed that drugs must act on ''receptor substances.'' It is the receptor substances, he argued, that determine the nature of the response to adrenaline, drugs, and neural input. As Langley (1921) phrased this idea,

> The known physical character of drugs are insufficient to account for the effects they produce; in consequence I consider that there is a chemical combination between the drug and a constituent of the cell—the receptive substance. . . . There are two broad classes of receptive substances: those which give rise to contraction and those which give rise to inhibition. I assume that both parasympathetic and sympathetic nerves can become either contractor or inhibitor and that the effect produced by stimulating them depends upon the relative amount of contractor and inhibitor receptive substance connected with them in the cells. (p. 44)

[1] Adrenaline was originally obtained as an extract from the adrenal medulla. It was often referred to as adrenalin. According to Innes and Nickerson (1955), the active principle in the adrenal extract was identified in 1899 by Abel, who named it epinephrine, and it was synthesized independently by Stolz and Dakin. What was most commonly used was a preparation obtained from oxen and sheep, which was patented as adrenaline by Parke Davis & Company.

For Langley, the same drug or identical neural impulses could evoke different responses because the response was determined by the particular receptor substance on which they acted. Langley saw no need to suggest that nerves secrete a chemical substance, let alone different chemical substances.

A student of Langley, Thomas Renton Elliott (1877–1961), is believed to have been the first to suggest that sympathetic nerves secrete adrenaline. Langley had encouraged Elliott to explore other instances where adrenaline mimicked the effects of sympathetic nerve stimulation, and he subsequently found that the effects were similar at all smooth muscles and glands innervated by sympathetic nerves. This led him to hypothesize at a meeting of the Physiological Society that ''adrenaline might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery'' (Elliott, 1904, p. xxi).

Elliott's presentation was followed by the publication of a 68-page article in which he described an enormous amount of experimental data demonstrating that adrenaline was effective on smooth muscles and glands innervated by sympathetic nerves, but it did not mimic any parasympathetic effects, nor did it excite the skeletal muscles innervated by spinal nerves (Elliott, 1905). He never specifically stated, however, that adrenaline is secreted by sympathetic nerves. It is conceivable that the adrenaline Elliott suggested was ''liberated'' when a sympathetic nerve impulse reaches the periphery might have originated in the responding muscle. This possibility had been proposed by several physiologists and pharmacologists at the time, and as described below, this was the view of Walter Dixon, one of the leading pharmacologists of the period, whom Elliott thanked for advice on his 1905 manuscript.

In Elliott's later articles, he appears to have concluded that postganglionic sympathetic cells may have at one time been capable of producing adrenaline, but as they became specialized they lost this capacity and became dependent on storing the adrenaline secreted by the adrenal medulla. In articles published in 1913 and 1914, Elliott described extensive studies of the splanchnic nerve innervation of the adrenal gland (Elliott, 1913, 1914). He was able to trace the path of degeneration after cutting the splanchnic nerve at the point it entered the adrenal gland all the way back into the spinal cord. There was no synapse in a sympathetic ganglion and Elliott correctly concluded the splanchnic nerve is a preganglionic nerve. That would explain why adrenaline did not duplicate the effect of stimulating the splanchnic nerve, which was to cause the adrenal medulla to secrete adrenaline. In his 1914 Sidney Ringer Memorial Lecture[2] on the adrenal gland, Elliott (1914) cited embryological evidence that the adrenaline secreting (chromaffin) cells of the adrenal medulla arise from the same ectodermal sympathoblasts from which postganglionic sympathetic neurons originate: ''We have seen how the ganglion cell and the adrenalin cell are both derived from what is almost a common cell with power to transmit a nervous impulse or to excrete adrenalin'' (p. 1395).

Elliott (1914) speculated that during the course of evolution, sympathetic neurons lost their power to produce adrenaline and became dependent on storing up some of the adrenaline secreted by the adrenal gland:

> It is conceivable that as the nervous cell developed its peculiar outgrowths for the purpose of transmitting and localizing the nervous impulse, it might lose its power of producing adrenalin and come to depend on what could be picked up from the circulating blood and stored in its nerve endings. Removal of the glands would cut off this source of supply, and paralysis of the nerves

[2] Physiologists might be familiar only with the Ringer solution that Sidney Ringer developed to provide an appropriate balance of salts to preserve living cells. Ringer was a highly respected physician, researcher, and teacher at the University College Hospital and Medical School.

> would result sooner or later in that territory where the nerves had been functionally most active and had consumed their stores. (p. 1395)

Elliott seems to have concluded that postganglionic nerves secrete adrenaline, but they no longer are capable of producing it themselves. Although he did not pursue these ideas, Elliott made a number of speculations that were uncannily prophetic. In his Sidney Ringer Lecture, for example, he hypothesized that parasympathetic nerves and the spinal nerves that innervate skeletal muscles may share a common biochemical connection similar to that which exists between adrenaline and the sympathetic nervous system. It would be more than 20 years before it would be proven that parasympathetic and spinal nerves both use acetylcholine to transmit their effects.

Despite their clarity and insightfulness, Elliott's articles had little influence and were only cited much later in a historical context. There are several reasons for this besides the fact that his ideas were too far ahead of their time and ran counter to the prevailing view that synapses were bridged electrically. Elliott's mentor, Langley, was said to be temperamentally skeptical and critical of theories, and he had this advice for those who worked with him: ''Make accurate observations and get the facts. If you do that, the theory ought to make itself'' (cited in Dale, 1961, p. 58). Langley opposed Elliott's speculation, and he may have edited them out of his initial manuscript. Moreover, Langley never mentioned the possibility of adrenaline secretions in his own papers or in his textbook on the autonomic nervous system (Langley, 1921).

A study by Walter Dixon (1871–1931) needs to be mentioned because it is often cited as providing the first experimental evidence that a parasympathetic nerve secretes a chemical transmitter. However, a careful reading of what Dixon actually wrote does not support this view. The experiment in question involved the innervation of the heart by the vagus nerve (Dixon, 1907b).[3] It was well known at the time that vagal stimulation produces a slowing of heart rate. What Dixon did was to extract a substance from the heart of a dog after its vagus nerve had been stimulated for 30 min. After some processing, a perfusate containing the substance was applied to a frog's heart.[4] Dixon reported that the perfusate caused the frog's heart rate to slow and that this effect was blocked by atropine, a drug known to also block cardiac deceleration induced by the drug muscarine. Dixon (1907a) concluded that it appeared that ''the ultimate effect is produced by the same mechanism in both cases,'' implying that the effects of vagal stimulation also involved muscarine or a similar substance. However, he never stated that this chemical substance was secreted by the nerve. What he actually concluded was

> that some inhibitory substance *is stored up in that portion of the heart,* to which we refer as a nerve ending, that when the vagus is excited this inhibitory substance is set free, and by combining with a body in the cardiac muscle brings about the inhibition. (p. 457, italics added)

Dixon was clearly suggesting that the chemical substance that he extracted came from the stimulated dog's heart, not the nerve. His views are made clear by the recording secretary who summarized his 1906 presentation:

> Professor W. E. Dixon gave an account of his experiments on vagus inhibition. He was of the opinion that the heart contains a substance—''pro-inhibitin,'' which as a result of vagus excitation

[3] Dixon presented the only study he did on vagal innervation of the heart at a 1906 meeting of the Therapeutic Society in London. The presentation was titled ''On the Mode of Action of Drugs.'' It was published the following year in a somewhat obscure journal (Dixon, 1907).

[4] The fluid was first extracted with alcohol and then dehydrated. The crystals were then rehydrated with 100% alcohol. There is no way that the highly unstable acetylcholine could have survived this treatment.

is converted into a chemical body—''inhibitin.'' This substance, combining with the heart muscle, results in cardiac standstill. (Dixon, 1907b, p. 1807)

THE CONTRIBUTION OF DALE AND LOEWI

Although the idea may have occurred to some from time to time, the possibility of neural secretions was not directly attacked until 1920 when Loewi had the inspiration for his landmark experiment. Before that, it was Dale and his collaborators who were responsible for discovering much of the basic information needed to eventually prove that nerves do indeed communicate by secreting chemical substances.

Henry Hallett Dale (1875–1968) had preceded Elliott as a fellowship student in Langley's laboratory. He was a good friend of Elliott's and certainly familiar with the similarities between adrenaline and sympathetic nerve stimulation. However, like most others at the time, he did not fully appreciate the importance of this observation. In fact, as he later recalled, he may have discouraged Elliott by telling him about several instances where the effects of adrenaline and sympathetic nerve stimulation differed. During the clinical year required to complete the medical degree, Dale was turned away by the authoritarian-style lectures and demeanor of the senior physicians, which was contrasted in his mind with the give-and-take exchanges he had with professors at Cambridge. He rejected the idea of practicing medicine, and in 1902 he was offered a research position in Ernest Starling's laboratory at University College in London.

In 1904, at Starling's recommendation, Henry Wellcome, who by that time had become sole proprietor of the Burroughs–Wellcome Pharmaceutical Company, offered Dale a position as a laboratory director in the Wellcome Physiological Research Laboratories in Herne Hill, a London suburb. According to Dale (1963), his friends tried to persuade him not to take the job, arguing that he would be selling his scientific birthright for commercial pottage, but he rejected their advice. He had decided that his academic prospects were bleak, and besides, he was anxious to have his own laboratory. Dale also admitted that he had welcomed the opportunity to make a marriageable income. It turned out to be a most fortunate move. Within 2 years, Dale was appointed director of all the laboratories, and by the end of the 10 years (1904–1914) that he spent there, Dale was recognized as a major figure in experimental pharmacology.

Henry Wellcome had encouraged Dale to look into the pharmacological properties of ergot. The ergot fungus had been used in folk medicine for hundreds of years to induce labor, and several pharmaceutical companies were exploring extracts for possible use in obstetrics. Ergot turned out to be a treasure trove of active pharmacological substances. One was a substance later identified as histamine, which produced a marked decrease in blood pressure because the increase in capillary permeability led to a vascular fluid loss. When Dale found a similar substance in the skin of animals, he recognized the relevance of histamine to anaphylactic shock. During World War I, Dale recommended that fluid, either whole blood or plasma, should be administered immediately to soldiers experiencing what was called ''secondary wound shock'' (Dale, 1919–1920; Dale & Laidlaw, 1919/1952).

More directly related to the current story were other extracts obtained from the ergot fungus. One of these was an amine with properties similar to adrenaline. Another was an extract called ergotoxine, which was later separated into three different active compounds with physiological properties similar to acetylcholine. And lastly, acetylcholine itself was found there. The presence of acetylcholine was due to bacterial contamination from the *Bacillus acetylcholini*, the same bacillus responsible for fermenting sauerkraut. Because ergot does not contain any cholinesterase, the enzyme

that rapidly degrades (inactivates) acetylcholine, the acetylcholine produced by the bacillus was relatively stable.

With the help of George Barger, a synthesizing chemist, and various other collaborators, Dale described the chemical structure and the pharmacological action of a number of drugs that mimicked either sympathetic or parasympathetic nerve stimulation (Barger & Dale, 1910–1911). Dale suspected that one of the ergot extracts was acetylcholine, and with Wellcome chemists, he was able to prove this to be the case. He replicated the findings of the American pharmacologist Reid Hunt, who had reported earlier that acetylcholine was enormously potent in decreasing heart rate and lowering blood pressure (Hunt & Taveau, 1906).

Dale also found that at a number of sites, acetylcholine and muscarine had the same effect. At other sites where acetylcholine was effective, however, muscarine was not effective, but at those sites, low doses of nicotine mimicked acetylcholine.[5] It was determined that atropine blocked transmission at the muscarinic sites but not at the nicotine sites.[6] Dale then classified acetylcholine active sites as either muscarinic or nicotinic, which implied that the substrate that responds to drugs must be able to distinguish between similar, but not identical, drugs. This finding foreshadowed the modern concept of receptor subtypes. Dale also introduced the terminology *parasympathomimetic* and *sympathomimetic* to classify the accumulating list of drugs that mimicked parasympathetic and sympathetic effects, respectively.

Dale observed that acetylcholine, more than any other drug, duplicated the effects of parasympathetic nerve stimulation. He described acetylcholine's action as ''immediate'' and ''intense'' but also ''extraordinarily evanescent.'' He anticipated by many years the discovery of cholinesterase when he suggested, in 1914, that the short duration of acetylcholine's action might be due to an esterase in the body that rapidly broke it down into acetic acid and an inactive choline (Dale, 1914a,b).

Despite all of these leads, Dale, being most cautious about what he wrote, did not speculate, at least in print, that parasympathetic nerves might secrete acetylcholine. He later wrote that not only was it due to a lack of a technique to capture acetylcholine before it was inactivated, but ''we had no evidence at all that acetylcholine was a constituent of any part of the animal body, and many years elapsed before we found it there'' (Dale, 1934, p. 836). Dale was doing traditional pharmacological research, with its emphasis on investigating the action of drugs and their potential therapeutic value. Determining how nerves communicate was not the primary purpose of the research.

In 1914, Dale was elected to the Royal Society in recognition of his many contributions to pharmacology and physiology. That year, he left the Welcome Laboratories for a position as director of the Department of Biochemistry and Pharmacology at the Institute of Medical Research in London (later named the National Institute of Medical Research). Barger joined him, and together they continued to investigate the properties of various sympathomimetic and parasympathomimetic substances. World War I also started that year, and Dale devoted considerable time during the next 4 years working on drug standardization, a critical problem for Great Britain given that Germany had been its main supplier of drugs.[7]

Although Dale had now to balance the roles of science administrator, contributor

[5] At high doses, nicotine paralyzes the nerve, allowing no further responses to be evoked.

[6] In general, muscarinic sites were located at the junction of parasympathetic nerves with smooth muscles, while nicotinic sites were found primarily at the autonomic ganglia synapses and at the junction of spinal nerves with skeletal muscles. There are, however, some ganglionic synapses that are muscarinic.

[7] Dale retained an interest in drug standardization, and in 1925 he chaired a League of Nations conference in Geneva on the subject.

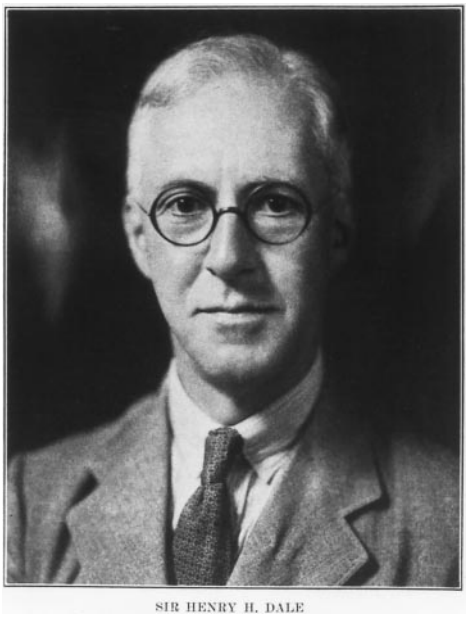

FIG. 1. Henry Hallett Dale.

to the war effort, and chair of several demanding national committees, his laboratory remained highly productive. In addition to the research on acetylcholine, the properties of a number of sympathomimetic compounds that Barger had synthesized were studied. Dale reported several such compounds that were more potent than adrenaline. The most potent proved to be noradrenaline—now generally called norepinephrine—but because it had not been identified as a natural substance in the body, Dale thought of it as only an interesting synthetic compound. Although Dale (Fig. 1) did not raise the possibility that sympathetic and parasympathetic nerves may secrete chemical substances, he had laid the foundation, if not for inspiring Loewi's landmark experiment, for the way to eventually prove that the initial speculative interpretation of the results was correct.

Otto Loewi (1873–1961) was born in Frankfurt, Germany, into a Jewish family of successful wine merchants. He attended an old-style gymnasium that offered a classical education, with a thorough grounding in Latin and Greek. He had wanted to study the history of art, but his father persuaded him to study medicine. Loewi matriculated in medicine at Strassburg, but being bored by the formal Germanic-style medical lectures, he often skipped classes to hear lectures on the arts instead. After Loewi barely passed the examination at the end of the third year, he arranged to spend a year in Munich to prepare for the final year and managed to complete his medical degree and some postgraduate courses in chemistry.

Loewi never practiced medicine. He was frustrated by the year he spent as an assistant at a city hospital. The high mortality among patients with tuberculosis and pneumonia, due to a lack of any effective treatment, turned Loewi away from medicine. He was able to get a research position in Marburg in the laboratory of Hans Meyer, an eminent German pharmacologist, and spent the next 6 years there doing research primarily on glucose metabolism and nutrition. His most notable work during this period was the demonstration, for the first time, that animals could synthesize proteins from amino acids.

In 1902, Loewi was able to spend several months in London visiting physiologists

in Starling's laboratory. It was on this occasion that Loewi and Dale first met and began what was to be a friendship and a sharing of interests that lasted nearly 60 years. Loewi visited England again in 1903 and spent time with Langley and Elliott in Cambridge before revisiting Dale in London. Dale later recalled that Loewi had especially commented on ''how much he had enjoyed his discussion with Elliott and what a high opinion he had formed of his promise'' (Dale, 1954). This meeting between Loewi and Elliott has raised the possibility, in the minds of some, that Loewi may have had the seeds of the idea of neurohumoral secretions planted in his mind at that time. Years later, Dale wrote about the meeting between Elliott and Loewi: ''The idea of chemical transmission from nerve endings might surely have had some mention between them, though we cannot expect either of them to have any memory now of such talk about it, a half century later'' (p. 9). What is known is that the following year, Loewi had speculated that the vagus nerve might inhibit heart rate by secreting something like muscarine. It was apparently only a casual remark given that Loewi completely forgot making it until he was reminded of it in 1929 by Walter Fletcher, a colleague who was working in Meyer's institute at the time (Loewi, 1954).

When Meyer became head of the Department of Pharmacology in Vienna, Loewi joined him. In 1908, Loewi was appointed professor of pharmacology at the University of Graz, the second largest city in Austria. He continued his research on carbohydrate metabolism and gained the reputation of a lively lecturer who was popular with students. He presumably was respected by the faculty that elected him dean of the medical faculty for 1912–1913. Although Loewi did no work on neurohumoral secretions, he recalled later that he occasionally thought about the possibility but saw no way of proving it and that ''it entirely slipped my conscious memory until it emerged again in 1920'' (Loewi, 1960, p. 17). It emerged in a dream, which Loewi subsequently described on many different occasions:

> The night before Easter Sunday of that year I awoke, turned on the light, and jotted down a few notes on a tiny slip of thin paper. Then I fell asleep again. It occurred to me at six o'clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. The next night, at three o'clock, the idea returned. It was the design of an experiment to determine whether or not the hypothesis of chemical transmission that I had uttered seventeen years ago was correct. I got up immediately, went to the laboratory, and performed a simple experiment on a frog heart according to the nocturnal design. (p. 17; see also Loewi, 1953, p. 33)

There are indications that Loewi could not resist making the story more dramatic than it actually was. Dale later commented that he must have been one of the earliest to hear an account of the remarkable story of the dream, but he recalled that Loewi had originally told him that when he awoke the second night at 3 o'clock, he made careful notes so that he would have no trouble deciphering his words the next morning (Dale, 1962). Loewi's version was clearly the more dramatic. According to Loewi's account, not only did he go directly to the laboratory at 3 o'clock, but by ''five o'clock the chemical transmission of the nervous impulse was conclusively proved'' (Loewi, 1953, p. 33). Moreover, the placing of the whole incident on Easter Sunday of 1921 could not be correct. Loewi's first manuscript was received on March 20, 1921, but Easter Sunday in 1921 occurred on March 27 (Davenport, 1991). The different versions have no historical significance except that they reflect a personality difference between Dale and Loewi. Dale would probably never stray even slightly from the facts, no matter how inconsequential, but Loewi, who had the more artistic temperament, probably could not resist some literary license to add drama to the story. When later questioned about some of these discrepancies, the physiologist van der Koot, who was a colleague of Loewi at New York University, said this about Loewi: ''He certainly loved a good story, and they certainly showed some normal

variation. He was also one of the most upright and decent men I have ever known'' (W. van der Kloot, cited as a personal communication by Davenport, 1991, p. 186).

The simple experiment that the dream inspired has also been described on many occasions by Loewi:

> The hearts of two frogs were isolated, the first with its nerves, the second without. Both hearts were attached to Straub cannulas filled with a little Ringer solution. The vagus nerve of the first heart was stimulated for a few minutes. Then the Ringer solution that had been in the first heart during the stimulation of the vagus was transferred to the second heart. It slowed and its beats diminished just as if its vagus had been stimulated. Similarly, when the accelerator nerve was stimulated and the Ringer from this period transferred, the second heart speeded up and its beats increased. (Loewi, 1960, p. 17)

Heart rate was both slowed down and speeded up because the nerve trunk stimulated contained sympathetic fibers as well as the vagus nerve. Loewi initially referred to the substances secreted as *Vagusstoff* and *Acceleransstoff*.[8]

Although Loewi later wrote that the experiment ''unequivocally proved that the nerves do not influence the heart directly but liberate from their terminals specific chemical substances,'' (Loewi, 1953) many physiologists were not at all convinced. Some would not accept that nerves could secrete chemical substances, let alone different substances. It was easier for them to believe that there were two substances in the heart: one whose release was triggered by the vagus and the other whose release was triggered by sympathetic nerve fibers.

Others simply claimed Loewi's results could not be replicated (Bain, 1932). There were many reasons why this was so. First, as it was later learned, the results could be obtained consistently only with so-called winter frogs. At that time of the year, the inhibitory nerves predominate, and it has also been suggested that at colder temperatures frogs may have less cholinesterase. Otherwise, it is unlikely that the acetylcholine would have remained stable long enough to have been transferred by a pipette from one heart to the other, each in a separate glass vial. Moreover, the physical disturbance of fluid being transferred could, by itself, have caused a change in heart rate. There were reports that any perfusate transferred in this way might make heart rate decrease. Heart rate might decrease when the perfusate came from a heart beating at a normal rate, from a heart whose vagus nerve had not been stimulated, or even from a heart stimulated mechanically. The recipient heart sometimes slowed down in response to a perfusate from an accelerated heart. Furthermore, it proved difficult at first to reproduce the results in mammals. Considering all that could have gone wrong, Loewi was clearly lucky that his initial experiment worked. Indeed, according to Davenport (1991), for a while Loewi also had difficulty replicating his initial results.

In 1926, Loewi was asked to respond to his critics by demonstrating the experiment at the International Physiology Congress held in Stockholm. It has been reported that he successfully demonstrated his results 18 times. Ulf von Euler, who was at the congress, later wrote that these demonstrations first aroused his interest in neurohumoral transmission. Later, von Euler spent 1930–1931 working with Dale, and in 1970 he shared the Nobel Prize for proving that the sympathetic neurotransmitter is noradrenaline (norepinephrine), not adrenaline (epinephrine).[9]

[8] The experiment was first published in a four-page article: ''Über humorale Übertragbarkeit der Herznervwirkung'' (On humoral transmission of the action of heart nerves) (Loewi, 1921).

[9] Loewi was hesitant to say that the sympathetic secretion was adrenaline. As late as 1935, he wrote, ''I do not feel justified as yet in assuming that the sympathetic transmitter is adrenaline, and I will therefore call it the adrenaline-like substance'' (Loewi, 1935, p. 300). The frog, however, is said to be an exception in that adrenaline may actually be the sympathetic secretion that accelerates the heart, but in most animals it is noradrenaline.

Not all critics were satisfied by the Stockholm demonstration. In 1927, Louis Lapicque, a neurophysiologist working in Paris and a strong proponent of electrical transmission theories, declared that the idea of humoral transmission of nerve impulses is ''unthinkable'' (cited in Minz, 1955). As late as 1932, Leon Asher of Basel, Switzerland, who had been disputing Loewi's findings for a number of years, just about accused him of being delusional when he wrote,

> Only the failure of the necessary experimental critique and a little ''autistic'' thinking by definition of Bleuler could allow one to consider experiments which in the present situation do not prove anything, much less than absolutely proving the existence of a vagus hormone. (cited in Minz, 1955, pp. 13–14)

According to Bacq (1975), before Loewi's first experiment on vagal slowing, Asher had used a similar technique, but he did not find any indication of a neurohumoral secretion.

Loewi must have been disturbed by these often acerbic criticisms, but he was not deterred. In his initial 1921 publication, Loewi recognized that there were two possible origins of the humoral substances capable of slowing down and speeding up heart rate:

> On the one hand, they may originate directly from the effect of nerve stimulation independent of the type of cardiac activity. . . . From a different viewpoint, there is also the possibility that these substances are only products of the specific type of cardiac activity which is released by the nerve impulse; under such circumstances, therefore the identification of their action with the nerve stimulus would be only accidental, so to speak. (Loewi, 1921, p. 241, translated from German)

Loewi was referring to the persistent problem of trying to determine whether any substance detected originated in the muscle that was inhibited or activated or from the innervating nerve.

In his second publication on neurohumoral substances, Loewi (1922) ruled out the possibility that the chemical substances came from the heart. He used high doses of nicotine to paralyze the donor heart and found that it was still possible to transfer the Vagusstoff and Acceleransstoff after stimulating the nerve. Following a series of experiments, Loewi and his collaborators were able to report that of all the known vagomimetic substances—muscarine, pilocarpine, choline, and acetylcholine—only acetylcholine satisfied all of the pharmacological tests (Loewi & Navratil, 1924a,b; 1926a,b; for a complete list of Loewi's publications, see Dale, 1962). The fact that atropine blocked the action of Vagusstoff eliminated most other possibilities except for choline. Choline was eliminated by determining that it was not present in a sufficient concentration to be effective and that, even in high concentrations, choline's effect on heart rate was relatively weak. The atropine effect was also specific in that it did not block the acceleration produced by sympathetic stimulation. Vagal slowing was also blocked or markedly reduced by small amounts of cholinesterase and was greatly enhanced and prolonged by eserine, which blocks the action of cholinesterase. Moreover, egotamine, a purified alkaloid substance known to block the effects of adrenaline, also enhanced vagal slowing of the heart.[10] Most persuasive was the fact that other laboratories had begun to confirm Loewi's results and were able to extend them to mammals.

Although by 1933 several neurophysiologists were willing to concede that Loewi was justified in speaking of neurohumoral substances controlling heart rate, others,

[10] Barger, Carr, and Dale isolated a pharmacologically active substance they called ergotoxine from the ergot fungus (Barger & Dale, 1907). It later was shown to be made up of three separate alkaloid substances. Later, Stoll obtained a purified extract of one of these, which he called ergotamine (Stoll, 1920).

FIG. 2. Otto Loewi.

including some of the more eminent members of that discipline, continued to express strong reservations. Many regarded Loewi's indirect pharmacological evidence as unconvincing. Others reluctantly conceded that he may have been right about the regulation of heart rate, but this was a special case that did not apply to other peripheral nerves, let alone central nervous system neurons. It is worth noting that almost up to 1935, Loewi was also reluctant to accept that the neurohumoral secretions he had demonstrated in the innervation of the heart applied elsewhere in the peripheral nervous system. He especially doubted that spinal nerves secreted chemical substances to innervate skeletal muscles. Dale (1954) described Loewi as seeming to be almost frightened by the idea in 1933:

> Loewi seems to have taken alarm, for the time being, at the thought of such a possible extension of his discovery, and to have gone to the length of establishing his own alibi by a public disclaimer of belief in chemical transmission at the motor nerve endings. (p. 11)

Loewi (Fig. 2) was also skeptical of neurohumoral transmission at autonomic ganglion synapses. At the first Harvey Lecture in New York in 1933, for example, Loewi expressed his doubt that acetylcholine was secreted at autonomic ganglia as well as at the junction of spinal nerves with skeletal muscles. This was at the very time Dale and his colleagues were collecting the evidence that this was indeed the case. By 1935, however, much of the evidence from Dale's laboratory had been published, and in Loewi's Ferrier Lecture to the Royal Society, he finally acknowledged that the evidence had convinced him that acetylcholine was secreted at these additional sites (Loewi, 1935).

For a number of years, Dale's research, by his own admission, had turned in other directions, but Loewi's research on chemical transmission

> had given such ideas for the first time an experimental reality. . . . And thus it was not until 1929, after an interval of some 15 years but then in a new atmosphere of generally awakened interest

> in its possible significance, that my direct participation in experiments with acetylcholine was awakened. (Dale, 1953, p. xv)

Dale was referring to the fact that he had speculated in 1914 that acetylcholine might be a natural substance, but it was not until 1929 that he and Harold Dudley actually found it in the spleen of the ox and the horse (Dale & Dudley, 1929). It was the first clear evidence that acetylcholine was a natural constituent of the body, and the occasion was used to review the similarities between the effects produced by this substance and parasympathetic nerve stimulation.

The next year, Dale and Gaddum (1930) investigated the phenomenon, reported earlier by Sherrington and others, that a denervated skeletal muscle contracted in response to the stimulation of a parasympathetic nerve not connected to the muscle. Dale and Gaddum also demonstrated that a denervated muscle contracts when acetylcholine is injected into the vascular system. After ruling out most other alternative explanations, they concluded that skeletal muscles normally contract in response to ''the peripheral liberation of acetylcholine'' by spinal nerves (p. 143). Dale seemed unwilling to explicitly state that spinal nerves secrete acetylcholine, but the implication was clear. However, the American neurophysiologist Herbert Gasser, who would share the 1944 Nobel Prize with his collaborator Joseph Erlanger, suggested an alternative electrical explanation and rejected Dale and Gaddum's conclusion that acetylcholine is the stimulus that normally makes skeletal muscles contract.

As noted, Dale had also surmised as early as 1914 that there was some esterase in the body that rapidly deactivated acetylcholine, but cholinesterase was not found there until 1930 when both Dale and Loewi independently reported finding it. It was known for a number of years that eserine enhanced and prolonged the action of acetylcholine, and Loewi and Navratil (1926a,b) had used it to enhance the cardiac response to vagal stimulation. However, neither Dale nor Loewi had developed a way to use eserine to capture acetylcholine released at nerve terminals before it was degraded by cholinesterase. What was needed was a sensitive method of detecting acetylcholine released at nerve terminals. The muscle preparation developed by Bruno Minz in Feldberg's laboratory proved to be the method needed at the time (Minz, 1932, 1955). This technique was brought to Dale's laboratory by Feldberg in 1933.

Wilhelm Feldberg (1900–1993) was born in Hamburg into a wealthy Jewish family. His father and uncle had started a business selling women's clothes, and this had been expanded into a successful department store. He attended the universities of Heidelberg and Munich and received his medical degree from Berlin University in 1925. Preferring research to practicing medicine, he accepted a laboratory position with Erich Schilf at the University of Berlin. Schilf had translated Langley's book, *The Autonomic Nervous System*, and he arranged for Feldberg to spend the year 1925 in Langley's laboratory. When Langley suddenly died, Dale invited Feldberg to work in his laboratory for the remaining 6 months of the visit. Feldberg later said that the experience working under Dale's tutelage had a lasting influence on the way he designed experiments. Dale's advice was as follows: ''Feldberg, you must work like an astronomer. Prepare for weeks, for months, if necessary for years, until your method is working to perfection, then do one experiment, perhaps two—and publish the results'' (Feldberg, 1982, p. 5).

In 1932, Dale was the guest of honor at a meeting of the German Pharmaceutical Society in Wiesbaden. At the meeting, Minz, who was working in Feldberg's laboratory, reported on the technique they were using for detecting the presence of minute quantities of acetylcholine (Minz, 1932; Feldberg & Krayer, 1933a,b). Dale was anxious to hear about it, and he arranged to have lunch with Feldberg. At one point in their conversation, Dale asked Feldberg what he thought about Hitler, whose Nazi

party was rapidly gaining strength in Germany but was not yet in control of the government. Feldberg replied, ''Sir Henry [Dale had been knighted that year], you need not worry, he will never win, and if he should, he will cook with water only,'' using the German expression that meant nothing would come of it. Dale, who was much more aware of political events, replied, ''Feldberg, you had better stick to your experiments'' (Feldberg, 1982, p. 45).

On January 30, 1933, the Nazis took over the government and the first anti-Semitic laws were promulgated. In April, while in the midst of an experiment, Feldberg was called to the director's office and informed that he would not be allowed to enter the institute after that day. Feldberg called his wife, and she volunteered to help him to finish the experiment. It was after midnight when they finished. Feldberg later commented that his departure was not totally ignored, as two Japanese colleagues who had been waiting at the door for their departure bowed silently as they left and bowed again when Feldberg and his wife turned back (Feldberg, 1982).

Feldberg had heard that the Rockefeller Foundation was providing assistance for some of the eminent scientists and intellectuals who had been dismissed from their positions, and he sought out their representative in Berlin.

> He was most sympathetic but said something like this: ''You must understand, Feldberg, so many famous scientists have been dismissed whom we must help that it would not be fair to raise any hope of finding a position for a young person like you.'' Then, more to comfort me, ''But at least let me take down your name. One never knows.'' And when I spelt out my name for him, he hesitated and said, ''I must have heard about you. Let me see.'' Turning back the pages of his dairy, he suddenly said, delighted himself: ''Here it is. I have a message for you from Sir Henry Dale, whom I met in London about a fortnight ago. Sir Henry told me, if by chance I should meet Feldberg in Berlin, and if he has been dismissed, tell him I want him to come to London to work with me. So you are all right,'' he said warmly. ''There is at least one person I needn't worry about any more.'' (Feldberg, 1977, pp. 67–68)[11]

Although Dale certainly benefited when Feldberg was able to join his laboratory, he was generally sensitive to the plight of the dismissed scientists in Germany and was successful in locating temporary positions for several of them.

The large number of scientists who had to leave Germany and went on to contribute enormously to the countries that adopted them has been described in the book *Hitler's Gift* (Medawar & Pyke, 2001). During the first year alone after the Nazis came to power, 2600 scientists left Germany. Of the refugees from Hitler, 20 received the Nobel Prize and 50 became fellows of the Royal Society. During the first 32 years of the Nobel Prize (1901–1932), Germany was awarded one-third (33 of 100) of the prizes in science. Great Britain received 18, and U.S. scientists were awarded 6. Those figures were turned on their head after 1933. One-fourth of all physicists, among them the most eminent, left Germany, and the majority of the early scientists working on the atomic bomb were refugees from the Nazis, not all of them German. Among those scientists who worked on synaptic transmission who left Germany between 1933 and 1937 were Herman Blasko, Edith Bulbring, Wilhelm Feldberg, Bernard Katz, Otto Krayer, David Nachmanson, and Marthe Vogt.

With Rockefeller Foundation support, Feldberg had left Germany in July. He recalled that shortly after arriving in London, Dale asked him, ''What do you think now about Hitler?'' The only reply that occurred to him was, ''Sir Henry, can I help it that history has made a mistake?'' Feldberg's wife, who was not Jewish, had insisted that he leave immediately while she remained behind to pack with their chil-

[11] A complication in getting Rockefeller support for Feldberg, which Dale found a way to resolve, concerned the fact that Feldberg technically was not discharged from a paying position. To devote full time for his research without having to do any teaching, Feldberg did not draw any salary, and he paid the salary of his technician and purchased his experimental animals.

dren. When they finally arrived in England, the immigration officer who had observed Feldberg's nervous pacing up and down for several hours, said, ''Mrs. Feldberg, you must never leave your husband again.'' Feldberg reported this incident to illustrate the sensitivity of the English to the plight of those persecuted in Germany (Feldberg, 1977, p. 69).

After his wife and children arrived, Feldberg immediately set up his preparation for detecting acetylcholine. The technique involved perfusing a leech muscle with the blood drawn from a vein located in the terminal region of a stimulated nerve. The leech muscle in a saline bath is not particularly sensitive to acetylcholine, but when eserine is added to the saline, the muscle's sensitivity is increased more than a million times.[12] Although this seems like a pretty straightforward bioassay, there were a number of additional steps necessary to make it work reliably. First, not all leeches are equally sensitive to acetylcholine. The Hungarian leech (*Hirudo officinalis*) worked best. Then the leech muscle, which was attached to a string gauge, had to be placed in an eserine–saline solution, the concentration of which needed to be adjusted separately for each muscle. The experimental animals (usually cats or dogs) had to be injected with eserine to protect the acetylcholine from degradation by cholinesterase The animals also had to be injected with an anticoagulant, such as heparin, to prevent blood clotting. The withdrawn blood needed to be passed through a cooling jacket to adjust it to a temperature suitable for the cold-blooded leech. Lastly, the stimulated nerve had to be gently lifted with a thread so that no surrounding tissue would be stimulated. When done properly, the leech muscle would contract only if there was acetylcholine present in the blood and it was capable of responding to a dilution of 1 part of acetylcholine in 500 million parts of solution.

In less than 3 years, between 1933 and 1936, Feldberg published 25 experimental articles, most in collaboration with Dale and, in different combinations, with an exceptional group of colleagues working in the laboratory.[13] Feldberg tended to be self-effacing about his contribution, perhaps out of gratitude for Dale's kindness. He once said in response to a remark that without his help, Dale might not have shared the Nobel Prize with Otto Loewi and that he ''may have brought a key that could open some doors, but Sir Henry and John Gaddum knew what doors needed to be opened'' (Feldberg, 1977, p. 71). However, even before Feldberg had joined Dale's laboratory, while still in Berlin, he had used the leech preparation to prove that acetylcholine was secreted by the vagus in innervating the heart of a mammal (Feldberg & Krayer, 1933) and by the splanchnic nerve when innervating the adrenal medulla (Feldberg & Minz, 1933). There can be little question that Feldberg had an enormous impact on the work in Dale's laboratory and on Dale himself. Not long after he had arrived in London, Dale's wife remarked to Katherine, Feldberg's wife, that she was so pleased they had come because her husband was so happy these days now that he was back in the laboratory.

The first experiments with the leech preparation in Dale's laboratory demonstrated that acetylcholine was also secreted by other branches of the vagus including the branch that innervates the stomach (Dale & Feldberg, 1934a,b). It was then demonstrated that acetylcholine was secreted by other parasympathetic nerves such as the

[12] The test was adapted from Fuhner (1918), who used the technique as a method for detecting the presence of eserine. Minz and Feldberg had turned the method around and used it to detect the presence of acetylcholine.

[13] Among others, Dale's collaborators at the time included, besides Feldberg, Harold Dudley, John Gaddum, Marthe Vogt, George L. Brown, A. Vartiaimen, and Z. M. Bacq. Brown, Dudley, Feldberg, Gaddum, and Vogt all were elected to the Royal Society. Brown and Gaddum were knighted in recognition of their scientific achievements.

chorda tympani when innervating the tongue. Dale and Feldberg (1934a,b) also found exceptions to the general rule that postganglionic sympathetic nerves secrete adrenaline. They reported, for example, that the sympathetic nerve that innervates the sweat glands on a cat's paws secretes acetylcholine. To avoid confusing pharmacology with anatomy, Dale recommended referring to autonomic synapses as either cholinergic or adrenergic.

Feldberg and Gaddum (1934) then demonstrated that acetylcholine was released by a presynaptic cervical nerve at its synapse in the superior cervical ganglion. To draw blood from the region of the ganglion, they employed a new method that had just been reported by a Russian physiologist, Anton Kibjakow, who worked in Kazan (Kibjakow, 1933). The demonstration by Feldberg and Gaddum was particularly significant because it showed for the first time that neurotransmitters were secreted not only to innervate smooth muscles and glands but also at synapses between neurons. Moreover, it demonstrated that acetylcholine was secreted at all autonomic ganglia synapses, sympathetic as well as parasympathetic.

The next important question to be attacked was whether there was neurohumoral transmission outside the autonomic nervous system. Although neurophysiologists offered many reasons why chemical mediation would be too slow for the innervation of skeletal muscles, there was, as mentioned above, an increasing amount of pharmacological evidence suggesting that spinal nerves do secrete acetylcholine. The leech muscle preparation provided additional evidence that this was indeed the case (Dale, Feldberg, & Vogt, 1936).

By 1936, Dale and his collaborators had extended Loewi's work by proving that neurotransmitters were secreted at all peripheral synapses. They had also provided seemingly persuasive evidence that all autonomic preganglionic nerves emerging from the spinal cord secrete acetylcholine at the synapse with postganglionic nerve fibers and that the spinal nerves innervating skeletal muscle are also cholinergic. Although there were a few exceptions, almost all postganglionic parasympathetic synapses are cholinergic, while postganglionic sympathetic synapses are adrenergic.

In 1936, Loewi and Dale shared the Nobel Prize in Physiology or Medicine for demonstrating neurohumoral transmission. The presentation of the award stated:

> You, Professor Loewi, first succeeded in establishing proof of such transmission and in determining the nature of the effective substances. This work was, in part, built up on earlier research to which you, Sir Henry, made an essential contribution. The results were consolidated and complemented in many important respects by you and your collaborators. You and your school have also greatly extended the range of the new conception by later discoveries. Through these various discoveries, which have stimulated research in innumerable parts of the world, therefore demonstrating once again the international character of science, pharmacology has been very considerably influenced, and physiology or medicine enriched to a high degree. (*Nobel Lectures, Including Presentation Speeches and Laureates' Biographies,* Physiology or Medicine, 1922–1941, Nobel Foundation, p. 401 Elsevier: Amsterdam)

In his Nobel Lecture, Dale briefly raised the question of whether there is chemical transmission in the central nervous system. He mentioned that the rich supply of acetylcholine found in the basal ganglia of the brain presumably must serve some purpose, and he alluded to some preliminary results by Feldberg demonstrating ''suggestive effects'' when acetylcholine and eserine are injected into the ventricles of the brain. Dale, however, concluded in his characteristically cautious manner, ''I take the view, however, that we need a much larger array of well-authenticated facts before we begin to theorize.'' (*Nobel Lectures, Including Presentation Speeches and Laureates' Biographies,* Physiology or Medicine, 1922–1941, Nobel Foundation, p. 412 Elsevier: Amsterdam) It would be another 25 years before neurotransmitters were generally acknowledged to be secreted by brain neurons.

OPPOSITION BY ECCLES AND OTHER NEUROPHYSIOLOGISTS

Despite the awarding of the Nobel Prize, many leading neurophysiologists rejected chemical transmission except in some limited capacity in the autonomic nervous system. John Eccles, was certainly not alone, but he often took the lead in opposing chemical transmission, particularly for the synapse between spinal nerves and skeletal muscles. He frequently engaged Dale and Feldberg in vigorous debates at meetings, but these were always conducted in a friendly and constructive manner. Feldberg later wrote, ''The strong opposition of Eccles and others, however, had a most beneficial effect. We were not allowed to relax but were forced to accumulate more and more detailed evidence in support of our theory'' (Feldberg, 1977, p. 72). Dale described Eccles as having been an ideal sparring partner who played an important role in honing the arguments of the proponents of chemical transmission, and Eccles, reflecting on this period, agreed with that characterization of his role.

Dale and Eccles exchanged many letters, informing each other of new work before it was published, and their disagreements were often softened by humor. On one occasion Dale and Feldberg met Eccles in Oxford right after the two of them had finished a vigorous tennis game during a break at a meeting. They had worked up quite a sweat, and Dale could not resist remarking to Eccles that their sweat was undoubtedly the result of acetylcholine secretion. Eccles replied that they needed more evidence, suggesting that they do a bioassay on their tennis socks (Karczmar, 2001).

One of the problems that neurophysiologists had difficulty in explaining was how inhibition could be propagated electrically, but they were not short of theories attempting to explain how eddy currents of different polarities could produce inhibition. Feldberg described an anecdote in this context that occurred in 1937. He had left England for a position in Sydney, Australia, and Eccles had recently returned to his native Australia. One day, a visitor to Feldberg's laboratory delivered a message from Eccles. The message simply read, ''Acetylcholine is all wet.'' Feldberg sent back a telegram stating, ''Prefer wet acetylcholine to dry eddy currents'' (Feldberg, 1982).

Throughout the 1940s and much of the 1950s, leading neurophysiologists continued to insist that transmission was primarily, if not exclusively, electrical in the spinal cord and certainly in the brain. Much later, Eccles (1976) described the sentiment of some of the most eminent neurophysiologists at the time:

> In 1939 a symposium on the synapse was published in the *Journal of Neurophysiology*, where Lorente de Nó, Gasser and Erlanger were strongly in favor of electrical transmission. In the Paris symposium of 1949, there was also good support for electrical transmission, but very largely for the central nervous system synapses. Even as recently as the Brussels symposium of 1951, there was substantial support for electrical transmission, particularly by Fessard. (p. 223)

Detlev Bronk and several other neurophysiologists were willing to accept a compromise in which acetylcholine was one of several factors capable of modifying the response to electrical transmission (Forbes, 1939). Dale commented that, in general, neurophysiologists seemed embarrassed to talk about chemical transmission in public, and he repeated an earlier comment by von Bruecke that ''transmission by chemical mediators was like a lady with whom the neurophysiologist was willing to live and consort in private, but with whom he was reluctant to be seen in public'' (Dale, 1954, p. 10).

Although neurophysiologists and pharmacologists might debate chemical and electrical transmission at meetings, the techniques and evidence employed by pharmacologists and neurophysiologists were so different that they frequently ignored the data

and arguments of the other side in their publications. In 1943, for example, both Feldberg and Eccles published articles discussing synaptic transmission. The articles were published in the same April issue of the *Journal of Physiology*. Eccles's article implied that local eddy currents completely explained neuromuscular synaptic transmission, and acetylcholine was never mentioned. Feldberg's article, on the other hand, presented the argument for acetylcholine without referring to any data or arguments from the neurophysiologists.

At a 1946 symposium sponsored by the New York Academy of Science, Eccles argued forcefully that electrical transmission could explain inhibition. The following year, he and Chandler Brooks published an elegant but, as it turned out, incorrect theory of how electrical transmission could explain inhibition based on non-impulse-generating interneurons (Brooks & Eccles, 1947). They acknowledged that acetylcholine might have a role in increasing the responsiveness to electrical stimuli, but it was clear that they believed that electrical transmission was the primary means of innervating skeletal muscles. They maintained, for example, that not enough acetylcholine is released by a single brief electrical pulse to account for the response produced. They also argued that the slow rate of hydrolysis by cholinesterase was inconsistent with the rapidity with which spinal nerves returned to their ''resting state.''

Eccles (1976) later described how this theory had occurred to him in a dream: ''On awakening I remembered the near tragic loss of Loewi's dream, so I kept myself awake for an hour or so going over every aspect of the dream and found it fitted all experimental evidence'' (p. 225). However, in this instance, the dream did not provide the right answer, although for a year or so the theory seemed to be supported by additional experimental evidence.

In 1949, John Fulton, who was editor of the *Journal of Neurophysiology* and one of the most influential neurophysiologists during the 1940s and 1950s, also opposed the idea that chemical transmission was responsible for innervating skeletal muscles:

> [Although] the theory of chemical mediation of nerve impulses appeared acceptable to many physiologists in the case of autonomic nerves acting on their effector organ, this concept, when applied to synapses and neuromuscular junctions, was less satisfactory and encountered increasing opposition. In addition to a great number of difficulties and contradictions, which were partly reviewed by John Eccles (in 1937) and have increased continuously since then, there are two main objections, the first being the time factor. This factor was of less importance in the case of the slowly reacting cells innervated by the autonomic nervous system. But the transmission of nerve impulses across the neuromuscular junctions and synapses occurs within milliseconds. No evidence was available that the chemical process can occur at the high speed required. . . . The second objection was still more fundamental. . . . The study of the electrical signs of nerve activity does not support the assumption that the transmission of the nerve impulse along the axon differs fundamentally from that across the synapse. The idea of a chemical mediator released at the nerve ending and acting directly on the second neuron or muscle thus appeared to be unsatisfactory in many respects. (Fulton, 1949, p. 73)

The criticisms of chemical transmission advanced by the neurophysiologists all were logical and reasonable. There were apparent problems with chemical transmission, particularly in regard to the latency of the response and thresholds. However, it is difficult to avoid the impression that many neurophysiologists were being hypercritical in defending their turf. They were the authorities on the nervous system, and they regarded their electronic amplifiers and oscilloscopes as far superior to the bioassay and smoked drum kymograph methodology typically used by pharmacologists. Neurophysiologists could measure the changes in threshold of postsynaptic neurons and the latency of their responses, while the pharmacologists typically did not collect any quantitative data on threshold changes or response latencies.

By mid-1951, microelectrodes capable of recording intracellular voltage changes were adopted in Eccles's laboratory. These electrodes could detect the voltage differ-

ential between the outer neuron membrane and the inner axoplasm of a neuron. When inhibition occurred, the voltage differential was increased and the threshold for exciting the neuron was raised. The reverse was true when the postsynaptic neuron was made more excitable. The critical experiment was done one day in mid-August 1951. As described by Eccles (1976), it was quite a day. In the middle of the experiment while Eccles was attending to the cat, the wife of one of his colleagues (Jack Combs) was delivered of a baby girl by another of his colleagues (Dr. Lawrence Brock). At the end of this long day, it was concluded that the level of inhibition (hyperpolarization) recorded with intracellular microelectrodes could not be induced electrically (Brock, Combs, & Eccles, 1951).

To his credit, Eccles almost immediately changed his mind, and he wrote to Dale about his conversion to a chemical explanation of inhibition for the spinal cord. Dale responded that he was indeed grateful and had derived great pleasure from reading the article, adding, ''Your newfound enthusiasm [for chemical transmission] is certainly not going to cause any of us any embarrassment'' (Eccles, 1976). Dale wrote that Eccles's change was like the conversion of Saul on the way to Damascus when ''the sudden light shone and the scales fell from his eyes'' (Dale, 1954, p. 11).

In 1953, Eccles's group in Camberra, Australia, made additional discoveries that further explained the role of acetylcholine in inhibition. The group demonstrated that collateral branches of spinal motor neurons secreted acetylcholine to excite the Renshaw interneurons in the spinal cord (Eccles, Fatt, & Koketsu, 1953). Renshaw cells had previously been shown to inhibit motor neurons and not to process any excitatory action. Eccles (1976) described these results in a letter to Dale, who responded, ''I do congratulate you all, not only upon the beauty of the observations recorded, but on the very attractively clear and concise account of them in the paper'' (p. 226).

It took quite a few years before chemical transmission was widely accepted by those not working directly on synaptic physiology. For example, in their widely adopted physiological psychology textbook, Clifford Morgan and Eliot Stellar described transmission across the synapse as occurring when the electrical changes in the presynaptic neuron induced a depolarization in the postsynaptic neuron. They made no reference to the possibility of chemical transmission even as an hypothesis, and the word *acetylcholine* was never mentioned even when describing synapses in the autonomic nervous system (Morgan & Stellar, 1950).

Opposition to chemical transmission continued into the mid-1950s, although by then it was mostly a rear guard action. Most neurophysiologists had retreated to the high ground of the brain, but not all of them. For example, Ralph Gerard, who is generally credited with having introduced the term *neuroscience*, would not accept that Eccles's 1951 experiment had completely ruled out electrical explanations of inhibition in the spinal cord (Gerard, 1954, p. 126).

During the second half of the 1950s, the possibility that neurons in the brain might use chemical transmitters to communicate was rarely considered even though there were several people, such as Feldberg and Vogt, who had started to study brain chemistry and to experiment with injecting acetylcholine and eserine into the ventricles of the brain. Although chlorpromazine and a number of other psychotropic drugs were being marketed during the first half of the 1950s, it was not until the 1960s that serious attempts to explain the action of these new drugs were based on their action on chemical neurotransmitters (Valenstein, 1998).

ADDENDUM: WALTER CANNON—A NEAR MISS

There were physiologists at the time who believed that the eminent Harvard physiologist, Walter Cannon, should have shared the Nobel Prize with Loewi and Dale.

Cannon and Dale were good friends. At the beginning of World War I, when Cannon came to England with a medical group, they had several discussions about histamine and anaphylactic shock and even went into the laboratory together to answer a question that arose. On numerous later occasions, Dale and Cannon expressed their mutual respect for each other's research. Cannon was regarded by many as the leading, and perhaps the most original, physiologist in the United States. Starting in 1921, he had been nominated for the Nobel Prize a number of times for his studies of the role played by the adrenals and sympathetic nervous system in emotional states.[14] Cannon's nominations in 1935 and 1936, however, made a special point of noting that he had used an original approach to arrive at the conclusion that sympathetic nerves release chemical substances. This work was going on at the very time that Loewi had started his research on the innervation of the heart. In a series of studies, Cannon and his collaborators demonstrated that even when the heart is denervated and the adrenals and other potentially relevant organs are removed, stimulation of a sympathetic nerve produces cardiac acceleration. This does not occur, however, if the nerve is isolated from the blood supply. Cannon concluded that there must, therefore, be a chemical substance released by sympathetic nerves that can be carried by the blood to the heart. Because not all of the effects of this sympathetic substance were identical to adrenaline, Cannon called it ''sympathin.'' Cannon was correct in concluding that the secretion was not adrenaline, as von Euler later proved that sympathetic nerves generally secrete noradrenaline (norepinephrine). There is some adrenaline (epinephrine) that is secreted, so Cannon was also correct in proposing that there were two different substances involved. However, Cannon's conclusion that there is both an excitatory Sympathin E and an inhibitory Sympathin I neurotransmitter was not correct, and this idea received considerable criticism. According to some, this may have prevented Cannon from winning a share of the Nobel Prize in 1936. In balance, it might be said that Cannon's contribution to the specific question of neurohumoral secretions was not of the same magnitude as that of either Dale or Loewi, although it would be difficult to overstate the value of his overall contribution to our understanding of the homeostatic mechanisms that regulate the internal milieu and to the physiology of emotional states.

POSTSCRIPT ON LOEWI

Circumstances prevented Loewi from remaining active in research for very long. There were many signs of the ensuing danger. Even in 1936, the Austrian Nazis were gaining strength, and they were especially active in Graz. In March 1938, shortly after the Anschluss, storm troopers broke into Loewi's home and arrested him at gunpoint. Loewi and two of his sons were put in jail, along with many other Jews, and were treated brutally. The news of Loewi's arrest reached those attending the International Physiology Congress in Zurich. Many wrote letters and signed petitions trying to get Loewi released. Dale and Cannon circulated a letter threatening that all British and American contact with German scientists would cease if Loewi were not released. Loewi was released after 2 months in jail and was permitted to leave Austria, but only after he transferred his assets, including the Nobel Prize money held in a Swedish bank, to the Germans. It is not clear whether the outside support for Loewi was responsible for his release because at the time there was a policy of ''enforced emigration'' in Austria. The policy was to make Jews feel so threatened that they were willing to surrender everything they owned if they were permitted to leave.

In September 1938, Loewi left Austria for England. He had cabled Dale, who

[14] Cannon's nominations for the Nobel Prize were made available through the Nobel Archives.

invited him to stay at his home. Loewi lived with Dale for several weeks until he found temporary positions at the Université Libre in Brussels and at the Nuffield Institute in Oxford. Luckily, he was in England when the Germans marched into Belgium. In 1939, Loewi accepted a position as a research professor in pharmacology in the Medical School of New York University (NYU).

At NYU, Loewi mainly occupied himself in tying up some loose ends of the problem on which he had previously worked. He was invited to give many lectures, and he received numerous awards and honors, but none pleased him more than being elected to foreign membership of the Royal Society. He became a U.S. citizen, and during the ensuing years he regularly spent summers at the Woods Hole laboratories in Massachusetts, where he had many friends. In 1958, Loewi had a serious fall, breaking his pelvis. Friends rallied around him and transported him to Woods Hole for several summers. Dale last saw Loewi during a visit to New York in October 1959. Loewi was pretty much confined to his New York apartment, having to push himself around with a walker, but Dale reported that Loewi's mind was as alert as ever.

Loewi died in his apartment in 1961 in a way that he would have approved. A friend from Woods Hole had sent him a lobster, and he consumed it all with much pleasure, sharing a bottle of fine wine with his nurse–housekeeper. The following morning, while engaged in a lively discussion with a friend, Loewi suddenly went silent. He died at the age of 89 years on Christmas Day. Six months later, Loewi's friends aranged to have his ashes buried in the churchyard at the Woods Hole laboratory. Loewi had retained his interest in the arts throughout his life. It is fitting, therefore, that his last publication was titled ''A Scientist's Tribute to Art'' (Loewi, 1958). The *New York Times* obituary quoted from an earlier *Saturday Review* article on Loewi: ''The years of Dr. Loewi have so overflowed with devotions to art, literature, music, mountain climbing, human fellowship, and the science of biology that only a book could tell his adventures'' (*New York Times*, 1961, p. 21).

POSTSCRIPT ON DALE

Although no longer actively engaged in research after 1938, Dale's interest in research was sustained at a high level, and he continued to attend professional meetings until he was 90 years old. It was said that his introductory comments or summarizing remarks at international symposia often did more to clarify the progress made and the important problems remaining than did any of the individual presentations. He remained director of the National Institute of Medical Research until 1942 and then became director of the Davy–Faraday Laboratory at the Royal Institute. He had been a trustee of the Wellcome Trust Fund since 1936, and from 1946 onward, as chairman of the fund, he devoted considerable time to awarding research grants and scholarships. During World War II, Dale served on the Advisory Committee to the War Cabinet, for which he received the Order of Merit in 1944. Dale also served as president of the Royal Society from 1940 to 1945.

Dale remained a prolific writer. He wrote 26 articles between 1937 and 1942 alone and kept up a steady pace almost until his death. His later articles were mostly on historical aspects of science, tributes to scientists, obituaries of colleagues, and comments on education. A complete list of Dale's publications is provided in a biographical memoir (Feldberg, 1970).

Dale was always concerned with world affairs, and after the war he wrote several articles, published in the *Spectator*, on the implications of the atomic and hydrogen bombs and his thoughts after reading an account of the bombing of Nagasaki, Japan.

In 1903, Dale had spent 4 months working in Paul Ehrlich's laboratory in Frankfurt. Ehrlich had received the Nobel Prize in 1910, and in 1912 the Germans, who took pride in another German winning this prize, named a Frankfurt street the Paul Ehrlich Strasse. Because Ehrlich was Jewish, the Nazis had removed his name from the street in 1938, 27 years after his death. When the war ended, Dale used his influence to have the street once again named after Ehrlich. Dale was always willing to express his objections to any political intrusion into science, and he later resigned his membership in the Academy of Science of the Soviet Union in protest over the persecution of geneticists during the Lysenko period. Dale died in 1968 at the age of 93 years.

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