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THE PHYSIOLOGICAL BASIS OF NEUROMUSCULAR DISORDERS*

BY

Sir HENRY DALE, O.M., G.B.E., F.R.C.P., F.R.S.

To-day's discussion is to deal with the bearing of recent physiological observations, and of the interpretations which have been applied to them, on clinical studies of neuromuscular disorders. The choice of myself as opener, and of subjects by those who are to follow me as invited contributors, would appear to direct our discussion chiefly to the clinical applications of the large and growing body of evidence for the transmission of effects from the endings of nerve fibres to contiguous responsive cells by the liberation of chemical transmitters.

Chemical Transmission at the Periphery

We may take for granted, as generally accepted, that the effects of impulses in the efferent post-ganglionic fibres of the autonomic nervous system are so transmitted to involuntary muscle and gland cells by the liberation of one of two known transmitters—adrenaline (sympathin) and acetylcholine. In this case the nature of the transmitter determines also whether the effect produced by the arrival of nerve impulses at the endings will be augmentation or inhibition of a spontaneous activity; and the two transmitters commonly produce opposite effects on the same layer of involuntary muscle. How they do so is one of the fundamental problems of pharmacology; but we might note in passing that it does not seem to have any intelligible relation to the hypothesis, which we shall meet later, according to which conduction of excitation along nerve fibres, and from their endings to receptive cells, is one uniform and electrically conducted process, everywhere due to the depolarizing effect of liberated acetylcholine.

For the purpose of the discussion I think that it must be said that these phenomena of chemical transmission from peripheral involuntary nerve endings, though we have been considering them as theoretical possibilities for nearly 45 years and have known them as well-established experimental facts for more than 25 years, have had relatively little influence on the understanding of clinical conditions. Their main effect on clinical practice has been, I think, to clarify understanding of the actions of well-known medicinal agents such as atropine, physostigmine, adrenaline itself, and the ergot alkaloids, and to guide the search for new ones such as carbachol, prostigmin, the fluorophosphonates and simpler organic phosphates, ephedrine, and others. It may be, however, that the application of this more intimate knowledge concerning the mechanism of the

control of such involuntary functions has not yet been exploited to its full value in clinical medicine.

I suspect that it may have been largely forgotten by now that Otto Loewi, many years before his epoch-making experiments on chemical transmission, had described in Graves's disease a supersensitiveness of involuntary muscle to adrenaline of such a kind that the mere instillation of it into the conjunctival sac of the patient produced abnormally a dilatation of the pupil. I wonder whether there may not be other abnormal reactions of involuntary muscle to the transmitters or their analogues yet to be discovered which might be useful in diagnosis or indicative of aetiology. I know, of course, that there has been a voluminous literature on what were called the "vagotonic" and the "sympathicotonic" diatheses; and I am not unaware of the perils to which a sprightly imagination may expose its owner when given the freedom of such clinical borderlands. I only raise the question, then, whether the positive knowledge and the specific reagents which we now possess might not be used to commit some of such conceptions to the crucible of experiment, and to enable us perhaps, with the refined product, to approach nearer to the ideal of that stimulating sceptic among my clinical teachers the late Mr. Barrett Lockwood, who used to offer to believe in a diathesis when one of his physician colleagues could show it to him "on a plate."

Before leaving the transmission of nervous effects at the periphery to involuntary effectors we ought to take note of the suggestion that histamine may act as a third transmitter. The evidence, not yet by any means so definite as for the two already mentioned, indicates that histamine may be released by the so-called "antidromic" nerve impulses at the endings of those terminal nerve branches which supply the networks of minute blood vessels to the skin.

Transmission at Ganglionic and Nerve-to-end-plate Synapses

I have no doubt that to-day's discussion will centre mainly on the interpretation of various defects of nervous and muscular function in the light of the evidence obtained in more recent years for a chemical transmission of the excitatory process from the endings of motor-nerve fibres to the motor end-plates of voluntary muscles, from the endings of preganglionic nerve fibres to the nerve-cells of autonomic ganglia, and perhaps at certain comparable synaptic junctions in the central nervous system. In all the cases concerning which we have clear evidence so far the

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transmitter is acetylcholine; and the actions of that substance on the motor end-plates and on the autonomic ganglion cells are those which are grouped together as its nicotine actions, because these structures are sensitive to acetylcholine after atropine has annulled its peripheral effects on the involuntary effectors and are, on the other hand, made insensitive to acetylcholine by excess of nicotine, by curarine, or, as we must bear in mind, by a persistent excess of acetylcholine itself.

Before we consider particular clinical abnormalities in relation to this conception we ought to have a clear idea of what it involves, and to note that it cannot yet be regarded as accepted by all who concern themselves with such matters. In the form in which my colleagues and I have advocated it the theory postulates chemical transmission at motor myoneural and ganglionic synapses as a process essentially different from that by which the excitatory process is conducted along either a nerve or a muscle fibre in continuity. It is incompatible, accordingly, with the facts that the excitatory transmission is irreciprocal only at these junctions and that it is blocked only at these by poisons such as curare or modified by such as physostigmine, contrasting sharply in these respects with conduction along continuous fibres, which goes equally well in both directions and is not elicited, stopped, or in any way modified by acetylcholine itself or by the alkaloids mentioned in concentrations even much greater than those which are so effective at the synapses. It may be said, further, that our conception restricts the transmitter function of acetylcholine to the sites where nervous impulses demonstrably liberate it, where it can be shown to act in the manner required, and where its action is annulled or modified by agents which comparably alter the effects of nerve impulses incident there.

We are content, while others seek further evidence, to accept the prevailing view of the momentary process of excitation at a point on a nerve or muscle fibre as due to a biochemical change, possibly the mobilization and reattachment of potassium ions, entailing a momentary loss or reversal of a resting surface-potential at each successive point as the process is conducted in either direction along the fibre by the successive closure of local electrical circuits. When this electrically conducted process reaches the ending of the nerve fibre we suppose that it releases a tiny charge of acetylcholine which during its momentary persistence causes an excitatory depolarization at the surface of ganglion cell or motor end-plate, presumably by a mobilization there of potassium ions, and that the process of excitation is then, as before, electrically propagated along the post-ganglionic nerve fibre or the muscle fibre. It is demonstrable by experiment that when ganglion cells or motor end-plates are treated with curarine they no longer respond to application of acetylcholine or to nerve impulses incident at the synaptic endings but still respond normally to the application of a potassium salt.

This conception, as I said, has not yet won general acceptance. There are those who find it difficult to resign the idea that electrical conduction will suffice to get the excitation across the junctions as well as along the continuous fibres; but they appear thus to become involved in complicated subsidiary hypotheses to account for the special character of transmission at the synapses and for the appearance there, on arrival of nerve impulses, of acetylcholine in a concentration adequate to excite ganglion cells or end-plates. Others, in particular Dr. Nachmansohn, have also tried to eliminate the special character of synaptic transmission by supposing that the release and disappearance of acetylcholine provide also the mechanism for the excitatory depolarization at each successive point on nerve

and muscle fibre. They wish to have excitation everywhere cholinergic and everywhere electrically conducted. There is a kind of attraction, especially for some minds, in such comprehensive master-key conceptions. But the proponents of this one have also the formidable task of upholding it against most of the direct evidence. Nobody has been able to show that acetylcholine excites nerve fibres: according to the most recent evidence they are as indifferent to it as to cane-sugar; while its excitation of effector and nerve cells first brought it to notice and has become a physiological commonplace. Even the fact that acetylcholine and the enzymes concerned with its formation and destruction are to be found along the course of nerve fibres and are not wholly concentrated at their endings applies only to cholinergic fibres, whereas others conduct impulses by a process not perceptibly different.

I think that it can also be claimed that this theory of the intervention of a special pharmacodynamic phase in the transmission of the excitatory process at these peripheral synapses, and especially from motor-nerve endings to muscle end-plates, has already contributed to a better understanding of certain neuromuscular disorders than any which could be offered by the older theory of a uniform electrical mechanism of conduction along fibres and across synapses. On the latter basis we could only discuss whether a particular anomaly was due to abnormal function of nerve or of muscle, and it offered no special explanation for defects at the synapse when excitability and conductivity of both nerve and muscle were normal. There are, of course, conditions affecting nerve fibres or muscle fibres as a whole—the neuritis caused by diphtheria toxin or by other poisons, the nerve degeneration and muscular atrophy following the attack of the poliomyelitis virus on anterior horn cells, or other so-called muscular dystrophies. None of these, however, are our concern. It is the functional defects or anomalies affecting transmission from motor nerve to muscle without anomaly of conduction in the fibres of nerve or muscle on which the evidence for a chemical mechanism at the junction throws light. Such anomalies may be produced by vegetable alkaloids or bacterial toxins, or by morbid conditions of obscure aetiology.

Action of Some Poisons

Perhaps the way in which the chemical theory can add detail and give a specific character to knowledge concerning transmission at a synapse can be most easily presented by considering first the actions of certain poisons. Since Claude Bernard we have known that curare blocks transmission from motor-nerve ending to muscle end-plate, leaving both nerve and muscle fibre normally excitable and conductile. On the electrical theory of synaptic transmission we could go no further; curare might stop conduction in the naked nerve terminals or make the end-plate inexcitable. Now we know by specific experiments, including those made by Buchthal with minute direct applications to the isolated unit, that curarine blocks the effect on the end-plate not only of an impulse reaching the nerve endings but also, and simultaneously, of the application of a minute quantity of acetylcholine, but leaves the same end-plate normally responsive to direct electrical stimulation and to the application of a small dose of potassium chloride. This enables us to conclude that the effect of curarine is to make the muscle end-plate specifically insensitive to acetylcholine; and the same can be shown to hold for the ganglion cell when curarine is appropriately applied to it. At the same time it makes clear the danger of applying such terms as “curarizing” or “curare-like” loosely to any action which interrupts or weakens transmission from motor nerve to voluntary muscle. There are obviously several ways in

which superficially similar effects of this kind could be brought about: paralysis of conduction in the naked terminal nerve branches; failure of the adequate liberation of acetylcholine from their actual endings either because the depots normally holding it ready there for release are not adequately replenished or because, though full, they do not yield their store when a nerve impulse reaches them; and excess of cholinesterase in such relation to the depots that acetylcholine, though normally released, is destroyed too quickly to produce its full effect. All these should, if possible, be eliminated by experiment before an action is described as "curare-like." An example has recently been provided by the action of the extremely potent toxin of *Bacillus botulinus*. This interrupts the transmission and leaves both nerve and muscle normally excitable, like curarine; but experimental analysis in greater detail has shown that the muscle end-plates remain normally responsive to acetylcholine and that it is the release of acetylcholine by nerve impulses which fails. The action is on the nervous side of the synapse, but whether on conductivity in the ultimate branches of the nerve fibres or on the filling or the stability of the depot for acetylcholine still remains to be discovered.

A more complicated problem is presented by the action of tetanus toxin when administered by injection into a muscle in such manner and dosage as to produce a local persistent tetanus of the muscle. As A. M. Harvey has shown, the condition seems to be accounted for by lowering of the retention-level of acetylcholine in the nerve-ending depots and a concomitant loss of cholinesterase from their neighbourhood so that acetylcholine leaks continuously on to the motor end-plates, while the arrival of a nerve impulse at the endings elicits only a weak but repetitive twitch from the muscle. When the tetanus is abolished by curarine the muscle responds normally to direct stimulation. It does not seem possible to account for such a condition by any elaboration of the electrical theory of neuromuscular transmission.

It is instructive to note the kind of explanation the chemical transmission theory offers for the previously obscure apparent antagonism between physostigmine (eserine) and a partially paralytic dose of curarine. There seems to be no reason to suppose that physostigmine directly interferes with the depression by curarine of the sensitiveness of the muscle end-plate to acetylcholine. The position seems rather to be that in partial curarization the amounts of acetylcholine making effective contact with the end-plates fall below the stimulation threshold for many of these, and for a greater proportion of them with the smaller, though normally still supraliminal, charges of acetylcholine which are released by the later stimuli of a series. When physostigmine is given and depresses the cholinesterase in the neighbourhood of the depots it allows the acetylcholine reaching the end-plates again to rise above the stimulation threshold for a larger proportion of them, and even perhaps by its persistence to cause a repetitive response of some and thus still further to raise the total tension of the resulting "twitch" towards the normal.

Anticholinesterases in Myasthenia Gravis

Probably the best example of the application of the chemical transmission theory to the elucidation of a morbid neuromuscular defect is afforded by myasthenia gravis. It was shown by Blake Pritchard that the response of a myasthenic muscle to rhythmic stimulation of its nerve was very similar to that of one which had been partially paralysed by curare. Some years later Mary Walker was led by descriptions of the alleviation of such effects of curare by physostigmine to try the latter and its synthetic analogue,

known by the proprietary name "prostigmin," in the treatment of myasthenia. The evidence for the transmitter function of acetylcholine and for the part played therein by cholinesterase was at the time becoming more generally known. It was natural, therefore, to connect the demonstrated relief of the myasthenic condition by physostigmine and its analogues with the action of these substances—their only common and specific action, indeed—in depressing cholinesterase and thus protecting acetylcholine. More recently other substances, such as diisopropyl-fluorophosphonate (D.F.P.) and tetramethyl-pyrophosphate, having nothing in common with physostigmine and its more immediate analogues other than a powerful anticholinesterase action, have proved to be similarly effective, and we shall hear more about these later this morning.

It was natural to look for evidence of an abnormal amount of cholinesterase in the blood of the myasthenic, but a number of such attempts failed to produce evidence of significant departure from the normal average in this respect; nor have muscle biopsies given any clearer indication. We seem at present to be left with several possibilities:—(1) It is not yet quite excluded that cholinesterase may be abnormally abundant in effective relation to the acetylcholine depots, though failure to find it in excess where it can be measured has rendered this unlikely; (2) there may be a defect of synthesis of acetylcholine and in consequence a defective replenishment of the depots at the nerve endings; (3) the sensitiveness of the muscle end-plates to acetylcholine may be lowered, as in partial poisoning by curarine—it may even be thus reduced by the action of an endogenous curarizing poison.

It will be clear that the remedial effect of the anticholinesterases could be accounted for by any one of these conditions, and thus affords no help in choosing among them. Experiments by Harvey and Lilienthal on the effects on the hand and forearm muscles of injecting acetylcholine directly into the brachial artery at the elbow seemed at first to suggest that the myasthenic muscles had an abnormally high sensitiveness to acetylcholine thus applied, giving a response to it which recalled that of a muscle at an early stage of the degeneration of its motor nerve. If this suggestion had been maintained it would have pointed to a defective storage of acetylcholine at the nerve endings, and thus to the causation of the myasthenia by a defect on the nervous side of the synapse. Later study of these responses has made it doubtful whether the contrast with that of the normal muscle is really of this kind—whether in fact the normal muscle is not more sensitive and in consequence more susceptible to the secondary depressant action of acetylcholine in the large arterial doses in which it was injected. If that were so the condition would be really like a partial paralysis by curare, causing a defect on the muscle side of the synapse, and the provenance of the poison causing it would become a matter of central interest. We could not, of course, overlook the possibility of the production of such a poison by an abnormally persistent thymus, the operative removal of which has been dramatically successful in a good proportion of severe cases of myasthenia though completely ineffective in others. We may hear something on the subject from Dr. Wilson, who has, I know, been examining thymuses removed by operation from myasthenics for the presence of a curarizing poison. Clearly, much more has to be discovered before we can regard the nature of this myasthenic defect as settled, but I do not think I go too far in claiming that the chemical transmission theory has helped to provide a new basis for its investigation.

Another condition, with which Dr. Brown is going to deal, is that of congenital myotonia (Thomsen's disease). I think

that the evidence which Dr. Brown will produce will show that the fault in this disease is not so much in the nature of the neuromuscular transmission as in the anomalous response of the muscle fibres to any kind of stimulus; but I do not think it can be doubted that the chemical transmission theory, and the experimental criteria based upon it, assisted a rapid analysis and clear decision in this case also.

Mention should be made also, perhaps, of the recurrent familial paralysis following a large ingestion of carbohydrate, first found by Aitken, Allot, Castleden, and Walker to be associated with a fall in the potassium content of the serum to about one-half its normal value or less and to be promptly relieved by giving potassium chloride. There was no evidence for failure of the excitatory process before it reaches the muscle, which during the paralytic crisis fails to respond to direct electrical stimulation.

Conclusion

I do not know whether anybody is going to deal in discussion with the recently advocated use of the quaternary tetraethylammonium salts in circulatory hypertension. Many years ago Dr. Burn and I pointed out that, whereas the tetramethyl compounds had a powerful nicotine action, their tetraethyl homologues had only a rather weak action of the curare type. Acheson recently confirmed this, emphasizing the fact that, in contrast to curarine, the tetraethylammonium salts depress the responses of the ganglion cells to preganglionic impulses and to acetylcholine more potently than they affect those of the muscle end-plates. If there is reason, then, to attribute a hyperpiesis to excessive outflow of preganglionic sympathetic impulses, tetramethylammonium salts might be expected preferentially to weaken their transmission through the ganglia.

Finally, the question which naturally excites a most urgent interest in the mind of anybody working in this field is that of the extent to which the evidence we have for a chemical transmission at the peripheral synapses provides an analogy for the mode of transmission at synapses in the central nervous system. If we are entitled to assume that cholinergic function at the synaptic endings will be marked by the presence of acetylcholine, and of the power to synthesize it, along the whole course of the fibres concerned, we can apparently conclude that the sensory fibres in the dorsal roots and tracts as far as the first synaptic connexions are definitely not cholinergic. Dr. Feldberg and Miss Vogt, on the other hand, have been finding by the same criterion evidence for a probable cholinergic function of the secondary neurones; Professor Miller is going to produce evidence of a more direct and functional kind for cholinergic transmission in the synapses of the hypoglossal nucleus and its connexions; and Professor Samson Wright is to deal with the action on central nervous functions of cholinesterases of various types. In the background, as it were, we have Professor Harvey's account of the very remarkable psychopathic effects, of the type of an anxiety neurosis, produced in man by continued treatment with D.F.P. in addition to the expected symptoms of excessive parasympathetic effects at the periphery. There is obviously a big field of possibilities here to be explored, and a need for great care and scientific discipline to prevent imaginative speculation from running ahead of the evidence. And we must not forget that, if we accept analogy as indicating, even as a working hypothesis, the probability of a chemical mode of transmission at *all* central synapses, then we must be prepared to look for transmitters other than those already recognized at the periphery and to be on the alert for their discovery.

FLOCCULATION TESTS: CHEMICAL AND CLINICAL SIGNIFICANCE*

BY

N. F. MACLAGAN, D.Sc., M.D., M.R.C.P., F.R.I.C.

Professor of Chemical Pathology in the University of London at Westminster Hospital Medical School

The application of the electrophoretic technique to pathological sera has led to the recognition of a relative increase in the serum gamma-globulin content as a fairly frequent event in certain diseases. These can be roughly divided into liver diseases—hepatitis, cirrhosis, chronic passive congestion—on the one hand, and infections on the other (Longworth, Shedolovsky, and MacInnes, 1939; Kekwick, 1940; Gray and Barron, 1943; Olhagen, 1947; Wuhrmann and Wunderly, 1947).

In the infective group the change is particularly prominent in bacterial endocarditis, malaria, lymphogranuloma, and certain cases of plasmocytoma, and probably reflects an increase of circulating antibody. In hepatic diseases it is thought to result from abnormal protein synthesis in the liver. In many of these conditions the demonstration of gamma-globulin excess could have considerable diagnostic and prognostic importance, as it often occurs without alteration of the total globulin level or of the albumin-globulin ratio. However, for technical reasons it is impracticable to rely on electrophoresis for routine diagnostic purposes and we may profitably inquire whether any technical short cut to the required information is available.

The short cuts proposed are of course the so-called flocculation tests, and the large number of these that have been invented is perhaps an indication of the need for a reliable method of demonstrating the change in question. There are now at least nine tests that need to be considered. Listed in chronological order they are:

Formol Gel (Gaté and Papacostas, 1920)
Takata-Ara Reaction (Takata and Ara, 1926; Jezler, 1930)
Weltmann Coagulation Band (Weltmann, 1930)
Cephalin Cholesterol (Hanger, 1939)
Colloidal Gold (Gray, 1940; MacLagan, 1944a, 1946)
Thymol Turbidity (MacLagan, 1944b)
Thymol Flocculation (Neefe, 1946a; MacLagan, 1947)
Cadmium Sulphate (Wunderly and Wuhrmann, 1945)
Sharlach Red (Maizels, 1946)
Zinc Sulphate (Kunkel, 1947)

Most of these tests were invented on purely empirical grounds some years before their mechanism was understood, and it is only comparatively recently that their common chemical basis has been recognized. Although there are considerable individual variations in the different tests, it is now clear from the work of Gray (1940), Kabat, Hanger, Moore, and Landow (1943), Olhagen (1947), MacLagan and Bunn (1947), and Wuhrmann and Wunderly (1947) that they all become positive in the presence of a sufficient increase in the gamma-globulin fraction, particularly if the albumin is also reduced. The tests are technically simple and rapid in execution, and are capable of yielding results of diagnostic value within a few minutes or hours. In each case the reagent has no pronounced effect on normal serum but produces an easily observable result such as turbidity, flocculation, precipitation, or gel formation with abnormal sera.

*Based on a paper read to the Section of Physiology, including Biochemistry, at the Annual Meeting of the British Medical Association, Cambridge, 1948.