II. THE BEGINNINGS AND THE PROSPECTS OF NEUROHUMORAL TRANSMISSION

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I am sure that you will wish me in the first place, on behalf of us all, to express our very warm thanks to the Physiological Society of Philadelphia, to Dr. Oppenheimer, Dr. Scott, the Smith, Kline and French Company, and to every other contributor, for the very generous hospitality which has been extended to us here, and for the very attractive arrangements which, following the International Physiological Congress in Montreal, have enabled us to gather now for a more intimate discussion of a subject of our common and special interest. We are particularly glad to be able to hold the Symposium here, in this historic city, where experimental science, among other things, first took firm root in the new soil of this American continent.

We have been given two days to discuss the transmission of excitation from nerve endings by the process for which the sponsors of our Symposium have used the term "neurohumoral." I am glad that they have done this, because it gives our discussions a certain link with the memory of a great American physiologist, a dear friend of mine, the late Walter Cannon, who first used it. As you will remember, Cannon's own contributions to our subject were chiefly concerned with the escape, in small quantities, of the transmitter of the effects of sympathetic—more strictly, we should now say of adrenergic—nerve-impulses, from the nerve-endings, or, perhaps, from the effector cells, into the blood stream which carried it to distant organs on which, if they had been sensitized by degeneration of their sympathetic nerve-supplies, it could be seen to produce definite, sympathomimetic effects. This observation was, of course, a sequel to Cannon's earlier work, in which he had used similar methods to detect the liberation of small doses of adrenaline (epinephrine) from the suprarenal medulla in response to various stimuli. And we may recall that the first of these later observations, in which a trace of something like adrenaline (epinephrine) escaped into the blood and was detected by its effects on distant, sensitized organs, following stimulation of sympathetic nerves which had no known connection with any chromaffin cells, was made by Cannon and Uridil in 1921. Earlier in the same year Otto Loewi had published the first of the classical series of papers in which he had given the subject of our discussion, for the first time, a firm, experimental basis.

Cannon, however, though he made so early an entry into this field, seems to have been held back from a more general exploration by concentrating his attention on the differences between the effects of the transmitter, carried by the blood from sympathetic nerve endings, and those of adrenaline injected artificially, in comparable doses, into the blood stream. So came, eventually, the theory of Sympathin, and its E and I combinations. And we are hearing now, in the Symposium, about the latest developments of the relatively recent discovery that the predominant component of the transmitter of the effects of adrenergic impulses

in a number of animal species, including the cat on which Cannon worked, is *nor*-adrenaline; and *nor*-adrenaline had for years been known to differ from adrenaline in its detailed effects, in just the direction which would enable its function as the transmitter to account for the discrepancies which Cannon and his co-workers had observed and emphasized. I can't suppress an impulse of admiration for the tenacity with which the last of Professor Cannon's distinguished collaborators, Professor Rosenblueth, in spite of all this recent evidence, still keeps the flag of the Sympathins, E and I, firmly nailed to the mast of his belief and his advocacy. Walter Cannon himself was, indeed, a tenacious champion of the theories which he framed or adopted, as well as an experimenter of outstanding skill and ingenuity, and an enthusiast for Horace Walpole's "serendipity," as he has told us in that delightful account of his own "Way of an Experimenter." I am very proud to have been honoured by his friendship, and I ask you to join me in a tribute to his memory.

Then I am sure that you will like me to tell Otto Loewi how much we all rejoice to have him with us. We were greatly saddened when his illness postponed the celebrations planned for his eightieth birthday a few months ago, and we are glad indeed to see him so fully restored and able to take, in this Symposium, the leading part which we should all of us recognize to be his as a matter of course. For me this year is one of anniversary celebrations; not only do I join in the cheers for Otto Loewi's 80th birthday; I celebrate for myself the completion of 50 years of an unbroken friendship and scientific comradeship with him. For it was in 1903 that I first met Loewi, when he came to work for some months in Starling's Institute in London; he went from there for some further months to Langley's Department of Physiology in Cambridge. Later in the same year, the late Walter Fletcher went from Cambridge to work for a spell in Hans Horst Meyer's Institute in Marburg, to which Loewi also had by then returned. And, as Loewi has told us in a recent lecture, Fletcher recalled in later years an incident of their meeting then in Marburg, which Loewi had himself forgotten. On a walk which they took together, Loewi appears to have put to Fletcher, already in 1903, the suggestion that impulses in the vagus nerve might possibly produce inhibition of the heart-beat by liberating something like muscarine from its endings. And I thought that you might be willing this evening, now that we can allow ourselves a little relaxation from the serious tension of our more formal, day-time discussion, to make a rapid survey with me of some of the landmarks in the general history of this central idea of our Symposium—the idea of a humoral, neurohumoral, or chemical transmission of the effects of nerve impulses from nerve endings. We might glance at its remoter origins, its first definite emergence, its temporary fall into disfavour and almost into oblivion, its experimental establishment for all peripheral autonomic endings by Loewi and those who followed his lead, its further extension to all peripheral nerve endings and junctions, its recent application to central synapses, and the points at which its further development may be expected or, at least, explored.

Evidence for some process of a special kind, for the transmission of excitation at the contact-junctions between nerve-fibres and excitable cells, goes back, I

suppose, to Claude Bernard, who observed that fatigue and the block produced by curare were located at such junctions. Du Bois-Reymond certainly recognized the possibility in 1877, when he suggested, and even definitely favoured, the idea of transmission from motor nerve-endings to motor end-plates by the liberation of a chemical stimulant, rather than by an electrical process, though of course he had no knowledge of a suitable substance for the function. Even before that, the action of extracts from *Amanita muscaria* in inhibiting the heart-beat in the same manner as vagus nerve impulses, had been recognized; and Schmiedeberg and Harnack had isolated Muscarine in 1869. When I was a student of Physiology in Cambridge, in the middle 1890's, one of our practical exercises, probably initiated by Walter Gaskell, was still to apply muscarine to the frog's heart, and to observe the similarity of its effect to that of vagus stimulation and the annulment of both by atropine. The only explanation offered was, of course, that muscarine presumably stimulated, and atropine paralyzed, the vagus nerve endings. That was the time when researches, first those of Gaskell and later those of Langley and Anderson, had been revealing the anatomical connections of the involuntary or autonomic nervous system, and leading to the recognition of its natural division into the thoracico-lumbar or true sympathetic, and the cranio-sacral or parasympathetic sub-systems, and the double innervation of many involuntary organs from these two main divisions, with their commonly antagonistic effects. Consequently when Oliver and Schafer, in 1895, described the astonishing activity of a suprarenal extract the way was prepared for Lewandowsky, and then Langley, to show how remarkably its effects corresponded with those evoked by stimulating the true sympathetic nerves, although its actions lasted longer. Later, of course, it became clear that they were accentuated by complete degeneration of the sympathetic nerve fibres. Then, when adrenaline was isolated and became available, T. R. Elliott, as a postgraduate research student at Cambridge, used it, at Langley's suggestion, to extend the comparison of its effects with those of sympathetic nerves over a wider range of organs and species, with the ultimate result that he published a remarkable paper on the subject, full of stimulating suggestions, in 1905. And I recall, in that connection, that Elliott had already begun that work in 1903, when Loewi was in Langley's department at Cambridge; and I remember that when Loewi came back to London, before he returned to Marburg, he and I had dinner together one evening, and he told me how much he had enjoyed discussion with Elliott and what a high opinion he had formed of his promise. The two had met and interchanged ideas; and the idea of chemical transmission from nerve endings might surely have had some mention between them then, though we cannot expect either of them to have any memory now of such talk about it, half a century ago. What we do know is that Loewi, later in the same year, threw off the suggestion that muscarine might be liberated to transmit vagus effects, though he did it so casually, it would seem, that only Fletcher remembered the mention of it; and that, early in the next year, 1904, Elliott committed himself in public and in print to a similar suggestion, with consideration in detail of the weight of evidence in its favour, for the sympathetic nerves and adrenaline. I was in Elliott's confidence at that time, and I am sure

that the idea had presented itself to him as entirely original and rather daring, though justified by the facts; while I myself was very doubtful then of his wisdom in launching it openly. To Loewi, I am sure, the vagus-muscarine idea, when he mentioned it, would have appeared entirely original with him. The only reasonable conclusion about such early happenings is, surely, that the atmosphere of thought and experience in Cambridge about 1903, was favourable to such an idea. Elliott was the first to regard it as worthy of public mention, and to summarize data of observation which it would explain.

This birth, perhaps a premature one, of the theory on which our discussions are centered, aroused little comment at the time, and its youth and adolescence were equally discouraging. Elliott seems early to have abandoned the attempt to win serious recognition for it. I must confess to having subjected it to a cold, or at best, a lukewarm douche, when, encountering the kind of differences which Cannon later emphasized, I observed that *nor*-adrenaline reproduced the effects of sympathetic nerve-impulses, without the systematic discrepancies seen with adrenaline itself. I ought, of course, to have seen that this told, not against Elliott's hypothesis in principle, but only against its perfection in detail. Later, as you know, came the recognition that acetylcholine had properties which would make it an ideal transmitter of parasympathetic effects; though I had no reason at that time to regard it as a natural constituent of the animal body, and though it exhibited, in addition, what seemed then to be rather gratuitous and embarrassing actions on ganglion cells and motor end-plates.

The general climate of physiological opinion, in any case, still remained hesitant and sceptical. The position was not unlike that to which v. Bruecke long ago compared the attitude of a physiologist to teleology: transmission by chemical mediators was like a lady with whom the neurophysiologist was willing to live and to consort in private, but with whom he was reluctant to be seen in public. And then, all that was changed when Loewi made his straightforward experiments, stood this egg of Columbus upright on its flattened end, and gave us experimental facts in place of half-discredited speculations. It was cheering, of course, to see how near those speculations had come to the truth in suggesting the identity of the transmitters.

When Loewi's discoveries had been thoroughly exploited and found to be applicable to all peripheral autonomic effects, in all classes of vertebrates, there remained for solution the puzzle of the other, nicotinic actions of acetylcholine. It is not without interest, I think, that Sherrington, already in 1925, regarded the intervention of a chemical agent as one of two possibilities to be considered to account for some of the characters of transmission at central synapses; and that Adrian, in 1933, showed that his mind was open to the possibility of such a process at the junction of motor nerve-endings and voluntary motor end-plates; and that both of them cited Loewi's observations on the frog's heart as furnishing a possibly valid analogy. Loewi, on the other hand, also in 1933, seems to have taken alarm, for the time being, at the thought of such a possible extension of his discovery, and to have gone to the length of establishing his own alibi by a public disclaimer of belief in chemical transmission at the motor nerve endings.

Later, when I first told Sherrington about our direct evidence for it, he was eager to emphasize its significance, by analogy, for chemical transmission at synapses in the central grey matter—the possibility of which he had himself so clearly foreseen some years earlier. "I have always assumed," he said to me, "that the process at the motor nerve-ending must provide a model, or paradigm, for that at the central synapses."

There were others, of course, whose reactions were the converse of this. Recognizing the force of the analogy, and finding its implication subversive of what they had long believed, they were led to doubt either the factual accuracy of our observations on the peripheral synapses, or the interpretation which we had given to them. I should not like to be misunderstood as having at any time resented this scepticism. On the contrary, I believe that it was, at least on balance, greatly to the advantage of the development of the subject. We used to call Jack Eccles our sparring-partner, and I remember writing to him on one occasion to protest against signs of an apparent weakening of his repudiation of cholinergic transmission at motor nerve endings. In any event, as you all know, Eccles, with Katz and Kuffler, together for a time and later separately, have produced an account of the manner of the intervention of acetylcholine, liberated from motor nerve endings in direct relation to the motor end-plate, much more convincing in its detail than any which we could have hoped to attain. Eccles, however, continued for some time to dig his toes in firmly in opposition to any suggestion of a cholinergic transmission at synapses in ganglia, still maintaining that it must there be purely electrical. One could hardly avoid a suspicion that he must still be unconsciously influenced by the somewhat closer analogy between these peripheral interneuronal synapses and those of the central grey matter; the suspicion was confirmed by the dramatic change in his attitude a little more than a year ago. Eccles and his co-workers had by then succeeded in recording the electrical potential between the outside and the inside of single motor-horn cells of the cat's spinal cord—a technical achievement, surely, of a very high order; they thus found that synaptic inhibition of such a cell entailed—appeared, indeed, to be due to—an increase, a positive variation, of this resting potential. This phenomenon recalls Gaskell's record, as long ago as 1887, of a positive variation of the resting potential of the quiescent auricle of a tortoise whenever its inhibitory vagus nerve was stimulated. Eccles and his team concluded that this positive variation in the motor horn cell could only be due to the release of a chemical agent from the endings of the afferent fibre making synaptic contacts with its surface, and that, if synaptic inhibition was thus chemically transmitted, synaptic excitation was unlikely to be transmitted by an essentially different process, though the transmitter might probably be a different one. By obvious analogy, it was to be supposed that some chemical agent or other would be effective at all central synapses, and that being accepted, Eccles was naturally ready to take cholinergic transmission in the ganglion in his stride. A remarkable conversion indeed! One is reminded, almost inevitably, of Saul on his way to Damascus, when the sudden light shone and the scales fell from his eyes. There had, of course, been scattered evidence already, suggesting cholinergic transmission at

some central synapses; but I think everybody will now agree that the indications are unfavourable to either of the known peripheral transmitters, acetylcholine or one of the adrenalines, as the agent responsible for the monosynaptic, reciprocal transmission to the motor horn cells. We seem rather to need to look for at least two others. The wide recognition of such a possibility, and of its importance, is indicated by the search, on the one hand, for possible candidates in extracts from dorsal nerve-roots, and experiments, on the other hand, which appear to indicate that the chemical transmitter of "antidromic" vasodilatation is something different from acetylcholine, or either of the adrenalines or histamine, and even to suggest that it might be ATP!

If all those indications prove to be justified, it seems likely that only the edge, the mere peripheral fringe, of a great problem has yet been touched. I feel it a great privilege to have had some contact with it during these opening stages. A big new advance may be due at any time; though, on the other hand, I hardly expect it to come in my time. I am remembering that 17 years passed between Elliott's first published suggestion of a process of this kind, and Loewi's first and completely convincing demonstration of its existence. I remember, even, that 37 years elapsed between Du Bois-Reymond's rather premature suggestion of a chemical agent for transmission from motor nerve endings, and the first hint that acetylcholine had properties which, surprisingly, might fit it for that function, as well as for transmission at peripheral parasympathetic endings; and that yet another 20 years passed before there was evidence fit for publication in support of its function as the transmitter of these rapid effects of voluntary motor nerve impulses. Perhaps the further pauses and latent periods will not be so long; on the other hand, the technical difficulties of further exploration seem likely to be disproportionately formidable.

There are several questions touching upon the subject of the Symposium, other than those which figure on its programme, which appear to interest and even to agitate the minds of some of our distinguished colleagues. There is one which I myself posed as long ago as 1937, in a lecture which I then gave in New York. "If," I said, "the liberation of a chemical mediator at a nerve ending should prove to be not a process peculiar and limited to that ending but merely a local intensification, to ensure transmission to a contiguous cell, of a process which actually figures in the propagation of the impulse along the nerve fibre, we should have to make yet a further revision of our existing conceptions. Some minds have undoubtedly felt difficulty in postulating a complete breach in the nature of the processes concerned in transmission, where the excitation passes from nerve ending to effector cell. This particular difficulty would then disappear, but only at the cost of a more fundamental change of conception concerning the nature of the propagated wave of excitation than any which has yet been seriously considered."

Since then, largely through the devoted researches of Dr. Nachmansohn, we have learned a good deal more about the occurrence of the transmitter, and in particular of acetylcholine, with the enzymes of its production and destruction, along the whole length of the neurone; on the other hand, I think that the histo-

chemical work of Koelle and his co-workers has made clearer than ever the concentration of cholinesterase at the nerve endings. The idea that liberation of acetylcholine is the first event in the depolarization process due to stimulation anywhere, and at each stage, therefore, of the electrically produced conduction of the impulse along the fibre, so that the whole procedure is continuous across the junction to the next cell, and there is no peculiar and specifically chemical phase of it there—such an idea must, indeed, be attractive to anybody with an instinct for simplification. I think we are all conscious of this liking for order and symmetry; but I believe that it can easily lead us astray in biology, by tempting us to choose the facts which fit a theory, and to ignore the awkward ones. In this instance, I believe that some of the trouble is due to an instinctive, though entirely fallacious assumption, that cholinergic function implies sensitiveness to acetylcholine. Of course it does not; there is no evidence that the motor neurones, or the preganglionic neurones ending in a sympathetic ganglion, are sensitive to acetylcholine. They are cholinergic; but it is the predominantly adrenergic ganglion cells and the muscle end-plates, in contact with which the cholinergic fibres end, which are sensitive, and exquisitely so, to acetylcholine. I have wondered sometimes if it would help our less biologically minded biochemical colleagues if one suggested a separate terminology—cholinoceptive and adrenoceptive, perhaps—to denote sensitiveness to these transmitters, and to distinguish it from activity in releasing them to act upon other, contiguous cells. I have suffered so much, however, from the action of colleagues in kidnapping my verbal offspring for what I regard as improper uses, that I am reluctant to beget any more.

Looking at the whole situation now as an increasingly distant, but still interested, observer and trying to keep myself in still intelligent contact with all the remarkable developments about which we have been hearing this morning, I am disposed to hope that we shall not forget that in transferring one phase of our problem so definitely, from the charge of electro-physiology to that of pharmacology (to what I have, on occasion, referred to as auto-pharmacology) we shall not begin to think that we have really solved the problem. We have, obviously, done no more than state it in new terms, allowing for a new order of facts. I still talk to my old friend, T. R. Elliott, who was so intimately concerned with the beginning of the present stage; he often says to me, "Dale, you won't have done anything towards an ultimate solution until you have discovered why acetylcholine and adrenaline should, each of them, augment the activity of one tract of involuntary muscle and inhibit that of another, which, in all other respects, appears to be an entirely similar tract." I have little doubt that he is right, and that our problem today is in a not very different position from that in which Schmiedeberg left it.

We shall be having recourse, no doubt, to Physical Chemistry, in our search for a more radical solution; ultimately, perhaps, those still concerned with an active investigation of the problem may find themselves driven, in turn, to call electro-physiology again in aid, when pharmacology by itself can make no further progress. I certainly do not expect that a final solution will appear in my time.