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# Henry Dale and the discovery of acetylcholine

E.M. Tansey

Wellcome Trust Centre for the History of Medicine, UCL, 210, Euston Road, London NW1 2BE, UK

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## Abstract

In 1936 Sir Henry Dale of London and Professor Otto Loewi from Graz shared the Nobel Prize in Physiology or Medicine for their work on chemical neurotransmission. This paper uses much unpublished archival material to augment an examination of Dale's work, from his discovery of naturally occurring acetylcholine in 1913, through to evidence of its role as a neurotransmitter at autonomic ganglia, post-ganglionic parasympathetic nerve terminals and the neuromuscular junction. **To cite this article:** *E.M. Tansey, C. R. Biologies 329 (2006).*

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## Résumé

**Henry Dale et la découverte de l'acétylcholine.** En 1936, Sir Henry Dale, de Londres, et le Pr. Otto Loewi, de Graz, se partagèrent le prix Nobel de physiologie et médecine pour leurs travaux sur la neurotransmission chimique. Cet article utilise, pour l'essentiel, des archives non publiées afin de dérouler l'examen des travaux de Dale depuis sa découverte de l'acétylcholine naturelle en 1913 jusqu'à la démonstration de son rôle de neurotransmetteur dans les ganglions du système autonome, dans les terminaisons nerveuses parasympathiques et à la jonction neuromusculaire. **Pour citer cet article :** *E.M. Tansey, C. R. Biologies 329 (2006).*

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## 1. Introduction

In 1936, Henry Dale of London and Otto Loewi of Graz shared the Nobel Prize in Physiology or Medicine “for their discoveries relating to the chemical transmission of nerve impulses”. The accumulation of evidence to suggest and to substantiate acetylcholine's role in neurotransmission, especially in Dale's laboratories, is the subject of this paper.

## 2. Acetylcholine and adrenaline before 1913

Acetylcholine was first synthesised in 1867, and forty years later it was shown to be an extremely potent physiological depressor “a hundred times more active in causing a fall of blood-pressure than is adrenaline in causing a rise.” [1] Adrenaline itself was isolated in 1894 by Edward Schäfer and George Oliver at University College London (UCL), and in 1904 a young Cambridge undergraduate Thomas Renton Elliott showed that in some circumstances adrenaline mimicked the effect of sympathetic nervous stimulation, his famous

E-mail address: [t.tansey@ucl.ac.uk](mailto:t.tansey@ucl.ac.uk) (E.M. Tansey).

Communication to the Physiological Society on the subject ending with a perhaps provocative, but certainly prophetic, sentence: “*Adrenaline might then be the substance liberated when the nervous stimulus reaches the periphery.*” [2] The precise contemporary meaning of these words is unclear, although they are usually interpreted as suggesting release of adrenaline from the nerve ending in response to the passage of a nervous impulse, and thus heralding the concept of chemical neurotransmission. At the time, however, they attracted little serious attention.

One of the few who was intrigued by Elliott’s work was his friend, Henry Dale. In 1904 Dale had accepted a novel opportunity to work for the pharmaceutical manufacturer Henry Wellcome, the sole proprietor of the firm Burroughs Wellcome & Co. and of laboratories devoted to bacteriological and physiological research, the Wellcome Physiological Research Laboratories (WPRL). After training in Cambridge and UCL, Dale was aware that professional scientific research was in its infancy in the UK, and the few full-time academic appointments then available all came with heavy teaching loads. Many men (there were few women) became pluralists, combining clinical practice, teaching and research activities as, when, and where they could. By accepting a position in the WPRL, Dale had practically unrivalled facilities for independent research – well equipped laboratories; professional colleagues, most notably the chemist George Barger, with complimentary skills and expertise; adequate support staff; no teaching duties; comparatively slight obligations to the company to test products for efficacy or quality; and considerable latitude to explore questions that interested him. Dale had no immediate research project of his own to follow, and readily acceded to Wellcome’s personal request that he work on the pharmacology of ergot of rye, then used in obstetric practice to hasten slow labour. Wellcome’s suggestion was based entirely on commercial considerations, as a rival, American, pharmaceutical company was successfully advertising and selling an ergot preparation with the innovative claim that it was ‘physiologically standardised’. Wellcome hoped that his new scientist would provide a preparation that was equally successful in the market place. It was to be an inspired suggestion. By the time he left the WPRL in 1914, recently elected to Fellowship of the Royal Society, the highest scientific honour in Britain, Dale’s work on ergot of rye had led him to the discovery of ergotoxine (an early adrenergic receptor blocker), histamine, acetylcholine and tyramine; the oxytocic properties of the posterior pituitary gland, and the basic mechanisms of anaphylaxis. Developing his own work on physiologically active chem-

icals, and inspired by Elliott’s experiments, Dale had also undertaken with George Barger a large study of the physiological actions of chemicals structurally similar to adrenaline, examining their effects on sympathetic nervous responses. For this group of chemicals, all of which mimicked endogenous neural stimulation to a greater or lesser degree, they coined the word ‘sympathomimetic’, and noted that of all the substances they tested one, noradrenaline, provided the closest ‘match’ to nerve stimulation [3]. Noradrenaline, however, like acetylcholine, was known only as a synthetic product and was not investigated further at that time.

### 3. Acetylcholine 1913

It was not until 1913 that naturally occurring acetylcholine was first isolated in the WPRL by Dale and his colleague Arthur Ewins [4]. Their discovery of acetylcholine as a rare contaminant in a batch of ergot was a ‘lucky accident’ that Dale was fond of claiming in later life [5]. Dale gleefully reported at the time to T.R. Elliott:

*“We got that thing out of our silly ergot extract. It is acetyl-choline and a most interesting substance. It is much more active than muscarine, though so easily hydrolysed that its action, when it is injected into the blood-stream, is remarkably evanescent, so that it can be given over & over again with exactly similar effects, like adrenaline. Here is a good candidate for the rôle of a hormone related to the rest of the autonomic nervous system. I am perilously near wild theorising.”* [6]

The following year Dale published an extensive paper on the pharmacology of an array of choline derivatives, and he noted two principal effects of acetylcholine: one that could be reproduced by injections of muscarine; and one reproduced by nicotine [7]. He offered no speculations to account for the functional differences, apart from commenting:

*“One may merely conclude that there is some degree of biochemical similarity between the ganglion cells of the whole involuntary system, and the terminations of voluntary nerve-fibers in striated muscle, on the one hand, and the mechanism connected with the peripheral termination of cranio-sacral involuntary nerves on the other. [...] There does not seem to be the same relation between the affinities of ganglion cells and of the terminal mechanism connected with the true sympathetic system.”* [8]

He listed several instances of close correspondence between the effects of acetylcholine and the parasympathetic nervous system, but noted:

*“The question of a possible physiological significance, in the resemblance between the action of choline esters and the effects of certain divisions of the involuntary nervous system, is one of great interest, but one for the discussion of which little evidence is available. Acetyl-choline is, of all the substances examined, the one whose action is most suggestive in this direction [of mimicking parasympathetic activity]. [...] On the other hand there is no known depot of choline derivatives, corresponding to the adrenaline [adrenaline] depot in the adrenal medulla, nor, indeed, any evidence that a substance resembling acetyl-choline exists in the body at all.” [9]*

By 1914 there was little direct evidence that acetylcholine was of any physiological significance. Wartime duties and responsibilities diverted much research interest and effort to other areas. Henry Dale took up a position at what would become the National Institute for Medical Research (NIMR), and after the war concentrated on other projects, most particularly the study of histamine [10]. It was work undertaken at the University of Graz in the early 1920s that re-kindled his interest in acetylcholine.

#### 4. Acetylcholine 1921–1929

In 1921 the Austrian pharmacologist Otto Loewi performed what is now regarded as a classic experiment, when he revealed that a denervated beating frog’s heart could be made to slow and stop by the passage over it of fluid taken from that surrounding an innervated heart that slowed and stopped in response to stimulation of its vagus. Loewi suggested that a chemical, which he called *vagusstoff*, was released from the vagus of the first heart upon stimulation and transferred to the second, denervated heart. Many were critical of his methodology, and Loewi demonstrated the experiment widely, his most notable success being at the 1926 International Congress of Physiologists, where his results provoked almost a ‘chain-reaction’ of renewed interest in the possibility of chemical involvement in neural functioning. Further work from Loewi and his colleagues showed that *vagusstoff* was a choline ester that was rapidly hydrolysed by an esterase, but he did not suggest that it was acetylcholine, which had still not been found to occur naturally in the animal body [11]. Although Dale was one of those intrigued and excited by *vagusstoff*,

and the possibility of its identification as acetylcholine, he remained concerned about acetylcholine’s apparent absence from the animal body, as he confided to a colleague at the beginning of 1929:

*“We are still struggling with the acetylcholine problem, which I mentioned to you when I saw you in the autumn. I am more and more convinced that the thing is there to be found, if only we can overcome the technical difficulties.” [12]*

Ironically, it was only a matter of months later that Dale and his chemist colleague at the NIMR, Harold Dudley did find acetylcholine as a natural constituent of the mammalian body [13]. It was particularly unexpected as they were actually looking for endogenous histamine. In the light of its natural occurrence, Dale and Dudley reviewed the suggestive evidence of an acetylcholine-like substance being involved in a range of physiological activities,

*“It appears to us that the case for acetylcholine as a physiological agent is now materially strengthened by the fact that we have now been able to isolate it from an animal organ and thus to show that it is a natural constituent of the body. [...] We feel, however, that its definite isolation from one organ has so far altered the position that, when an extract from, or a fluid in contact with the cells of an animal organ can be shown to contain a principle having the actions, and the peculiar instability, of acetylcholine, it will be reasonable in future to assume the identification.” [14]*

It was a momentous discovery, as Dale clearly acknowledged some years later:

*“This direct proof of the occurrence of acetylcholine, as a natural constituent of the animal body, provided an important stimulus to our renewed interest in the possibilities of its functional significance, and in the extension of this in directions previously unexplored.” [15]*

Despite Dale’s eagerness to pursue ‘directions previously unexplored’, limited resources, other research priorities and staff commitments meant it that it was to be some time before the next decisive steps were taken in his laboratory. These were heralded by the arrival of a refugee from Nazi Germany, Wilhelm Feldberg, in 1933.

## 5. The eserinated leech muscle: the key to acetylcholine

Less than a year later, Henry Dale wrote enthusiastically to Otto Loewi:

*“I cannot close this letter without saying what a joy it is to have Feldberg back here, however much one deplores the conditions, which have driven him out of his own country. His importation of the leech test, which he based on Fühner’s earlier observations, seems likely to be as stimulating for my own work on chemical transmission, as the expulsion of the Huguenots from France was for the British textile industry.”* [16]

The leech test revolutionised the analysis of the role of acetylcholine. The principal problem that continued to bedevil physiological studies on acetylcholine was the extreme transiency of its effects, as it was quickly hydrolysed by circulating acetylcholinesterase [17]. What Feldberg’s leech-muscle preparation did was to provide a simple, reliable method to detect that acetylcholine. As he himself acknowledged:

*“To make use of a metaphor: perhaps it was that I had brought with me a key that would open the doors. Dale and Gaddum seemed to know what lay behind them, but I had the key.”* [18]

That ‘key’ was the use of eserine, which inhibited the activity of the enzyme acetylcholinesterase. The technique was based on the discovery by the German pharmacologist Fühner, that when eserine was added to an organ bath in which a leech muscle was suspended, the muscle became extremely sensitive to acetylcholine, and he suggested the preparation as an assay system for eserine [19]. Feldberg merely reversed the procedure and used the eserinated muscle as a sensitive and simple assay for acetylcholine. The use of intravenous eserine in vivo work or in in vitro experiments by adding it to the perfusion fluid, reduced the circulating levels of acetylcholinesterase, increased the amounts of acetylcholine that survived in the venous effluent, circulating blood or perfusate, and thus made the accurate identification and measurement of acetylcholine possible. The contractions of the leech muscle, itself made super-sensitive to acetylcholine by treatment with eserine, in response to acetylcholine were recorded on a kymograph and could be calibrated and measured. The impetus of this powerful new technique provided the

major stimulus in Dale’s laboratory for several avenues of investigation to be followed.

A great deal of the work on acetylcholine emerged from Dale’s laboratory between late 1933 and early 1937. These included papers by Dale himself and by his co-workers, especially George Lindor (always known as ‘GL’) Brown, Marthe Vogt, John Gaddum, and Frank (always known as Hank) MacIntosh. Most notable, however, was Feldberg, whose name appeared on all twenty-four full publications that emerged from Dale’s laboratory between 1933 and 1937. The first direct experimental evidence for the role of acetylcholine in ganglionic transmission, at the parasympathetic post-ganglionic junction and at the neuromuscular junction of the voluntary nervous system was soon available. Their work showed that nervous stimulation liberated acetylcholine; that acetylcholine was not produced in the absence of such stimulation; and that injection of acetylcholine reproduced the effects of nervous activity (e.g., [20–24]). Laboratories around the world extended their examinations of every aspect of acetylcholine: its chemistry, physiology and pharmacology, and its anatomical localisation. Contrarily, as its almost ubiquitous presence was revealed, doubts began to be expressed as to whether it could indeed have a specific role in neurotransmission, rather than performing a general role as a neural metabolite.

However, one of the most significant scientific contributions from the laboratory was a Communication to the Physiological Society. This was not a report of new experimental results, but a contribution by Dale alone, entitled ‘Nomenclature of fibres in the autonomic nervous system and their effects’ [25]. Feldberg recalled the preparation for the paper:

*“I remember well Dale having gone on several days to the Library, which was not usual for him, and then his happiness one day when he came to show me the typescript of his communication. He was fully aware of its importance.”* [26]

‘[I]ts importance’ was the introduction of the terms ‘cholinergic’ and ‘adrenergic’ to designate nerve fibres by the nature of the chemical that they used, or might possibly use, as a transmitter, rather than by the anatomical classification of sympathetic and parasympathetic divisions of the autonomic nervous system. The final sentence of Dale’s communication *“I think such a usage would assist clear thinking, without committing us to precise chemical identifications, which may be long in coming”*, [27], indicates why his suggestion was of immediate major importance: he permitted leeway about

the precise identification of the chemical transmitter, the semi-neutral terms ‘adrenergic’ and ‘cholinergic’ allowed that their respective chemical neurotransmitters might be ‘adrenaline-like’ and ‘acetylcholine-like’ but were not necessarily either of those two chemicals.

## 6. Acetylcholine: beyond 1936

From the mid-1930s onwards, when Dale and his colleagues had produced so much evidence to support the theory of chemical transmission, questions about the role, if any, of acetylcholine in neural functioning assumed a central place in physiological debates. The idea of chemical neurotransmission promoted vigorous opposition from many prominent physiologists, most notably John Eccles, who queried the interpretation of the experimental evidence and maintained that neural transmission was purely an electrical phenomenon [28]. Known jocularly as ‘soup versus spark’ (chemical versus electrical transmission) the debates and discussions were principally conducted at meetings of the Physiological Society, as an eye witness from the time recalled:

*“One of the main controversial topics of these meetings of the English Physiological Society in the 1930s was the role of acetylcholine (AcCh) in nerve activity. Dale and his associates had proposed that AcCh acts as a mediator of nerve impulses across nerve junctions (synapses) between nerve and nerve, and nerve and muscle, in contrast to the electrical currents that propagate impulses along nerve and muscle fibers.” [29]*

In 1951, in quite spectacular fashion, the main opponent of chemical neurotransmission, Eccles finally admitted that he did accept the theories put forward by Dale and his colleagues, and on the occasion of Dale’s ninetieth birthday in 1965 offered the following tribute:

*“My memory takes me back more than three decades when Dale and his colleagues literally staggered us neurophysiologists by the hypothesis that even the fast synaptic transmissions were mediated chemically. After many years of resistance to this hypothesis, I came in 1951 to accept it unreservedly by what Sir Henry regards as the scientific equivalent of a religious conversion. I reciprocate appropriately by saying that he is one of my scientific saints.” [30]*

For very many years the eserinated leech muscle assay, which had made such an enormous contribution

to the study of acetylcholine, remained the most reproducible, sensitive method of identifying and quantifying acetylcholine. It was not until the 1960s that more sophisticated chemical methods of detecting and measuring acetylcholine were developed [31]. And it was towards the end of that decade that a collaborative project between staff of the University of California and the Karolinska Institute in Stockholm, using a gas-chromatography/mass spectrometry technique, was able to determine acetylcholine in sub-microgram amounts. To mark the occasion, the authors wrote to the elderly Dale in his retirement home in Cambridge:

*“Stockholm, June 7, 1968*

*Dear Sir Henry,*

*It is now forty years since you and Dudley reported your classical work in the first rigorous identification of acetylcholine in animal tissue. Although most of us have relied on a large volume of circumstantial evidence for its occurrence in mammalian brain, no positive identification in fresh brain has so far been reported.*

*We therefore believe it may be of interest to you to know that [...] we succeeded on the fourth of this month in conclusively demonstrating the existence and identity of acetylcholine in a single rat brain. [...]*

*In view of the tremendous technological advances in recent years we cannot but feel humble at your great achievements with such simple means forty years ago.*

*Yours respectfully,*

*Bo Holmsdedt      Donald J. Jenden”*

Dale had just celebrated his 93rd birthday, and his reply must be one of the last letters he ever wrote; he died in his sleep a month later:

*“You will agree that one of the interesting and puzzling questions still to be answered, is the apparently random distribution of acetylcholine in the organs of different species, from which it can be easily obtained by methods far less subtle and conservative, than those which you have now been able to apply. As you are aware, my late colleague, Harold Dudley and I came across it almost by accident, in the spleens of the large ungulates, and actually managed to isolate and identify it from the spleen of the horse. [...] I do again send you my heartiest thanks for your most interesting communication and your kindly reference to what were, necessarily, in those earlier days, the*

*relatively crude methods which my colleagues and I were then obliged to employ.” [32]*

## 7. Conclusions

In 1936 Dale shared the Nobel Prize in Physiology or Medicine with Otto Loewi “*for their discoveries relating to the chemical transmission of nerve impulses*”. That year also marked the end of his personal involvement with laboratory research. He increasingly accepted onerous duties and responsibilities elsewhere (for example, he served as President of the Royal Society for most of the Second World War) and these consumed most of his time although not his interest. It was his younger colleagues, most notably Brown and Feldberg who continued to amass evidence about the role of acetylcholine in the nervous system. Over the next three to four decades they and others extended the concept of chemical neurotransmission to include the central nervous system; sophisticated theories of drug-receptor interactions in the cholinergic system were developed; and ultimately came an explosion of theories and knowledge about the role(s) of several endogenous chemicals in the nervous system, both experimentally and therapeutically.

Henry Dale kept a close watch on the plethora of evidence that accumulated about chemical neurotransmission, and in 1958 wrote to his old colleague Elliott about “a happy morning” that he had spent with Bernard Katz, the Professor of Biophysics at University College London (and future Nobel Laureate), discussing possible mechanisms of cholinergic transmission. Dale’s explanation and comments on the material he had been shown provide a revealing picture of the changes in physiology that had come about during his lifetime:

*“I feel almost bewildered by the kind of detail which such people are now elucidating with the aid of electron-ultramicroscopy, and also with an electrical recording which they can now achieve of the transmitted excitatory process at the motor end-plate of a single muscle fibre. [...] A great deal indeed has happened since you first suggested a chemical mechanism for the transmission of the excitatory process from a nerve ending; and it goes on happening with a constant acceleration.” [33]*

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