Section of Therapeutics and Pharmacology

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WALTER ERNEST DIXON MEMORIAL LECTURE

Pharmacology and Nerve-endings

By Sir HENRY DALE, C.B.E., M.D., F.R.S.

ABSTRACT.—A brief account is given of the scientific career of Walter Ernest Dixon, and of the importance of his work and his influence for the development of Pharmacology in England. It is suggested that the Memorial Lecture may appropriately deal with some matter of new interest, from one of the fields of research in which Dixon himself was active. Special mention is made of his work with Brodie on the physiology and pharmacology of the bronchioles and the pulmonary blood-vessels, as probably showing the beginning of Dixon's interest in the actions of the alkaloids and organic bases which reproduce the effects of autonomic nerves.

An account is given of Dixon's early interest in the suggestion, first made by Elliott, that autonomic nerves transmit their effects by releasing, at their endings, specific substances, which reproduce their actions; and of his attempt to obtain experimental support for this conception. After the War it was established by the experiments of O. Loewi; and it is now generally recognized that parasympathetic effects are so transmitted by release of acetylcholine, sympathetic effects by that of a substance related to adrenaline.

Very recent evidence indicates that acetylcholine, by virtue of its other ("nicotine-like") action, also acts as transmitter of activity at synapses in autonomic ganglia, and from motor nerve to voluntary muscle.

The terms "cholinergic" and "adrenergic" have been introduced to describe nerve-fibres which transmit their actions by the release at their endings of acetylcholine, and of a substance related to adrenaline, respectively. It is shown that Langley and Anderson's evidence, long available, as to the kinds of peripheral efferent fibres which can replace one another in regeneration, can be summarized by the statement, that cholinergic can replace cholinergic fibres, and that adrenergic can replace adrenergic fibres; but that fibres of different chemical function cannot replace one another. The bearing of this new evidence on conceptions of the mode of action of "neuromimetic" drugs is discussed. The pharmacological problem can now be more clearly defined, and Dixon's participation in further attempts at its solution will be sadly missed.

RÉSUMÉ.—Courte description de la carrière scientifique de Walter Ernest Dixon, de l'importance de son travail, et de son influence sur le développement de la pharmacologie en Angleterre. L'auteur suggère que la conférence dédiée à sa mémoire peut à juste titre traiter de quelque nouvelle matière intéressante dans un des sujets dont Dixon s'occupait. Une mention spéciale est faite de son travail avec Brodie sur la physiologie et la pharmacologie des bronchioles et des vaisseaux pulmonaires, comme étant probablement le point de départ de l'intérêt de Dixon dans l'action des alkaloïdes et des bases organiques qui reproduisent les effets des nerfs autonomes.

L'auteur décrit l'intérêt précoce de Dixon dans l'idée présentée d'abord par Elliott, que les nerfs autonomes produisent leur effet en déchargeant à leurs extrémités des substances spécifiques qui reproduisent leurs effets, ainsi que ses efforts pour obtenir une confirmation expérimentale de cette conception. Après la guerre les expériences de O. Loewi établirent cette idée, et on reconnaît aujourd'hui que les effets parasympathiques sont produits par la décharge d'acétylcholine, et les effets sympathiques par la décharge d'une substance alliée à l'adrénaline.

Des travaux très récents indiquent que l'acétylcholine, par son autre action (" semblable à la nicotine "), agit aussi comme transmetteur de l'activité dans les synapses des ganglions autonomes, et des nerfs moteurs aux muscles volontaires.

Les termes " cholinergique " et " adrénergique " ont été introduits pour décrire respectivement les fibres nerveuses qui transmettent leurs effets par la décharge d'acétylcholine ou d'une substance alliée à l'adrénaline. Il est démontré que l'évidence de Langley et Anderson, existant depuis longtemps, sur les espèces de fibres efférentes périphérales qui peuvent se remplacer dans la régénération, peut être résumée ainsi : Les fibres cholinergiques peuvent être remplacées par des fibres cholinergiques et les fibres adrénergiques par des fibres adrénergiques, mais des fibres de fonction chimique différente ne peuvent pas se remplacer. Les rapports de ces nouvelles connaissances sur notre conception de l'action des médicaments " neuro-mimétiques " sont discutés. Le problème pharmacologique peut être plus clairement défini, et la perte de la collaboration de Dixon dans les efforts futurs pour le résoudre se fera grandement sentir.

ZUSAMMENFASSUNG.—Kurzer Bericht über Walter Ernest Dixons wissenschaftliche Leben und über die Bedeutung seiner Leistungen und seines Einflusses in der Entwicklung der englischen Pharmakologie. Es scheint zweckmässig zu sein dass ein Vortrag zu seinem Andenken von einem neuen interessanten Gegenstand auf dem Gebiet wo Dixon selbst arbeitete handle. Seine Arbeiten mit Brodie über die Physiologie und Pharmakologie der Bronchiolen und Lungengefässe werden besonders besprochen, denn sie zeigen wahrscheinlich den Ausgangspunkt Dixons Interesse über die Wirkungen der Alkaloiden und der organischen Basen welche die Wirkungen der autonomen Nerven wiedergeben.

Dixons frühes Interesse in dem zuerst von Elliott geäusserten Begriff, dass die Wirkung der autonomen Nerven durch die Absonderung an den Nervenenden von spezifischen Stoffen die ihre Wirkung wiedergeben zustandekommt, wird besonders besprochen, ebenso wie seine Versuche experimentellen Beweis dafür zu bringen. Nach dem Krieg wurde diesen Begriff experimentell von O. Loewi bewiesen, und es ist heute allgemein anerkannt dass parasympathische Wirkungen durch Freiwerden von Azetylcholin, und sympathiche Wirkungen durch Freiwerden von einer adrenalinähnlichen Substanz, zustandekommen.

Sehr neue Untersuchungen weisen darauf hin dass Azetylcholin, durch seine zweite (" nikotinähnliche ") Wirkung, auch als Leiter der Wirksamkeit an den Synapsen in den autonomen Ganglien, und vom Motornerven an den willkürlichen Muskel wirkt.

Die Wörter " cholinergisch " und " adrenergisch " sind in die Sprache gekommen um die Nerven zu beschreiben die ihre Wirkung durch Freiwerden von Azetylcholin bzw. von einer adrenalinähnlichen Substanz zu bezeichnen. Es wird gezeigt dass Langley und Andersons Befunde über die Arten von peripheren efferenten Nervenfasen die sich in der Regeneration ersetzen können, die schon lange zugänglich sind, in folgender Weise zusammengefasst werden können : cholinergische Fasern können cholinergische und adrenergische Fasern können adrenergische ersetzen, aber Fasern mit verschiedenen chemischen Wirkungen können sich nicht ersetzen. Die Beziehung dieser neuen Befunden mit unserem Begriff der Wirkungsweise der " neuromimetischen " Mittel wird besprochen. Das pharmakologische Problem kann jetzt genauer bestimmt werden, und die Teilnahme Dixons an den weiteren Versuchen es zu lösen werden wir sehr vermissen.

INTRODUCTORY

THOSE who are responsible for the administration of the Fund which was raised as a Memorial to Walter Ernest Dixon have greatly honoured me by the invitation to deliver this, the first Dixon Memorial Lecture. In later years, I think that my successors will feel that they can best honour Dixon's memory by considering some new and progressive phase of activity in the field of research and teaching which received so strong an impulse from his life and work. It is thus, I believe, that Dixon would himself wish us later to remember him. We should pay but a poor and partial tribute to the memory of any man of science if we were satisfied merely to recall at intervals the state of knowledge during his lifetime in the field where he himself was active, and newly to assess the value of each item of the harvest of discovery which fell to his own reaping. We can better keep his memory alive among those who come after by studying some new and interesting growth from the

ground where he dropped the seed, or, it may be, only prepared the soil for later sowing. So it will be with Dixon; and to-day I shall later ask your attention to some recent experimental data, which throw new light on the meaning of a group of pharmacological actions, which once claimed a central position in Dixon's thoughts and in his research activities.

But on the occasion of this first Memorial Lecture, when the loss of our friend is still fresh in memory, we cannot feel content to pay only such an impersonal tribute to him. The creation of this Memorial signifies much more to those who have raised it than a desire to perpetuate the memory of Dixon's researches, or to provide for the presentation, in the years to come, of new developments in related spheres of investigation. The memory is still vivid with us of Dixon, as a vigorous and inspiring personality, who, more than any other, was responsible for the awakening of interest, here in England, in pharmacology as a progressive science, and as a necessary item in training for the practice of medicine. You will notice that I said England, not Britain, for at the time when Dixon began his work at Cambridge the Scottish medical schools had already their long-established and active departments of pharmacology. In England, on the other hand, pharmacological teaching was limited to a few, often somewhat perfunctory lectures, by one of the physicians in each of the medical schools. At Cambridge, pharmacology did not figure as an obligatory item in the pre-clinical studies. When the Downing Professorship of Medicine fell vacant, Dr. Bradbury, a physician at Addenbrooke's Hospital practising in Cambridge, had been appointed to the Chair, which he held till he died in extreme old age, only a few months before Dixon. But Sir Michael Foster, originator of so much in the Schools of Experimental Medicine and Biology at Cambridge, had seen the need for pharmacology as an experimental science, and it was arranged, when Bradbury became Professor, that an assistant in that subject should be supported from the emoluments of the Downing Chair. It was in this capacity that Dixon came to Cambridge in 1899, and he was still there, as Reader in Pharmacology, at the time of his death, thirty-two years later. Cushny, by some years Dixon's senior, a pupil of Cash at Aberdeen and later of Schmiedeberg, was then away in Ann Arbor, as Professor of Pharmacology in the University of Michigan; and when the creation of a Chair of Pharmacology in University College, London, brought Cushny to England, Dixon had already been six years in Cambridge, and the new stimulus of his activities there had made itself felt far outside his own school. I myself missed the direct contact there, for Dixon came to Cambridge just as I was about to leave; so that whatever piecemeal knowledge of pharmacology may since have come to me has been acquired for special needs, and largely by later friendship with Dixon and Cushny. But from my immediate juniors I soon heard of the new life which had been breathed into pharmacology at Cambridge, where it could be studied, no longer now in terms of traditional *materia medica* and empirical therapeutics, but as a living body of experimental science, closely linked with physiology. Through them I had some share in Dixon's stimulating influence, before I made any personal contact with him, and for some years before I had the great privilege of his close friendship. To the yearly groups of the students who followed them, for more than thirty years, Dixon's lectures and classes continued to give a conception of pharmacology, not as a collection of facts to be learned for examination, but as a lively scientific adventure, in which skill and mental enterprise could hope to win new knowledge, of interest for its own sake, as well as for the part which it might play in building the foundations of rational therapeutics. A number of such students were attracted to obtain a first-hand experience of research in his laboratory; and the results of that experience are in some instances to be measured, not so much by the permanence of the direct contributions to science which resulted from such short-term apprenticeships, as by the lasting effect of an acquaintance with conditions in which observation can be quantitative, and experiment deliberate and controlled.

This attraction which he had for young workers was a part of Dixon's charming and generous personality. His kindliness and his robust humour endeared him to students and colleagues alike. Mrs. Dixon and he made their beautiful old house, in the village of Whittlesford, near Cambridge, a centre of charming hospitality, and a rallying-point for those from many countries who shared his interests. He had real gifts as a raconteur, and his simple and vivid presentation of scientific matter made him an effective popular lecturer, and gave authority to his opinions far beyond the circle of those having expert knowledge of his subject. For a number of years, until his promotion to a Readership at Cambridge with effective control of the department there, Dixon held the additional post of Professor of Pharmacology at King's College, London. This widened the range of his contacts, and brought him into fruitful collaboration with the late W. D. Halliburton and others. On the other hand, apart from the additional teaching obligation, the double duty used much of his time in travelling, and the arrangement probably tended still further to diffuse his already wide interests.

This memorial lecture is not the proper occasion for a systematic review of Dixon's contributions to science. The admirable obituary notice, which Professor Gunn (1932) contributed to the *Journal of Pharmacology and Experimental Therapeutics*, gives a full bibliography of his scientific publications. His earliest papers, on mescal (1899*a*) and *Cannabis indica* (1899*b*), show already his interest in the action of drugs of addiction, to the study of which he returned at intervals throughout his career, and on which he acquired more than a national authority. Nobody who looks at this list of Dixon's publications can fail to be struck by the wide variety of the subjects on which he worked and wrote. He had an almost exuberant interest in any new line of knowledge touching on pharmacology, and a desire to share in its exploration; and his conception of the scope of pharmacology tended to expand well beyond the study of drugs and their action, and to include any procedure finding application in therapeutics. His researches were, accordingly, characterized by range and variety, rather than by intensive and deep exploration in a limited field. It would be presumptuous at this stage to attempt any estimate of the relative permanence and ultimate importance of Dixon's different direct contributions to knowledge. One can already more safely judge of their influence on the work and the ideas of his contemporaries and immediate juniors, and I am inclined to think that this influence was strongest in the cases of his work with Brodie (1903, 1904) on the physiology and pharmacology of the bronchioles and the pulmonary vessels, and his work with Halliburton (1910) on the conditions governing the formation of the cerebrospinal fluid. If I to-day select, from these two, the former for more particular mention, it is because it seems to have provided a starting point for the development of one of Dixon's predominant pharmacological interests.

Bronchioles and Pulmonary Vessels

Brodie and Dixon's (1903) work on the contractility of the bronchioles provided the experimental foundation for a rational conception of the mechanism of asthma. It also, unless I am mistaken, first brought vividly to Dixon's notice the remarkable resemblance between the actions of certain alkaloids and those of autonomic nerves. In this case his attention was, by a curious accident, limited to the resemblance between the effects of vagus stimulation and those of alkaloids like muscarine and pilocarpine, both readily annulled by small doses of atropine. There was waiting for later discovery the equally striking resemblance between the effects of adrenaline and sympathetic nerve stimulation on the bronchioles, both relaxing the tone of the plain muscle which vagus stimulation or muscarine enhanced. Brodie and Dixon seem just to have missed the appropriate conditions for demonstrating this particular sympathetic effect. The remarkable resemblance between the effects of adrenaline

and those of sympathetic nerves must, however, by that date have been in Dixon's mind ; for he was working in Cambridge, where this similarity was already a matter of lively interest, through Langley's (1901) researches, and through the remarkable series, then in its initial stages, by which Elliott later (1905) established the general validity of this correspondence, and explored its meaning. In 1903, indeed, Dixon himself published experiments which showed that the actions of adrenaline and those of sympathetic nerves disappeared together, in the rather complex series of paralytic effects produced by the alkaloid apocodeine ; and in 1904 his work with Brodie on the pulmonary vessels provided a negative example of the correspondence between the two types of action, the arteries of the lungs, which were not significantly affected by sympathetic stimulation, being shown to be similarly unresponsive to adrenaline (Brodie and Dixon, 1904).

Site of Action of Neuromimetic Drugs

In this paper on the pulmonary vessels Brodie and Dixon included a long discussion of the evidence, including some new items of their own, as to the nature of the reactive structure on which adrenaline produces its effects. Chiefly on the basis of the action of apocodeine, which left the effector cells responsive to other kinds of stimuli when the effects of sympathetic impulses and adrenaline had been simultaneously annulled, they concluded that adrenaline acted on sympathetic nerve-endings. With regard to the direct evidence of Lewandowsky and of Langley, who had both shown that adrenaline retained its full action after the nerves had been cut and allowed to degenerate, Brodie and Dixon were doubtful as to whether degeneration of the nerve-endings, as they used the term, could be assumed, and whether, in any case, a sufficient period had been allowed to elapse, after section, to assure full degeneration. In their own experiments of this kind, the results of which entirely confirmed Langley's, they do not seem to have waited long enough to eliminate this objection to their own satisfaction. Evidence beyond criticism in this respect was given, however, about a year later, by the publications of Elliott and of Anderson. Elliott (1905) completely denervated the pupil by removal of both the superior cervical and the ciliary ganglion, and showed that it was still fully sensitive to adrenaline many months after the operation ; while Anderson (1905) obtained corresponding evidence with regard to pilocarpine and the parasympathetic nerve-supply, showing that the reaction of the pupil to pilocarpine had an unlimited persistence after removal of the ciliary ganglion.

Different workers and writers in this field appeared to give somewhat different interpretations to this evidence, though, since there was no difference of opinion as to the available facts, the differences were, perhaps, more verbal than real. It was generally agreed that the impulses in autonomic nerves, and the drugs, such as adrenaline and pilocarpine, which simulated their effects, must act on some structures which completely survived degeneration of the nerves ; but that, on the other hand, the annulment of the responses of these structures to nerve impulses or to the mimetic drugs, produced by selectively paralytic alkaloids such as ergotoxine or atropine, left the effector muscle or gland cells still normally responsive to stimuli of other kinds. In view of the evidence from degeneration, the specifically sensitive structures could only be termed "nerve-endings" in a special and doubtfully admissible sense ; and though the use of the term has lingered, in pharmacology especially, we shall see that, in the light of the evidence more recently made available, its employment is now even more difficult to justify. Elliott spoke of the structures as "myoneural junctions," and Langley as "receptive substances." Both recognized that they belonged trophically to the effector cells, and the difference in terminology corresponded to relatively small theoretical differences of conception, as to their mode of origin and as to the extent to which they were localized in the

neighbourhood of the true nerve-endings. With his then collaborator, Dr. Fred Ransom, Dixon published in 1912 (Dixon and Ransom, 1912) a systematic and reasoned review of all the evidence concerning this "Selective action of drugs on the peripheral nervous system."

Early Hints of Chemical Transmission

Before these discussions arose Elliott (1904) had already indicated what we can now recognize as the real clue to the meaning of these remarkable similarities, between the actions of certain drugs and those of the two main anatomical divisions of the autonomic nervous system. In a short note published in 1904 he had suggested that the resemblance between the effects of sympathetic nerves and those of adrenaline might mean, that sympathetic impulses, on arriving at the nerve-endings, released small quantities of adrenaline, or of something like it, in immediate relation to the effector cells, which would then give the same responses as to adrenaline artificially applied. Dixon saw that, if this were a true conception, an analogous mechanism would almost certainly be used by parasympathetic nerves, and he pictured the substance transmitting their effects as something like muscarine. Muscarine is a very stable alkaloid, and a knowledge of this fact may well have suggested to Dixon the possibility of obtaining experimental evidence of its release, when the vagus nerve was stimulated. In 1906 and 1907 he published results which seemed to him to give positive support to the conception. In a dog bled as completely as possible, he subjected the vagus nerves to strong and protracted stimulation, removed the heart, boiled it briefly in water, and made an alcoholic extract. This he evaporated to dryness, took up the residue with absolute alcohol to remove salts, and finally brought the soluble portion into saline solution. On applying this solution to the beating heart of a frog, he observed an inhibitor action which atropine removed. With a similar extract from a heart not subjected to vagus stimulation, a similar inhibitor effect was obtained, but a somewhat weaker one. With our present knowledge that the vagus transmitter is not muscarine, but an extremely unstable ester of choline, we can feel no certainty that Dixon had any of it in his extracts. The most recent evidence further suggests that, if he had succeeded in preserving it, the quantity in such artificial extracts from the whole organ would have been no greater with vagus stimulation than without it. Probably the substance responsible for the effects which Dixon observed was free choline. In any case, the experiments were described with so little quantitative detail that they appeared to be of a purely preliminary and tentative nature. From what Professor Gunn tells us, however, Dixon appears to have been discouraged by the scepticism with which the evidence was received. The statement cannot fail to excite the sympathy of anyone who has tried to break new ground in research. If Dixon had felt encouraged, however, to follow the same line of experiment beyond this preliminary stage, we may feel pretty certain that his early evidence would have broken down under his own criticism. What is beyond doubt is that he had already, in 1906, grasped a true conception, with characteristic conviction and enthusiasm. The evidence which really established it, however, came many years later, from a much simpler type of experiment than that which Dixon had tried. Dixon and Ransom's review, published in 1912, and mentioned above, does not consider this explanation of the phenomena there discussed, and we must suppose that Dixon had lost full confidence in it by that date.

At the meeting in 1906 of the British Medical Association in Toronto, at which Dixon made the first mention of his heart-vagus experiments, Reid Hunt with Taveau (1906) described the action of certain esters of choline, and, in particular, the extraordinarily intense activity of acetylcholine, many thousands of times as potent as that of the parent choline. Nobody at the time suspected the possibility

of any thread of connexion between the observations presented in these two entirely independent communications, by pharmacologists from different countries. Seven or eight years later, having come across acetylcholine by accident as a constituent of an ergot extract, and having had experience of the directly physiological interest of other substances which that remarkable drug had brought to our notice, I made a thorough investigation of its actions (Dale, 1914). I was immediately struck by the remarkable fidelity with which it reproduced the effects of stimulating parasympathetic nerves, and with the extraordinary intensity and rapid evanescence of its action, which, I supposed, might be due to its rapid hydrolysis by an esterase —a speculation which later evidence has justified. I made a comparison between the close reproduction of parasympathetic effects by acetylcholine and that of sympathetic effects by adrenaline; both being, for different reasons, highly unstable substances, acting with a brief intensity. I remember very well that, when I first demonstrated some of these effects of acetylcholine to the Physiological Society in January 1914, Dixon came to me and discussed the possibility that this might be his vagus substance. I agreed with him that, if clearer evidence could be produced for the transmission of parasympathetic effects by peripheral release of a chemical agent, acetylcholine appeared to be a more promising candidate for the rôle than muscarine, and one more likely to appear in the animal body. In the event, we had to wait another fifteen years before acetylcholine was found in an animal organ, in such quantity that my colleague, H. W. Dudley, was able to isolate it and identify it chemically (Dale and Dudley, 1929). But one can hardly help wondering whether the new interest excited by its remarkable action might not have encouraged Dixon to renew his attempt to find evidence of a chemical mechanism for the transmission of vagus effects. For him, as for all of us, however, all thought of such academic inquiries was rudely brushed aside by the outbreak of war within less than six months. Dixon spent the following years abroad in the service of the Admiralty, and he never picked up again the thread of what had, for a time, been one of his central scientific interests.

These peripheral effects of acetylcholine, reproducing so closely those of parasympathetic nerves—constituting what I termed its "muscarine action"—were very easily annulled by atropine. Then, with somewhat larger doses, acetylcholine produced another series of effects, due to what I called its "nicotine action," since I traced them to a general stimulation of autonomic ganglion cells (Dale, 1914). Later, other investigators showed that acetylcholine also shared the stimulating effects of nicotine on voluntary muscle, which Langley had described in such detail. At the time, this secondary "nicotine" action seemed rather difficult to reconcile with any suggestion that acetylcholine might be concerned with the transmission of purely peripheral, parasympathetic effects. We shall later consider the significance which recent evidence now enables us to accord to it.

Loewi's Proof of Chemical Transmission

Elliott had made a brilliant suggestion; Dixon had grasped it with characteristic eagerness and enthusiasm, had extended it, and had attempted to verify it experimentally; but we had to wait till 1921 for Otto Loewi, in Graz, to establish, as an experimental fact, the transmission of the effects of autonomic nerve impulses by the peripheral release of specific chemical stimulants (Loewi, 1921). By the simplest imaginable procedure, using the isolated hearts of frogs, Loewi showed that vagus stimulation released into the fluid filling the heart a substance which would transfer the vagus inhibition to another heart; and that when the accelerator action, due to the sympathetic component of the frog's vagus, predominated, an accelerator substance could be similarly detected. When the action of one or the other type of nerve-fibres was paralysed by atropine or ergotoxine, the chemical transmitter of

nervous activity was still released: only its action on the effector cells had been abolished. Loewi and his co-workers were further able to use, to brilliant purpose, the hint, which had not been available to Dixon, as to the kind of substance which the vagus transmitter might be. They showed that it was an unstable choline ester, indistinguishable, in all the properties which a minute scale of experiment allowed them to examine, from acetylcholine. The heart contained an esterase which rapidly destroyed it, and eserine specifically inhibited this esterase, and thereby produced its potentiating action on the effects of the vagus and of other parasympathetic nerves.

Further Evidence

As to Loewi's accelerator substance, the transmitter of sympathetic effects, our further knowledge has come largely from Cannon and his school (Cannon and Bacq, 1931, Cannon and Rosenblueth, 1933). Whether it is adrenaline itself is still open to question. Cannon and his co-workers believe that they have evidence that it is not, and that, in any case, it is capable of appearing in two forms of combination, one producing only the augmentor, the other only the inhibitor effects of sympathetic nerves. They call it "sympathin," to avoid any premature implication as to its chemical nature; though there is already evidence that it is a substance chemically related to adrenaline, even if it is not identical with it. With regard to the parasympathetic transmitter, the evidence which has come, in increasing volume, from the laboratories of many different countries, has given evidence, as was to be expected, of its appearance in connexion with a wide range of parasympathetic effects. This accumulating evidence, moreover, has so strengthened the case for regarding the substance as acetylcholine itself, that there can be hardly any further doubt as to its identity.

The detailed evidence for these two kinds of chemical transmission of autonomic effects has been frequently reviewed. Twice during the present year I have reviewed it myself, dealing on the same occasions with more recent evidence which, rather surprisingly, indicates that a chemical mechanism of this kind also effects the transmission of nervous activity at the synapses in peripheral, autonomic ganglia, and at the motor nerve-endings on voluntary muscle-fibres (*see* Dale, 1934) To-day I must be content with the briefest sketch of chemical transmission in the whole efferent peripheral nervous system, as it now appears in the light of our most recent evidence.

Recent Developments

While in general the distinction holds that peripheral parasympathetic effects are transmitted by the liberation of acetylcholine, and peripheral sympathetic effects by the release of something like adrenaline, this rule is not without exceptions. There are certainly some postganglionic fibres of the true sympathetic system which transmit their effects by means of acetylcholine, as Feldberg and I (1934) have demonstrated in the case of the cat's sweat-glands. Feeling the need of terms to describe nerve-fibres, or their impulses, in terms of a chemical function, which we can no longer regard as corresponding to their anatomical origin, I suggested (1933) the term "cholinergic" to describe those which transmit their action by release of acetylcholine, and "adrenergic" for those which employ a substance resembling adrenaline. The use of such functionally descriptive terms became more necessary when we were led, by stages which I have elsewhere described, to consider whether those actions of acetylcholine, which I classed as its "nicotine" actions, had also a physiological significance. Feldberg and Gaddum (1934), perfusing the superior cervical ganglion with Locke's solution containing a very small proportion of eserine, found that, when the preganglionic nerve was stimulated, but only then, acetylcholine appeared in the venous effluent, in quantity sufficient to enable it to be

identified with practical certainty. Further experiments by Feldberg and Vartiainen (1934) have definitely located at the synapses this liberation of acetylcholine in the ganglion, and have shown that when the ganglion cells are paralysed by nicotine or by excess of eserine, to the stimulus either of drugs such as acetylcholine or of preganglionic impulses, the latter still liberate acetylcholine in undiminished amount, though the ganglion cells can no longer respond to it. There is an obvious and close parallel to the effect of atropine in preventing the action of vagus impulses on the heart, as described by Loewi. I think it is no longer possible to doubt that the liberation of a small quantity of acetylcholine, when a preganglionic impulse arrives at a synapse, plays an essential part in the transmission of the excitation to the autonomic ganglion cell, and that the postganglionic impulse is essentially a separate physiological event; though the correspondence of a single postganglionic impulse to each preganglionic impulse, and the shortness of the delay (not more than 2σ) at the synapse, had given the impression of conduction across the synapse by unbroken propagation. The recognition, however, of acetylcholine as the chemical transmitter at ganglionic synapses, does not mean that its appearance will account for all the phenomena associated with such transmission; and the shortness of the delay at the synapse, leaving no time for diffusion, indicates that the arrival of the preganglionic impulse must liberate a small charge of acetylcholine practically in contact with the ganglion cell, where, having caused the discharge of a postganglionic impulse, it must immediately disappear. Though we may, accordingly, class the preganglionic fibres and their impulses as "cholinergic," the process by which their effects are transmitted to the ganglion cells differs widely in detail from that by which postganglionic parasympathetic impulses use acetylcholine, to produce their modifying actions on the spontaneous activities of plain muscle and gland cells.

The same considerations apply to the evidence suggesting a similar function for acetylcholine, as transmitter of somatic motor nerve impulses to voluntary striated muscle-fibres. The pharmacological affinities between ganglion cells and striated muscle-fibres have often been pointed out. The action of acetylcholine on such fibres again resembles that of nicotine, and is similarly susceptible to paralysis by curare; it is also similarly irregular in its incidence, and conditioned, in the case of most mammalian muscles, by degeneration of the motor fibres. These and other complications have to be borne in mind. On the other hand, the analogies between a ganglionic synapse, and the ending of a motor nerve-fibre on a muscle-end plate, are many. When, therefore, we find, as Feldberg and I (1934) have done, that the effective stimulation, of a purely motor somatic nerve-supply to a perfused voluntary muscle, regularly causes the appearance of acetylcholine in the perfusion fluid, it is difficult to resist the implication, that the liberation of acetylcholine is concerned with transmission of excitation from motor nerve-ending to muscle end plate, as from preganglionic nerve-ending to ganglion cell. These experiments on muscle, begun much later, are far less complete than those on the ganglion; but, so far as they have gone, they have yielded evidence only in favour of such a conception. The difficulties of time relation and repetitive response are the same in the one case as in the other.

Evidence from Regeneration of Nerves

Instead of presenting again the details of such direct evidence, I will ask you to consider the bearing upon it of data, long available, which display the functional similarities and differences between different fibres of the peripheral nervous system, by the method of regeneration after artificial cross-suture of nerves. If we rightly interpret the direct evidence, the preganglionic fibres of the whole autonomic system, the motor fibres to voluntary muscle, and the postganglionic fibres of the para-

sympathetic system can be classed together as cholinergic; only the postganglionic fibres of the true sympathetic system forming a separate class, as being predominantly adrenergic. Look now at the results of the cross-union of nerves, as revealed in a series of papers published by Langley and Anderson between 1897 and 1904. They cut such nerves as the vagus, the cervical sympathetic, the recurrent laryngeal, the hypoglossal, and the fifth cervical. In varying combinations they joined the central cut end of one nerve to the peripheral cut end of another, and awaited the results. And the results showed clearly that the preganglionic fibres of any autonomic nerve, whether sympathetic or parasympathetic, could grow down the degenerating peripheral end of any other preganglionic nerve, and make functional synaptic connexion with the cells of its ganglion, to whichever division of the system it belonged. All preganglionic fibres, therefore, were functionally interchangeable. They showed, further, that any motor fibre to a voluntary muscle could similarly replace any preganglionic autonomic fibre, and make functional synaptic connexion with its ganglion cells; and that, conversely, any preganglionic fibre could replace any motor voluntary fibre, and make effective functional connexion with the corresponding striated muscle-fibres. On the other hand, no replacement could ever be effected of postganglionic sympathetic fibres by preganglionic fibres of either system, or by voluntary motor fibres, and postganglionic sympathetic fibres would make no synapses with ganglion cells or motor endings with voluntary muscle. Any postganglionic sympathetic fibres, however, would replace other fibres of the same type. You will see that, so far, the results seem to fit well with our classification in terms of functional chemistry. They can be simply summarized by stating that any cholinergic fibre will functionally replace any other cholinergic fibre, and that any adrenergic fibre will replace any other adrenergic fibre, but that neither can assume the function of the other.

There remains one case to be considered, and it is one of special interest. The postganglionic fibres of the parasympathetic system are cholinergic, though the conditions under which the acetylcholine, released by the arrival of impulses at their endings, reaches the effector cells, must be widely different from those which obtain at a ganglionic synapse or at a motor nerve-ending in voluntary muscle. The question necessarily arises, then, whether preganglionic or voluntary motor fibres will grow down the track of degenerated postganglionic fibres of the parasympathetic system, and effect any kind of functional replacement of their action on the peripheral effector organ. There is only one case in which the anatomical conditions make it practicable to put this question to the test of operative experiment. The case is that of the ciliary ganglion, and the effects of impulses in the postganglionic fibres, running from it to the sphincter of the pupil, are, of course, peculiarly accessible to observation. The experiments in question were made, and their results recorded in perfect detail, by the late H. K. Anderson, nearly thirty years ago. Anderson (1905) observed that, when the ciliary ganglion was removed from the orbit of a kitten, the sphincter of the pupil perfectly retained its response to pilocarpine, but that the normally potent constrictor effect of eserine disappeared entirely with degeneration of the postganglionic fibres. We may note that Anderson, using the terminology and the conceptions of that time, regarded this as showing that eserine acted on a part of the nerve-ending which degenerated with the fibre, while pilocarpine acted on a more peripheral portion of the neuromuscular mechanism, which survived. To-day I think we can more reasonably attribute the effect of eserine to the accumulation of acetylcholine, the liberation of which, by the play of impulses in postganglionic fibres, is normally balanced by the destructive action of the cholinesterase, which eserine inhibits. When the nerve-fibres are gone, no acetylcholine is liberated, and eserine no longer acts. The point of special interest for our present purpose, however, is that Anderson found that the action of eserine returned, if the animal was kept for some months after operation, and that

the pupil then, under partial eserine action, recovered its response to illumination of the eye. Regeneration appeared to have taken place. The fact puzzled Anderson, as being in apparent conflict with Langley's evidence, obtained from true sympathetic nerves, that postganglionic could never be replaced by preganglionic fibres. He tested the possibility further, however, by a second operation, in which he cut the scar tissue which had formed between the preganglionic stump and the former postganglionic bundle; with the result that the action of eserine again disappeared. In one experiment Anderson was able to trace the origin of the fibres which had grown across the gap and had made at least a partially functional connexion with the pupil, and he found that they came not only from the preganglionic stump, but from motor branches of the oculomotor nerve to extrinsic ocular muscles, which had been inevitably injured at operation. The functional union with the sphincter was not quite normal, since artificial stimulation of the oculomotor trunk had little effect; but it was sufficient to puzzle Anderson, who honestly recorded his beautiful observations, and confessed himself unable to explain them. To-day I think we may look on them as providing the missing item of evidence required to justify the general statement that any cholinergic fibre can functionally replace any other. Alternatively, we might regard them as affording independent, confirmatory evidence of the cholinergic function of preganglionic and voluntary motor fibres.

The Case of Sensory Fibres

If we attribute such evidential value to the results of positive regeneration experiments, we cannot properly ignore the significance of others which were negative in result. We thought, at one time, that the vasodilator peripheral axon branches from sensory fibres were cholinergic (Dale and Gaddum, 1930), but the most recent observations of Hinsey and Cutting (1933) seem to have disposed of the evidence on which that view was based. Dikshit (1934), on indirect evidence, has suggested that sensory impulses in fibres of the vagus produce their effects at central synapses by liberation of acetylcholine. Some experiments, by Feldberg and Schriever, in my own laboratory, have given direct evidence, indeed, of the appearance of acetylcholine in the cerebrospinal fluid when the vagi are centrally stimulated; but our knowledge of the factors involved in this phenomenon is not yet complete enough to warrant an interpretation of its meaning. Langley and Anderson, we must note, were unable to obtain any functional replacement of preganglionic or voluntary motor nerve-fibres by sensory fibres, whether these were growing peripherally or centrally from a sensory ganglion; so that regeneration experiments give no support to the suggestion of a cholinergic function of sensory fibres, either in the case of their peripheral, vasodilator axon-branches, or in that of their endings in central synapses. On the other hand, the antidromic vasodilatation so closely resembles that produced by autonomic nerves, and central synapses have so many points of analogy with those in autonomic ganglia, that it is reasonable to expect that some definite evidence of chemical transmission may yet be found in these cases also. Other specific chemical transmitters of nervous action, in addition to the two already known, may yet await discovery. It is to be noted, further, that in the cases for which direct evidence is already available, the phenomena of regeneration appear to indicate that the nature of the chemical function, whether cholinergic or adrenergic, is characteristic for each particular neurone, and unchangeable. When we are dealing with two different endings of the same sensory neurone, the one peripheral and concerned with vasodilatation and the other at a central synapse, can we suppose that the discovery and identification of a chemical transmitter of axon-reflex vasodilatation would furnish a hint as to the nature of the transmission process at a central synapse? The possibility has at least some value as a stimulus to further experiment.

A Change in Pharmacological Conceptions

The general conception of the mode of transmission of the effects of nerve impulses, which is even now taking shape, will obviously entail some revision of pharmacological conceptions and terminology. It no longer has any scientific meaning to say that acetylcholine and adrenaline reproduce the effects of parasympathetic and true sympathetic nerves, because they act on the respective types of " nerve-endings." It is truer to say that parasympathetic nerve impulses reproduce the peripheral effects of acetylcholine, because, when they arrive at the nerve-endings, they liberate that substance in relation to the effector cells; and the same is true of sympathetic nerve impulses and adrenaline, with the still necessary reservation as to the chemical identity of the transmitter. In either case the action of the chemical substance must be on the effector cells, and not on the nerve-endings. When atropine or ergotoxine produces its specific paralysis, it does so by rendering the effector cell specifically insensitive to acetylcholine or to adrenaline. Similar conceptions, *mutatis mutandis*, apply to the actions of acetylcholine on ganglion cells and striated muscle-fibres, and to the annulment of these actions, with blockage of the corresponding nervous excitations, by nicotine and curare respectively. We still have to account for the fact that, when a substance like acetylcholine is artificially applied, the effector cells responding to its action are predominantly those in relation to which it is normally liberated as the transmitter of nerve impulses. The correspondence, in this case, cannot be regarded as exact; acetylcholine causes, for example, arterial dilatation widely outside the limits of any cholinergic nerve supply yet demonstrated. It is close enough to have an important significance, but its meaning is by no means clear. We must remember that the effector cells having a parasympathetic innervation, and accordingly habituated to responding physiologically to acetylcholine, also show a highly selective response to other substances, such as pilocarpine and arecoline, which have no recognizable similarity, in chemical structure or properties, to acetylcholine. It is similarly difficult to trace more than a general chemical similarity between adrenaline and some of the substances which share, to varying degrees, its selective action. I doubt whether the use of such terms as " myoneural junctions," or " receptive substances," to describe hypothetical components of the effector cells, to which their selective responses may be attributed, will serve any longer to clarify the issue. Elliott's term " myoneural junctions " was introduced with reference to involuntary muscle cells and their autonomic innervation, but there is no evidence in this case for a localization of the specific excitability in the neighbourhood of the actual nerve-endings, such as the term might imply. Langley's term " receptive substances " may be used so as to imply nothing more than the existence of the specific excitability, which it is supposed to explain, but as Langley himself used it, it connotes a chemical fixation of the stimulating substances, for which there is no evidence, and with which, indeed, the lack of chemical similarity between substances having a closely similar action is hardly compatible. In the special case of the voluntary muscle-fibre, the receptive or excitable structure may be histologically distinguishable from the contractile elements, and localized near the nerve-ending as the end-plate; and it is interesting that Langley, in seeking evidence for an unlocalized " receptive substance," should have chosen voluntary muscle for his experiments with nicotine, so that he was obliged to describe a preëminence of sensibility to that substance in the neighbourhood of the nerve-endings. We cannot generalize, however, from such a highly specialized and complex structure as that of the voluntary muscle-fibre, and I do not think that, in most cases, we are entitled to draw or to imply any more exact conclusion than that the action of the specific transmitters, and of other similarly active bases, is on the effector cells and not on the nerve-endings.

How does the nerve impulse, on reaching the nerve-ending, cause the chemical transmitter of its action to appear? The evidence is meagre as yet, and not wholly

consistent. The latest results (Vartiainen, 1934) support the view that the transmitter is not newly formed by synthesis as each impulse arrives, but held in some inactivating and protective complex, from which the nerve impulse releases it, and from which it is easily separated by ordinary methods of chemical extraction. Experiments by Engelhart (1931) show that, in the one case yet investigated, this depot is dependent for its maintenance on the integrity of the nerve-endings, and that it disappears or becomes depleted when the nerve-fibres degenerate. (We may note, in passing, the probability that the exaggerated sensitiveness of the denervated effector cells, to the artificial application of the chemical transmitter, may be conditioned by this disappearance of its depot and failure of its normal release.) In accordance with the interpretations given to earlier evidence, we should take this disappearance to mean that the depot belongs to the nerve-ending; but it may merely mean that its maintenance is dependent on the arrival of nerve impulses at a normal rate, and that its depletion with nerve degeneration is comparable to an atrophy of disuse. The permanent association, however, of a particular neurone with one kind of transmission would be more easily interpreted, if the transmitting mechanism were actually a part of the nerve-ending. On either conception it seems possible to give a clearer interpretation to the actions of the only two specifically stimulant bases, for which an action on nerve-endings appears to be really supported by evidence. One case, that of eserine, I have already discussed in describing Anderson's observations. The other is that of tyramine, certain sympathomimetic actions of which were found by Burn and Tainter (1931) to disappear with nerve-degeneration and under the action of cocaine. The fact that its vasoconstrictor action was found by Burn (1930) also to disappear during artificial perfusion, and to be restored when adrenaline was added to the perfusing blood, suggests that tyramine may act by liberating the transmitter from the depot, cease to act when this is depleted, and act again when it is replenished. The same may be true of the sympathomimetic effects of ephedrine, which is chemically not distant from tyramine, and loses its action in the cat similarly with nerve-degeneration (Pak and Tang, 1933). In no other instances known to me, and in no other sense, does the description of a specific effect as due to action on nerve-endings seem yet to be justified by the evidence now available.

To the fundamental pharmacological problem, why a particular type of chemical structure, or, more mysteriously, several apparently unrelated types, should be associated with a specific action on particular types of reactive cell, we have made no nearer approach. The newer evidence merely exposes the nature of the problem, and clears the ground for an eventual attack upon it. I can picture the eager interest with which Dixon would have welcomed this clarification, which he, indeed, had in part foreseen. As pharmacology approaches one of its fundamental tasks, it will sadly miss his exuberant fertility in ideas, and the stimulus of his buoyant optimism.

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