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Sir Henry Hallett Dale and the Acetylcholine Story

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Living organisms depend upon their nervous systems to provide integrated responses to environmental stresses. Due to Charles Sherrington we recognize that the key to understanding nervous integration resides not in the body of the cell or in its long filamentous axon, nor in the ionic shifts involved in electrical propagation, but rather in the subtle events occurring at the gap between nerve cells. Similarly, coordination of muscle and gland function depends on the junction between the nerve and the effector organ. Sherrington applied the term "synapse" to the region of contiguity of two nerve cells. I use the term with its more general connotation, which includes neuromuscular and neuroglandular junctions.

In December 1970, Bernard Katz, Ulf von Euler, and Julius Axelrod shared the Nobel Prize for their role in unraveling the intricacies of synaptic transmission. Fresh excitement has pervaded this field of research as evidence emerged suggesting that the synapse may play a critical role in longterm learning and memory. No matter how molecular or organismic the investigations, they all take for granted the mechanism of chemical transmission.

Sir Henry Hallett Dale deserves the credit for establishing chemical transmission as the core of synaptic theory. Before his work the synapse was considered a region where electrical currents simply jumped from a nerve to an effector cell. Dale, along with Otto Loewi, demonstrated that, in general, electrical information crosses synaptic gaps only indirectly via a chemical intermediary.

Dale's neuropharmacological work was only one facet of an incredible career that included many areas of medical and physiological research as well as the training of a school of younger scientists. W. S. Feldberg, in his enchanting biographical memoir on Dale recalls Dale's experimental attitude, one that he inculcated in his students: "You must work like an astronomer. Prepare for weeks,

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for months, if necessary, for years, until the method is working to perfection, then do one experiment, perhaps two—and publish your results"(1).

THE STATE OF THE ART BEFORE DALE

Peripheral Synapse

Claude Bernard provided the first evidence that transmission across the synaptic junctions might involve forces different from simple electrical transmission. In fact, proponents of electrical transmission always recognized that Bernard's experiments with curare in 1854 highlighted an inadequacy in their theories. Bernard's preparation involved a peripheral muscle and its nerve in each of the hind legs of a frog(2). By ligation, he blocked the circulation to one of the legs. He then injected curare anteriorly. The frog went limp. Pinching an anterior part of the body elicited no local response; it did, occasionally, result in twitching of the leg protected from curare by ligation. This showed that the curare had not affected the sensory but rather the motor nerves. The tissue of the muscle itself also was unaffected; the curarized leg muscle still twitched in response to electrical stimulation. And, although stimulating the nerve in the curarized leg produced no response, the nerve to the ligated leg was still functional, despite exposure to curare for most of its length. Bernard concluded that curare affected the motor nerve, exclusively, at its peripheral connection to skeletal muscle, implying that this region was pharmacologically different from the rest of the nerve. Electrical transmission would not require, nor could it explain, this specialization.

Since it was known that both nerve and muscle were electrically excitable, it was tempting to ignore Bernard's evidence and continue to explain neuromuscular transmission as a direct electrical discharge between nerve and muscle. The first critical evaluation of electrical transmission was made by Emil Du Bois-Reymond in 1877. He saw no way for the electrical transmission theory to explain localization of the electrical discharge or the latency between nerve stimulation and muscle reaction. He postulated chemical transmission as the only possible alternative and supposed that ammonia or lactic acid was the transmitter(3,4). Little attention was paid to this new suggestion.

Meanwhile, Louis Lapique, Bernard's successor at the Sorbonne, offered his variation on the electrical transmission theory. Though wrong, it was quite ingenious, and survived for many years as the core of several other electrical transmission theories. Using many types of excitable tissues, he plotted voltage necessary to overcome the threshold of the tissue as a function of the duration of the stimulus. To his joy, the results in all tissues were fit by exactly the same hyperbola; the time axis just had to be divided into different units for different tissues. This characteristic unit was represented by the tissue's "chronaxie"(5). A nerve could stimulate only the muscle with which it was "isochronic." So, in terms of chronaxie, "slow" muscles were innervated by "slow" nerves. Paralysis resulted from an imbalance of chronaxies. Thus, curare lengthened the chronaxie

of a muscle until it no longer matched that of its own motor nerve. Lapique's theory was considered by many a brilliant breakthrough until it was finally discredited in the 1940's.

Central Synapses

The early work had focused only on peripheral nervous connections. No difficulty was encountered in explaining transmission across cellular gaps in the central nervous system since all cells were considered continuous. According to the reticular theory which Joseph von Gerlach proposed in 1871 and Camillo Golgi, in particular, espoused, the central nervous system was a net in which cells anastomosed freely with each other. Wilhelm His and August Forel soon challenged this concept, but not until Ramon y Cajal performed his beautiful histological and embryological studies did neurophysiologists begin to accept each nerve cell as an independent unit. Even then Golgi's views were considered authoritative and, since Cajal and Golgi shared the podium for the Nobel Prize of 1906, and since the anatomical evidence was not conclusive, the debate lingered.

However, the neuronal theory did receive forceful and convincing support from Sherrington who delineated the pregnant physiological conclusions that could be drawn from it. He realized that an intercellular gap could explain many of his observations on the spinal reflex. In the 1897 edition of Michael Foster's *Text Book of Physiology,* Sherrington clearly stated his opinion of the separateness of nerve cells:

> "So far as our present knowledge goes we are led to think that the tip of the aborescence (at one end of a nerve cell) is not continuous with but merely in contact with the substance of the dendrites or cell body on which it impinges"(6).

He defined this special connection as a synapsis, from the Greek words implying "a process of contact"(6,7) and recognized this region as possibly responsible for unidirectional flow of impulses, as well as through valving mechanisms, an area to maintain order in the anatomically chaotic spinal cord. In his Silliman lectures of 1903–04, Sherrington emphasized the role of the synapse in integration, illustrating the final common path as a field for play from many synapses. Here, the motor neuron could be controlled by the summing of excitatory inputs and the antagonization of inhibitory effects. He envisioned latency as "explicable by the minimal quantity of transmitted influence necessary to give detectable effect"(8) [ideas compatible with later chemical transmission theories]. Similarly, fatigue and after-discharge could be ascribed to synaptic characteristics. And in 1925, he proposed that "the arrival of (the impulse) . . . at the central terminals of the afferent fibre is an essential element in the central excitation process . . . There the nervous impulse resulting directly from the external stimulus may be regarded as ending . . ."(9). Sherrington's emphasis on the neuron theory, using physiological synaptic centers, strongly buttressed Cajal's interpretation.

There was little doubt about the mechanism of transmission between the cells of the central nervous system: because of the requisite speed of impulse propagation, electrical "sparks" were the only feasible answer. Many years elapsed before techniques that were suitable to test this obvious explanation were devised and used to show that it was wrong. Understanding synaptic connections to skeletal muscle motor nerves also had to await better techniques of isolation and perfusion. So the story of chemical transmission begins with the peripheral synapses of the autonomic nervous system.

The Autonomic Nervous System

The labyrinthian complexities of the autonomic nervous system were first unraveled by two disciples of Michael Foster, who were later to be Dale's mentors. Walter Holbrook Gaskell discovered the general nature of the sympathetic system and John Newport Langley delineated the functions of its specific sections. In the history of chemical transmission, Langley is especially remembered for detailed work showing that sympathetically innervated glands and muscles responded identically to either adrenaline (epinephrine) or to stimulation of the sympathetic nerves(10). Later workers noticed several exceptions to this rule. Langley tendered no explanation for either the rule or the exceptions.

Thomas Renton Elliott made the first attempt to combine Langley's pharmacological and neurophysiological observations into one picture, that of chemical transmission. In 1904, Elliott postulated that Langley's work, and his own extensions of it, might be more than a pharmacological idiosyncrasy of adrenaline, and might in fact indicate a mechanism by which nerves transmit information to end organs. Noting that adrenaline's action could be limited to the peripheral ends of the nerve, and:

> ". . . since adrenalin does not evoke any reaction from muscle that has at no time of its life been innervated by the sympathetic, the point at which the chemical excitant is received . . . is perhaps a mechanism developed out of the muscle . . . the function of which is to receive and transform the nervous impuse. Adrenalin might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery" (11).

Although Elliott designated the wrong compound as sympathetic transmittor (it is really the closely related norepinephrine), he had foreseen the role of chemical transmission in unifying nervous action.

Unfortunately, some staid colleague must have shaken Elliott's faith in his idea, because his paper in 1905, dealing again with the correlation between adrenaline's action and the stimulation of the sympathetic chain, entirely avoided the issue(12). Elliott's delayed impact was duly recognized by Dale, who inscribed his book *Adventures in Physiology* "To T. R. Elliott, who had so much to do with the beginning of these adventures, and, long after they have ended is still my counsellor and friend"(13).

Walter Dixon attempted to use Elliott's logic and extend Elliott's arguments to the other half of the autonomic system, the parasympathetic nerves. He did an

experiment that, in its reincarnated form, won Otto Loewi a Nobel Prize 30 years later. Dixon isolated from a heart under vagal stimulation a compound that could slow another heart(14). He was unable to establish the identity of his active substance; he had no bodily compound to serve his purpose as adrenaline did Elliott's. Dixon guessed that it might have been similar to muscarine, a drug isolated from mushrooms, and which later figured in Dale's writing. In all probability, Dixon's compound was choline, a substance found in animals that like muscarine can mimic some parasympathetic actions. With Dixon's attempt, the chemical transmission theory was laid to rest, not to be revived until Loewi's work in 1921.

THE YOUNG HENRY HALLETT DALE

Meanwhile, another Cambridge student, H. H. Dale, had finished his first neuroanatomical research with Langley. When, despite this work, Dale was refused a fellowship, he moved to London, where in 1903 he qualified for a medical degree. He was immediately given an opportunity to move back to the laboratories as the George Henry Lewes student, working with Ernest H. Starling and William M. Bayliss. In 1904, Dale, as he explains himself, "at the age of 29, (and) faced with what then seemed a rather bleak academic prospect"(13), succeeded, with Starling's recommendation, in joining the Wellcome Physiological Research Laboratories. Within a year and a half he became its director. Although he approached the job with trepidation, he accepted, motivated "not only by a conscious desire to earn a marrying income, but also by an instinctive feeling that it would be a good thing for me, at that stage, to be obliged to stand scientifically on my own two feet, to find my own problems, to plan my own experimental attacks upon them . . . and to make my own mistakes"(13).

To give him a chance to fulfill all of these objectives, Wellcome assigned Dale a pharmacological problem–to alleviate confusion surrounding the drug ergot. Dale was none too happy. Pharmacological research was then quite confused and primitive, a state epitomized by research on ergot, a fungus that grows on rye. Since 600 B.C. it had been used as an oxytocic drug. Although the Greeks and Romans noticed some problems with its use, it was not until the Middle Ages that epidemics of poisoning were recognized to be due to ergot. By Dale's time it had begun to fall from favor, but its chemistry remained obscure(15). Dale was among the first to isolate an active compound, ergotoxine, from ergot. His initial studies at the Wellcome consisted of testing this and other extracts of ergot on the blood pressure of the cat. And he would have finished this dull project in short order had he not been given the opportunity to study the effects of adrenaline on his preparation. After observing results so curious that the young investigator returned the samples of adrenaline as unfit(13), Dale yielded to facts and published(16). He found that ergot specifically antagonized adrenaline. Wherever Langley and Elliott had observed identical reactions to adrenaline and to sympathetic stimulation, ergot reversed both reactions. Synapses im-

mune to adrenaline, in the ganglia, skeletal muscles, and the parasympathetic system, were equally unaffected by ergot or its extracts. Although Elliott participated in the experiments, Dale did not use the work to renew Elliott's view that adrenaline served as transmitter of sympathetic stimuli; he did state that his effects were "confined to the myoneural junction"(16). His careful observations also indicated that adrenaline and sympathetic stimulation did not elicit quantitatively equal results, and that adrenaline's effects were far more easily abolished by ergot. In his retrospective critique of this paper Dale blamed himself for not concluding from his work that Elliott's chemical transmission theory must have been right in principle, but that quantitative differences between adrenaline and sympathetic stimulation indicated a close analog, rather than adrenaline itself, as transmitter(13). Be that lapse as it may, this work launched Dale into further efforts at elucidating functions at the myoneural junction.

In 1913, an argument with Walter B. Cannon led Dale to elaborate his earlier work on ergot. The substance of the paper simply reaffirmed his conviction that adrenaline acted at the myoneural junction. It is interesting to note that Dale had swung even further from Elliott's suggestion, and entangled himself in a morass explaining the effects of adrenaline and ergot as due to qualities of hidden motor nerves.

ACETYLCHOLINE

In 1906, Reid Hunt and Rene Taveau of the U. S. Public Health Service announced that while running a gamut of tests on choline derivatives they had discovered that the artificial acetyl derivative of choline had incredibly potent physiological effects. It was a "hundred times more active in causing a fall of blood pressure than is adrenaline in causing a rise"(17). They postulated, incorrectly, that it affected the force of the heart beat.

A paper by Dale in 1914 drew on these observations in studying "The Action of Certain Esters and Ethers of Choline, and Their Relation to Choline"(18). Even though the next chapter in the chemical transmission story was 7 years away, I believe that this was Dale's most important work. It outlined the effects of acetylcholine at various types of peripheral synapses. These observations served as the basis for the future analysis of acetylcholine's role in chemical transmission. Once again, chance originated the research, and once again, ergot was the protagonist. Dale recalled later that ". . . what

> was supposed to be an ordinary liquid extract of ergot had been sent to me for a routine control of its activity. When a conventional dose of this had been injected into the vein of an anaesthetized cat, it caused a profound inhibition of the heart beat; I suspected, indeed, a fatal accident of injection . . ."(13).

But repeated trials confirmed his results and indicated "the presence in it (the ergot) of an unusual constituent, with actions suggestively resembling those of

muscarine"(13). The unknown substance, apparently an ester, was too unstable to survive purification procedures. Noting the similarity between his observations and those of Hunt and Taveau, Dale compared his substance to a fresh batch of acetylcholine. On the basis of physiological effects they appeared identical. At this time Dale was not sure how acetylcholine got into his ergot. In retrospect he recognized his debt to some errant bacterium, excreting acetylcholine as a waste product(13).

Dale began an investigation of acetylcholine's effects. He found that he could divide them into two groups: (1) the "muscarine-like" effects and (2) the "nicotine-like" ones.

He summarized the muscarine effects

> "as a reproduction of the effects of stimulating the [parasympathetic] nerves . . . the constrictor effect of the third cranial nerve on the pupil [for example] . . . the inhibition of the heart, constriction of the bronchioles, and contraction of the muscular walls of the alimentary canal produced by the vagus . . ."(18).

The potency of acetylcholine in eliciting these responses was quite remarkable; for example, "complete stoppage of the ventricle being obtained with one part of the ester in 100 million of solution"(18). However, the parallelism to the parasympathetic nerves was incomplete. For example, the sweat glands were innervated only by the sympathetic system, but could be activated by acetylcholine or muscarine. In general, several common features characterized the muscarine-like effects: they were always peripheral in nature (often mimicking stimulation of parasympathetic nerves), they were annulled by atropine, but unaffected by massive doses of nicotine that destroyed the brain and the spinal cord.

To observe the nicotine-like effects, Dale had to suppress the muscarine-like ones with atropine. Acetylcholine then caused phenomena similar to those seen by Langley when mapping the bodily response to nicotine, including a rise in blood pressure that was blocked by doses of nicotine large enough to destroy the ganglia. Later workers showed that other nicotine-like effects of acetylcholine, as Dale had guessed but not directly observed, included stimulation of voluntary muscle and the adrenal medulla, as well as the ganglionic synapses.

Dale ascribed the tremendously large doses of acetylcholine needed to produce effects in the intact animal, as opposed to isolated organs, to the rapid hydrolysis of the ester by natural esterases. The consequent evanescence of pharmacological action was later to play a critical part in the chemical synapse hypothesis.

There seemed to be strong evidence that acetylcholine had a role:

> ". . . its action surpasses even that of adrenine, both in intensity and evanescence, when considered in conjunction with the fact that each of these two bases reproduce those effects of involuntary nerves which are absent from the action of the other, so that the two actions are in many directions at once complementary and antagonistic. (It) gives plenty of scope for speculation. On the other hand there is no known depot of choline derivatives" (18).

Many years later Dale realized that this

> ". . . discussion of the 'biological similarity,' as shown by their common responsiveness to acetylcholine, between ganglion cells and "nerve-endings," voluntary motor as well as cranio-sacral (parasympathetic) involuntary, would have gained much by reference to the very penetrating suggestions made many years earlier by Elliott . . . I must have read this discussion . . .; but I cannot have had it in conscious memory when I wrote this one on the choline esters. . . . Even more curious, however, is the fact that, by this time, both Elliott and I seem to have become shy of any open allusion to the 'chemical transmission' theory, which had originated ten years earlier. . . . As I have elsewhere suggested, the stage was now set for it, and only a piece of direct evidence was needed to ring up the curtain . . . produced, in 1921 and the following years, by another friend of ours, Otto Loewi, in Graz"(13).

So by 1914 two substances, adrenaline and acetylcholine, were known to faithfully mimic the functions of the sympathetic and parasympathetic autonomic systems respectively. But, of the two, only adrenaline had been shown to exist in the body. It was not until 1929 that Dale demonstrated that acetylcholine was also a natural bodily constituent.

Dale spent the First World War working for the Medical Research Committee. While resettling afterwards, he received news of Loewi's outstanding, but simple, demonstration of chemical transmission of vagal impulses to the heart(14). Loewi suspended two beating hearts filled with Ringer's solution. One heart had its vagus nerve and the other did not. He stimulated the vagus to one heart and collected the reservoir of Ringer's solution, which he transferred to the second heart. This solution inhibited the heart as potently as did vagal stimulation itself, meaning that stimulation of the vagus to the first heart released some chemical transmitter from the nerve. The more that Loewi tested his "Vagusstoff," the more that it appeared to be acetylcholine. Atropine antagonized its effects, just as it did acetylcholine's. An atropinized heart produced as much Vagusstoff as did a normal heart; the action of the transmitter itself was inhibited. The Vagusstoff was an ester, easily hydrolyzed, but protected by the action of eserin, which seemed to inhibit the cholinesterases present in muscle and in blood.

Loewi's findings were soon extended to other peripheral parasympathetic nerve endings. For example, his student, Erich Engelhart, found Vagusstoff in the vitreous fluid of an eye after stimulation of the oculomotor nerve(14). All evidence indicated that the Vagusstoff was acetylcholine. Nevertheless, acetylcholine was known only as a synthetic drug so many hesitated to accord it a role in the natural bodily economy.

SUBSTANTIATION OF THE NEW THEORY

As tempting as it was to equate Loewi's Vagusstoff with acetylcholine, the latter had never been isolated from an animal organ. It was known only as an artificial derivative of choline or as a byproduct of plant metabolism. Loewi obtained samples too small for positive identification.

Dale, capitalizing on another accident, demonstrated the presence of acetylcholine in animals. While mapping the distribution of histamine in various

organs of the body, Dale and Harold W. Dudley found potent choline action in their alcohol extraction of horse spleen(20). The action derived from an unstable choline derivative whose physiological effects (e.g., depressor activity) could be matched by proportional doses of acetylcholine. Further work confirmed its identification as acetylcholine.

Dale had no idea why horses store acetylcholine in their spleen. But its presence in living animals convinced him of the plausibility of acetylcholine playing a critical role in chemical transmission. He picked up the flag tentatively waved by Loewi and others and began to extend the concept of chemical transmission to the entire parasympathetic system. He started by explaining several pharmacological anomalies plaguing advocates of the new concept.

One such problem involved a discrepancy in the effects of atropine. The drug always blocked reactions to extrinsically applied acetylcholine (even by injection) but often was ineffective in blocking actual parasympathetic stimulation. Theoretically, if atropine blocked effects of acetylcholine, it should also block those of the nerve. Without evidence at hand, Dale postulated in his Croonian Lecture of 1929 that often atropine simply could not get at the area of acetylcholine's release. The transmitter released by nerve stimulation might be "peripherally liberated by nerve impulses in such intimate relation to the receptive structures that atropine is relatively ineffective in hindering its action"(21). Loewi accepted Dale's explanation of the atropine anomaly. "Daring as this hypothesis at first may seem," Loewi suggested, "it alone enables us to conceive the working mechanism of the parasympathetic nerves as uniform"(14).

Another anomalous behaviour explained by Dale as part of the acetylcholine story was the "Vulpian" response, first observed by Alfred Vulpian and Jean Marie Philippeaux in 1863. They cut the motor nerves to the voluntary muscles of the tongue and found that the tongue became newly sensitized, exhibiting a strange, slow response to stimulation of the chorda tympani, a parasympathetic nerve usually producing only vasodilatation or secretion from salivary glands. Others noticed similar behavior in skeletal muscle from other parts of the body. Sherrington, for example, caused degeneration of the motor nerves to the hind leg; stimulation of parasympathetic sensory nerves in the sciatic, normally responsible for vasodilatation in the leg, elicited an abnormal slow contraction of voluntary muscles there as well(22).

Several efforts had been made to explain these phenomena. Some investigators thought that a muscle deprived of its nerve simply became over-sensitized to impulses travelling along the axons of nearby nerves. Langley made some vague statements about effects of metabolites on voluntary muscle.

Dale and his colleagues suspected humoral agents, specifically their new baby, acetylcholine. All these observations became intelligible when it was assumed that: (1) acetylcholine was released at all peripheral endings of the parasympathetic nerves, and (2) that muscles, when deprived of their motor nerves, acquire a new sensitivity to acetylcholine released nearby. Indeed, all tests indicated just such an explanation. Acetylcholine itself, produced similar contractions from

denervated muscle. Eserine prolonged responses either from stimulating the vasodilator nerves, or from injecting acetylcholine. Atropine blocked the effects of the applied acetylcholine(23).

By 1930, a strong case had therefore been made for humoral transmission in at least one group of nerves, the peripheral ends of the parasympathetic system. Acetylcholine seemed the most likely candidate for transmission. However, isolation of acetylcholine at the point of release was rarely possible in quantities great enough for positive identification. So, in 1933, H. Chang and J. H. Gaddum postulated the six criteria that henceforth were used to differentiate acetylcholine from other similar substances(24). They made use of all the characteristics mentioned above: its instability, potentiation by eserine, and muscarine-like and nicotine-like effects. Their chief contaminant was choline, which could mimic acetylcholine's actions, but with far less potency. In general, the most convincing proof involved an assay of the extract for acetylcholine by different means, all indicating identical concentrations. For example, Dale would look for similar ratios of extract activity:control amount of acetylcholine activity on cat's blood pressure and on isolated leech muscle.

EXTENSIONS OF THE THEORY

It was becoming obvious that more than the parasympathetics operated via acetylcholine. As Dale had pointed out in 1914, some end organs innervated by the sympathetic system, such as the sweat glands, responded to acetylcholine. The possibility that acetylcholine's nicotine-like effects on skeletal muscle and in ganglionic synapses also indicated such humoral transmission was under investigation by Dale's students. To attempt to resolve some of the complications, Dale suggested a new classification of nerves, based on chemical transmission. Nerves using acetylcholine were called "cholinergic"; nerves transmitting by the (still unidentified) adrenaline-like substance were called "adrenergic"(25).

In the absence of direct chemical evidence of acetylcholine's presence, and in the light of several parasympathetic effects that were resistant to atropine, many investigators were reluctant to credit the ester with a critical role. An often-cited example of inconsistent behavior involved vagal innervation of the stomach. Unlike the branch of the vagus to the heart, the branch to the stomach was immune to atropine.

Dale repeated previous investigators experiments on the stomach using first an isolated perfusion network and, second, an *in vivo* protocol. In both cases eserine was added, and then the vagus stimulated. The effluent contained high concentrations of an active substance, indistinguishable from acetylcholine(26). This reinforced Dale's lack of faith in the atropine reaction as an absolute criterion for acetylcholine release.

A second apparent inconsistency tackled by Dale was how the sweat glands, innervated only by the sympathetic system, responded so well to the parasympa-

thetic transmitter, acetylcholine. Similarly, vasodilatation in the cheeks, although innervated by the sympathetics, showed no response to adrenaline. Dale guessed that the anatomical division into sympathetic and parasympathetic simply did not always follow adrenergic and cholinergic lines. By perfusing a cat's foot artificially with Ringer's solution, Dale and Feldberg collected acetylcholine in the venous effluent after stimulation of the sympathetic nerves to the sweat glands. So some fibers that were part of the sympathetic system were found to be mediated by acetylcholine(27). As obvious as that explanation seems now, an observer present at the conclusion of these experiments recalled Dale's sense of joy and triumph at their outcome, when he exclaimed, "and if the others don't believe it, let *them* repeat the experiment"(28).

SOUP VS. SPARK

Proponents of the electrical synapse still survived, and throwing off the clumsy mantle of Lapique, began to raise their heads. Grudgingly they accepted the fact that peripheral stimulation of parasympathetic nerves caused release of acetylcholine. But, V. E. Henderson and M. H. Roepke, for example, found two phases of contraction in the bladder—a tonic one, mediated by acetylcholine, and a quick contraction, mediated in some other manner(29). Similar responses were found in several other muscles. In his review of the subject(30) Eccles pointed out that the quick responses often seemed to be independent of eserine. He postulated an action-current hypothesis whereby the close contact of the nerve to local receptors on the effector organ allowed a direct electrical connection. Eccles, however, saw that such a mechanism could not explain the synaptic delay, nor account for, teleologically, the release of acetylcholine. Not until Paul Fatt and Bernard Katz, in 1950, introduced the method of recording intracellular neuromuscular potentials was there a technique to settle the dispute. Acetylcholine could then be shown to alter the postsynaptic potential in milliseconds, rapidly enough to account for the quick reaction seen in some muscles.

In the 1930's the question of speed became especially important as Dale attempted to extend the theory of chemical transmission to ganglionic synapses and voluntary muscle. The muscarine responses of long latency, slow rise, and slow fall had been easy to conceptualize in the framework of chemical transmitters. This was not true of the nicotine responses. But, as Dale observed, it

> ". . . was difficult to suppose that ganglion cells and voluntary muscle fibres would be endowed with this sensitiveness to acetylcholine, if the only physiological function of the latter were the transmission of the effects of autonomic nerves to involuntary muscle and gland cells. On the other hand, the transmission of the excitatory process across ganglionic synapses, or at voluntary motor nerve endings had the appearance of a direct, unbroken, physical propagation. . . . If acetylcholine were to intervene at all in the transmission of these rapid and individual excitatory events, it could only do so by appearing with a flash-like suddenness . . . and (must) . . . vanish almost as quickly as it appeared"(31).

CENTRAL GANGLIA AND SKELETAL MUSCLE

E. D. Adrian seems to have been the first to propose that humoral agents were involved in the synaptic connections of the central nervous system. He employed arguments from Sherrington's work on the unique nature of the reflex, including refractory period, summation, and inhibition(32), features that later suggested the same possibility to Sherrington(9).

The first experimental proof came from Dale's students, Chang and Gaddum(24). In a careful analysis of the distribution of acetylcholine in the body, they found surprisingly high concentrations in the sympathetic ganglia. W. S. Feldberg, one of Dale's most distinguished disciples, found that acetylcholine appeared after electrical stimulation of nerves to the adrenal medulla, causing an adrenaline discharge. Since the cells of the adrenal are morphologically identical to postganglionic cells, Feldberg's experiment showed that acetylcholine could be involved in ganglionic transmission(33). In the next article in the same journal, Feldberg and Gaddum, using a method devised by A. W. Kibjakow, extended the observations to include the synapses of the superior cervical ganglion. All pharmacological tests indicated that stimulation of the preganglionic nerve (the cervical sympathetic) caused release of acetylcholine. They concluded that preganglionic synapses belong to the cholinergic system(34).

The chief problem was speed. John Eccles, for example, doubted the ability of cholinesterase to destroy the ester quickly enough(30). Also eserine did not seem to potentiate a ganglionic volley, as it should if it protected the acetylcholine. There was no technique to deal with the first question; Dale dismissed the second as a function of the pharmacological quirks and inconsistencies of eserine. He did not convince Eccles.

And Eccles had strong support, especially when criticizing chemical transmission theories applied to the central nervous system. As late as 1939 at the neurophysiological congress, bitter argument still raged. For example, Joseph Erlanger, citing his work by which stimulating electrodes were placed at various nodes along a nerve axon, argued in favor of electrical transmission: "If an inactive stretch of fiber over 1 mm in length does not stand in the way of electrical transmission of the impulse, is it reasonable to maintain that the discontinuity at a synapse will stop such transmission"(35). Rafael Lorrente de No wryly prefaced his discussion of the old Wedensky theory of electrical propagation, commenting that "the action currents of nerve impulses arriving at the synapse may prove not to be the agents for synaptic transmission, but everything happens as if they were"(36). Detlev Bronk, on the other hand, adopted the straddle position. "I have no desire," he worried, "to defend either the acetylcholine hypothesis or the theory of excitation by circulating currents. . . . If it be necessary to do more at this time than describe the phenomena of transmission . . . , I would argue for a pluralistic theory"(37).

Meanwhile, Dale's laboratory found increasing experimental proof for aceytlcholine intervention in central synaptic systems. Until the 1950's, however, and the era of intracellular recordings, they convinced mainly their friends.

Dale's last personal contribution proved that skeletal muscle motor nerves also were cholinergic. Dale, with Feldberg and Vogt, used an artificially perfused, eserinized muscle preparation. They succeeded, for the first time, in collecting enough acetylcholine from a voluntary muscle to be able to characterize it as due to stimulation of the motor nerves. Dale later recalled the tremendous technical difficulties hindering work on voluntary muscle:

> "It might have been expected . . . that it would be easier to detect the release of acetylcholine at motor nerve endings in a large muscle than at synaptic endings in a tiny ganglion. In fact, however, . . . the experiment on the ganglion was much the easier. . . . In the case of the bulky muscle, the perfusion had to be rapid to keep the tissue alive, the motor endings were widely scattered, oedema set in early and, . . . excitatory transmission from nerve to mucle soon failed . . ."(13).

He had trouble, however, convincing the doubters that the acetylcholine itself could account for the strength and speed of the characteristic skeletal muscle twitch. Direct application of acetylcholine gave inconclusive results. Some muscles from reptiles and birds responded with inappropriately weak contractions. Mammalian muscle was completely unaffected.

Dale believed that this failure was due to the nonspecific application of acetylcholine to the excised muscle. He expected that extrinsic application could not mimic the coordinated release to restricted areas that resulted from proper nerve stimulation. Through the efforts of G. L. Brown, Dale's team succeeded in a sudden injection of acetylcholine into the empty vessels of the excised muscle; it reached all nerve endings almost simultaneously. Dale described the results colorfully in his Nobel oration in 1936:

> "If 1γ of acetylcholine, for example, dissolved in 0.1 cc of Ringer's solution, is thus injected suddenly into the artery supplying the frog's gastrocnemius, the surface of the muscle, covered with its glistening aponeurosis, shows immediately the ripple and shimmer of innumerable, unsynchronized contractions, propagated along the fibres and fascicles of the muscle; at the height of the effect a tension of several hundred grams is developed; and the electrical record gives decisive evidence that this response is . . . asynchronous . . ."(38).

Dale demonstrated potentiation by eserine, reemphasizing the role of cholinesterases in a possible mechanism for rapid termination of the effects of acetylcholine at the end of the impulse.

Dale completed these acetylcholine studies in 1936, the year that he and his friend, Otto Loewi, were awarded the Nobel Prize. By the time of his retirement from active research, his humoral transmission theory was well on its way to general acceptance. Later workers confirmed and refined his revolutionary paradigm. Histological and physiological studies showed a considerable specialization at the synapse, where the transmitted "spike" was transduced into a chemical messenger. This transmitter, acetylcholine, norepinephrine, or one of several amino acids, in turn instituted a postsynaptic potential, a local membrane activation that either caused or prevented spike propagation in the postsynaptic cell. There was no need to invoke electrical coupling.

In 1938 Dale was chosen as director of the National Institute for Medical Research. Even though he had retired by the end of the war, Dale took up a new crusade—against the scientific secrecy that had been initiated by war-time atomic research.

Meanwhile, Dale continued to receive international recognition for having chaperoned the humoral transmission theory through its most difficult days, when it was considered a radical and unsubstantiated theory. In his own country Dale was accorded the supreme scientific honor, the presidency of the Royal Society, which he held from 1940 to 1945. At the time of his death in 1968 at the age of 93, Dale had seen his "radical" ideas accepted as the basis for the most conservative of neurophysiological research.

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