ACETYLCHOLINE AND THE PHYSIOLOGY OF THE NERVOUS SYSTEM

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IN a text-book, "Physiology of the Nervous System,"[1] published in 1938, the opinion was expressed that evidence then available to support the theory of "chemical mediation" in the central nervous system was unsatisfactory. Since the problem appeared to be at that time (early in 1938) still open to discussion, only a few short paragraphs were devoted to it. This view was criticized by Forbes[2] and later by Sir Henry Dale[3] both in the columns of SCIENCE. Sir Henry was particularly opposed to the statement that there was "no evidence fit for critical examination that would place the liberation of acetylcholine as a *primary* event, central or peripheral."[4]

Five years have now elapsed. Recent developments have changed many aspects of the problem and its reexamination is desirable. In the text-book[1] it was emphasized that the work of Eccles and Sherrington and that of Lorente de Nó indicates that the excitable properties of the central neuron do not differ fundamentally from those of the peripheral axon, except in the degree of polarization. Hence, it appears unnecessary to assume a mechanism at synapses fundamentally different from that in the axon. At the Symposium on the Synapse in 1939, Gasser and Erlanger[5] reviewing the problem arrived at the same conclusion, *i.e.,* that conduction along fibers and across synapses differ only quantitatively.

Electrical recording instruments have a high degree of perfection. With the oscillograph changes of potential are recorded virtually without inertia. It is difficult to conceive that conclusions based on electrical signs of nerve activity can be contradicted by observations based on methods which are slow, and therefore less appropriate to follow the rapid events accompanying nerve activity. The theory of Dale and his associates that acetylcholine might be the transmitting agent of nerve impulses across synapses and neuromuscular junctions, was based essentially on the type of evidence adduced previously by Otto Loewi in studying the action of autonomic nerves on effector organs: *i.e.,* liberation of acetylcholine by stimulation of motor nerves and preganglionic fibers, establishment of the stimulating action of acetylcholine in small amounts, and the potentiation of the acetylcholine effects by previous eserinisation. Although there remained some contradictions and difficulties, well summarized by Eccles[6] in his review, these were the facts that aroused suspicion and caused criticism. In the case of autonomic nerves the evidence appeared adequate for the assumption that a liberated substance acted as the "mediator"; but transmission of nerve impulses across synapses and neuromuscular junctions are rapid events which occur within milliseconds or even within a fraction of a millisecond. Sir Henry and his associates admitted that this time factor was the chief difficulty encountered by their theory. As long as the time course of the reaction was not established evidence that the liberation of acetylcholine was the *primary* event remained unsatisfactory. The liberated acetylcholine could be a byproduct of nerve metabolism, however essential for the mechanism of nervous action, but *following* the passing of the electrical change. The attempt of MacIntosh[7] to determine the correspondence between the period of preganglionic stimulation and the period during which acetylcholine is present in the perfusate by collecting the venous effluent in 30 second-samples can not be considered as an adequate approach to this essential problem of the time-relation: for 30 seconds constitute an interval some 10,000 times longer than the period during which the impulse passes through those foci.

An entirely different approach to the problem was made by Nachmansohn and his associates. They investigated the problem whether at synapses and neuromuscular junctions the rate of acetylcholine metabolism is as high as required by the assumption that it is a transmitter of nerve impulses. For, if it is the primary event in this transmission, one must postulate that it appears and disappears at least at the same speed as the electrical changes. Little is known about the mechanism of acetylcholine appearance during stimulation; but the ester is inactivated by the specific enzyme, choline esterase, which splits it into its two components. Studies on the concentration and distribution of this enzyme have revealed that at neuromuscular junctions and ganglionic synapses, as well as at all synapses of the central nervous system choline esterase is sufficiently high in concentration to split within milliseconds amounts of acetylcholine which, if released at those foci, would be in sufficient concentra-

[1] Fulton, "Physiology of the Nervous System." Oxford University Press, 1938. Pp. xv + 675.
[2] Forbes, SCIENCE, 90: 17, 1939.
[3] Dale, SCIENCE, 90: 393, 1939.
[4] Fulton, SCIENCE, 90: 110, 1939.
[5] Symposium on the Synapse, *Jour. Neurophysiol.,* 2: 361–474, 1939.
[6] Eccles, *Physiol. Rev.,* 17: 538, 1937.
[7] MacIntosh, *Jour. Physiol.,* 94: 155, 1938.

tion for a stimulating action. And if a substance can be hydrolyzed in a cell at such a high rate it is possible, and even probable, that it can appear at a similar high rate. The work on isotopes has shown that in living cells enzymes are continuously active. A high concentration of a specific enzyme can therefore be accepted as indication of the high rate of the metabolism of its substrate. The results have recently been reviewed by Nachmansohn.[8] They make possible the assumption that acetylcholine intervention is a *primary* event essential in synaptic transmission. The physiological significance of the enzyme mechanism is, moreover, emphasized by the observation that during embryonic development the time when the high enzyme concentration appears coincides with the time when the function of those foci begins.

Further investigations on the activity of choline esterase has led, however, to a modification of the original concept. The new concept is easily compatible with the conclusions of the American and Australian electrophysiologists cited above. According to this concept[9,10] acetylcholine metabolism is intrinsically connected with the electrical changes occurring everywhere at the neuronal surface. Hence, it is only quantitatively more important at the synapse where the neuronal surface increases considerably due to the extensive end-arborisation. The new concept is based essentially on two lines of evidence.

(i) *Localization of choline esterase inside the nerve cell.* In the experiments on the rate of acetylcholine hydrolysis in nervous tissue it was early noticed that the enzyme activity is high everywhere in nervous tissue and the difference between synaptic regions and axon is only a quantitative one. This was particularly obvious in non-myelinated fibers like the sympathetic chain of mammals or the abdominal chain of lobsters.[11] Hence it was concluded that acetylcholine metabolism differs only quantitatively between axon and synapse.[12] This is in agreement with the observation of Lorente de Nó[13] that acetylcholine is liberated in preganglionic fibers as well as at synapses. This observation, contested at one time by the Hampstead school, has meanwhile been confirmed by Lissak.[14] The situation became clearer when experiments on the superior cervical ganglion of cats (after section of preganglionic fibers) indicated that the enzyme might be concentrated at or near the neuronal surface.[15] Further evidence favoring this assumption has emerged from experiments on the giant axon of squids in which it was shown that the enzyme is localized almost wholly in the sheath, the amount in the axoplasm being negligible.[16]

Bio-electrical phenomena occur at surfaces. The localization of the enzyme at the surface and the high rate of acetylcholine metabolism are particularly pertinent in view of experiments in which a close parallelism could be established between the electromotive force of the action potential and the concentration of choline esterase.

(ii) *Electromotive force and concentration of choline esterase.* This parallelism has been brought out in experiments on the electric organs. The discharge of these organs is in principle identical with the action potential of ordinary nerves. The high voltage is obtained by the fact that the electric plates are arranged in series. In the electric organ of *Electrophorus electricus* (Linnaeus), the species with the most powerful electric organ as yet known, there are several thousand electroplaxes arranged in series. The maximal discharge of some specimens rises to more than 800 volts.

High concentrations of choline esterase are found in the strong electric organs of *Electrophorus electricus* and *Torpedo*. These organs hydrolyze in 60 minutes amounts of acetylcholine equivalent to 1–3 times their own weight. Since in the larger specimens the organs have a weight of several kilograms the amount of acetylcholine which can be split in these organs may amount to many kilograms in 60 minutes, *i.e.,* several milligrams in one millisecond. This high rate of metabolism makes possible the assumption that acetylcholine is closely connected with the discharge. The high enzyme concentration is particularly significant in view of the low protein (2–3 per cent.) and high water content (92 per cent.) of these organs.

In the weak electric organ of *Ray* the enzyme concentration is relatively low. If, in the three species, electromotive force per cm and number of plates per cm are compared with the concentration of choline esterase a precise relationship becomes obvious.[17] In the electric organ of *Electrophorus electricus* volts per cm, number of plates per cm, and concentration of choline esterase decrease from the head to the caudal end of the organ in an S-shaped form.[10] If the electrical changes are recorded on the same specimen and at the same section as the chemical values a close parallelism is obtained between voltage and enzyme concentration.[18] This parallelism exists, not

[8] Nachmansohn, *Yale Jour. Biol. and Med.*, 12: 565, 1940.
[9] Nachmansohn and B. Meyerhof, *Jour. Neurophysiol.*, 4: 348, 1941.
[10] Nachmansohn, Coates and Cox, *Jour. Gen. Physiol.*, 25: 75, 1941.
[11] Nachmansohn, *Compt. Rend. Soc. Biol., Paris,* 127: 894, and 128: 516, 1938.
[12] Nachmansohn, *Bull. Soc. chim. biol.*, 21: 761, 1939.
[13] Lorente de Nó, *Am. Jour. Physiol.*, 121: 331, 1938.
[14] Lissak, *Am. Jour. Physiol.*, 127: 263, 1939.
[15] Couteaux and Nachmansohn, *Proc. Soc. Exp. Biol. and Med.*, 43: 177, 1940.
[16] Boell and Nachmansohn, SCIENCE, 92: 513, 1940.
[17] Nachmansohn, SCIENCE, 91: 405, 1940.
[18] Nachmansohn, Cox, Coates and Machado. *Jour. Neurophysiol.*, 5: 499, 1942.

only in regard to the variations which occur in the same specimen, but even in absolute amounts for the variations between the individuals which are quite considerable.

The localization of choline esterase and its correlation with the electromotive force appeared to be specific. Other enzyme systems and substances studied so far—the investigations are still in progress—do not show a similar distribution nor a parallelism with the electromotive force.[18,19] An exception is the localization of vitamin B_1, determined as diphosphothiamin, which is considerably more concentrated in the sheath of the giant axon than in its axoplasm.[19] This again appears significant. Since the breakdown of pyruvic acid, which requires vitamin B_1, is probably important for the formation of acetylcholine, the high concentration of this coenzyme is in agreement with the assumption of a high rate of acetylcholine metabolism at or near the surface. On the other hand, since pyruvic oxidation is of general importance it could not be expected that the vitamin is concentrated at the surface as exclusively as is choline esterase.

The new concept removes the chief difficulty for conciliating the "electrical" and "chemical" theories of transmission of nerve impulses. For it was not the question whether the process is chemical or electrical, but whether there is or is not a special mechanism at the synapses different from that in the axon, which was the basis of the controversy. A satisfactory answer has now been found to this question. There are certainly other factors and reactions involved in the propagation of nerve impulses, but the new investigations indicate that acetylcholine in any case is an essential link in the generation of the electrical changes recorded during activity. Since $V = E - IR$ the ester may act on the surface by producing E.M.F. or decreasing the resistance, or by both. Resistance and electromotive force are closely related properties of the membrane. If an impulse reaches the polarized surface the resistance breaks down. The resting potential disappears or is even reversed. It can easily be envisaged that a polarizing or depolarizing substance which appears and disappears within milliseconds is responsible for these changes.

OBITUARY

MONT ROGERS REID

THE untimely death of Mont Reid on May 11, 1943, is mourned by the community in which he lived and by his many friends throughout the country. Rarely has a man so universally won the love and affection of his fellow citizens. That, during his illness, those in higher walks of life should have awaited daily reports of his progress; that telephone operators, taxicab drivers, hotel clerks and news-stand attendants should have inquired how he fared; that churches should have invoked special prayers for his recovery; that, on his death, the flag on the City Hall of Cincinnati should have been flown at half-mast for one not connected with government; that civic organizations should have attended his funeral services in a body; that the American Surgical Association, meeting at the time in Cincinnati, should have interrupted its scientific program to do him honor—these are but a few of the indications of the extraordinary hold he had established upon his fellows. He was in a true sense the beloved physician.

Mont Reid was born on a farm near the small town of Oriskany, Virginia, on April 7, 1889. His elementary education he obtained largely from his father, who acted as schoolmaster for his six sons and daughter. He attended the Daleville Normal School for two years, then entered Roanoke College, from which he graduated with an A.B. degree in 1908. He entered the Johns Hopkins Medical School in the fall of that year and on graduating in medicine in 1912 was appointed an intern in surgery by Dr. W. S. Halsted, the distinguished chief of the surgical department of the Johns Hopkins Hospital and professor of surgery in the Johns Hopkins Medical School. The following year, 1913 to 1914, he was an assistant resident in pathology, then returned to surgery and held the position of assistant resident surgeon from 1914 to 1918. In 1918 he was appointed resident surgeon of the Johns Hopkins Hospital, a post he occupied for three years. Following this period he was an associate surgeon of the hospital until his departure from Baltimore in 1922. Academically, he was successively instructor in pathology, instructor in surgery and associate in surgery in the Medical School.

In 1922 he accompanied the writer to Cincinnati as his associate in the newly organized department of surgery of the University of Cincinnati Medical College. It was a period of transition with all the difficulties inherent in such a period; and there is little doubt that his loyalty, sound judgment and winning personality contributed greatly to such success as was achieved in the development of the department. He became active and successful in practice and quickly won a host of friends. In 1925 he was appointed visiting professor of surgery to the Peking (later Peiping) Union Medical College of China and spent a

[19] Nachmansohn and Steinbach, *Jour. Neurophysiol.*, 5: 499, 1942.