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CARBON DIOXIDE PRODUCTION FROM NERVE FIBRES WHEN RESTING AND WHEN STIMULATED

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF SCIENCE IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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BY

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CARBON DIOXIDE PRODUCTION FROM NERVE FIBRES WHEN RESTING AND WHEN STIMULATED; A CONTRIBUTION TO THE CHEMICAL BASIS OF IRRITABILITY.[1]

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Introduction

THERE have been two theories of the nature of conduction — one upheld among others by Hermann, that it was a propagated chemical change; the other, at present the dominant view, that it is a propagated physical change.

In 1901 Professor Mathews suggested [2] that it was in the nature of a coagulative wave propagated along the fibre; this coagulation of the nerve colloids leading either directly or indirectly to the electrical disturbance accompanying the impulse. At the time, there was no evidence of chemical change in the nerve fibre, and its indefatigability seemed to point to an absence of metabolism. Certain facts were known, however, which were difficult to reconcile with this physical theory. Darwin had observed that in Drosera,[3] conduction occurred only if the protoplasm had oxygen; and Mathews [4] observed that salts would not stimulate a nerve, or, at any rate, their power of stimulation was much reduced if the nerve remained in the body for a time after death, or if the nerve were brought into the salt solution in an atmosphere of hydrogen. This clearly indicated a dependence of the irritability on oxygen.

[1] The preliminary report of these investigations was given in part in Biochemical section of Eighth International Congress for applied chemistry, September, 1912. See original communications, Eighth International Congress of applied chemistry, xxvi, p. 163. See also this Journal 1913, xxxi, p. xxii.

[2] Mathews: Century Magazine, 1902, pp. 783–792; Science, 1902, xl, p. 492.

[3] Insectivorous Plants, p. 57.

[4] Unpublished observations.

This fact lead to a search for evidence of the chemical nature of irritability and in a number of papers [5] it was clearly pointed out that the anaesthetics were probably acting directly in a chemical manner instead of indirectly, by affecting permeability, and that probably the anaesthetics acted by uniting with the protoplasm where O_2 usually took hold. This view was strengthened by the temperature coefficient of conduction, which is nearly that of a chemical reaction; by the importance of O_2 for artificial parthenogenesis; and by many other facts some of which have recently been collected by Haberlandt, Buijtendijk and others.

Although it has been established by repeated demonstrations, that the nerve does not fatigue under ordinary conditions, as measured by the method used in muscular studies, yet Fröhlich [6] observed that the nerve undergoes certain changes by long activity. Gotch and Burch discovered [7] in 1889 that if two stimuli are successively set up within $\frac{1}{500}$ of a second, only one negative variation is produced. This critical interval, or refractory period, is found to be altered by temperature changes, by drugs, asphyxiation, and anaesthetics.[8] Thus by prolonging the refractory period by partial anaesthesia, Fröhlich easily demonstrated that with a frequency of stimulation less than this normal refractory period, stimulation of the attached muscle no longer occurred. He interprets this as a phenomenon of fatigability of the nerve. Thöner's [9] observation seems to lead to a similar interpretation, for he found recently that fatigability is less effective when the refractory period is shortened by high temperature. There seems, then, to be fatigue in the nerve, but it cannot be measured by an ordinary scale.

After the complete failure of the chemical detection of CO_2 and

[5] A. P. Mathews: Biological bulletin, 1904–5, viii, p. 333; this Journal, 1904, xl, p. 455; *ibid.*, 1905, xiv, p. 203; Biological Studies by the pupils of William Sedgwick, 1906, p. 81; Journal of pharmacology and experimental therapeutics, 1911, ii, p. 234.

[6] Fröhlich: Zeitschrift für allgemeine Physiologie, 1903–4, iii, p. 445. *Ibid.*, p. 75.

[7] Gotch and Burch: Journal of physiology, 1899, xxiv, p. 410.

[8] See Tait and Gunn, Quarterly journal of experimental physiology, 1908, i, p. 191; Tait, *ibid.*, 1909, ii, p. 157.

[9] Thöner: Zeitschrift für allgemeine Physiologie, 1908, viii, p. 530; *ibid.*, 1912, xiii, pp. 247, 267, 530.

acids in the excited nerve, Waller still believes that it must give off CO_2 when stimulated. In 1896, he showed, with an electro-physiological method, that among other reagents, CO_2, in minute quantities, increased the excitability of the isolated nerve of the frog, and that the normal nerve, when excited, also increased its activity.[10] From this he ingeniously formed the hypothesis that every activity in the nerve fibre must be associated with CO_2 production.

That there may be CO_2 production in the nerve, but too small to be measured by ordinary methods, is shown by the following calculations: A frog (Rana temporaria) gives off 0.355 gram of CO_2 per kilogram per hour at. 19 – 20° C.[11] A small piece of the nerve fibre of the same animal, say 1 cm. in length, will weigh in the neighborhood of 10 milligrams. Now, if the mass of the nerve respires at the rate of the whole animal, it would give off about 0.0000007 grams of CO_2 during ten minutes. This calculation at once suggested that the lack of positive evidence of metabolism in the nerve fibre was not at all conclusive that such metabolism did not occur, in view of the limitation of the methods for the estimation of CO_2. It was evidently necessary to devise methods for the detection of very minute quantities of CO_2. Thus at Professor Mathews' suggestion a new method for CO_2 analysis was first devised, and then, under his direction, I have undertaken to go back once more to the question of CO_2 production in the nerve fibre during the passage of a nerve impulse.

To study the nature of metabolism involved in a tissue, one should at least determine the oxygen consumption and the carbon dioxide production. Inasmuch, as the present problem, however, is concerned only with direct evidence for the existence of metabolism in the nerve fibre, I have attempted to measure CO_2 production only, for it is true that the lack of oxygen consumption may not necessarily indicate the absence of chemical changes, while the production of CO_2 will surely prove the presence of metabolism. Furthermore, as CO_2 production is the only sure universal expression of the respiratory activity in anaerobic and aerobic plant and animal tissue in normal condition, the inquiry of CO_2 production in an excited nerve will not only concern the problem of the nature of the nerve impulse

[10] WALLER: Croonian lecture, Philosophical transactions, London, 1896.

[11] Taken from Pott's figures. See figures in Table ix, p. 129.

itself, but may, also, aid in forming a fundamental conception of the tissue respiratory mechanism. In this way, if the protoplasmic irritability has a direct connection with the cellular respiration, then our idea of the general nature of the pharmodynamics of many reagents on a living tissue may be essentially modified.

Methods and Materials

Two new apparati were constructed which will detect CO_2 in as small quantities as one ten-millionth of a gram and estimate it with quantitative accuracy. The detailed method has been described in a separate article.[12]

Preliminary experiments with these new apparati showed that the sciatic nerves of dogs gave too large quantities of CO_2 for my method so that I was compelled to use a smaller nerve of a cold-blooded animal for quantitative estimation. For exact measurements of CO_2 production, I have used only two kinds of nerve, although I have used a large variety of nerves in qualitative experiments. For a non-medullated nerve fibre, Prof. G. H. Parker[13] was so kind as to suggest to me that I use the nerve trunk of the claws of the spider crab (Labinia Caniliculata) which is a bundle of mixed sensory and motor fibres. The frog, whose sciatic was used as a representative for medullated nerve, was exclusively Rana pipiens, obtained from Indiana.

As my apparati in the present form cannot be used for a muscle nerve preparation nor for the normal nerve in situ, the use of an isolated nerve could not be avoided. Experimental factors thus introduced should be carefully considered before we interpret the observation as a normal metabolism. This serious objection, however, can be overlooked, as far as our fundamental question of different metabolic activities before and after a stimulation is concerned, for Waller[14] has demonstrated that the presence of excitability in an isolated nerve persists as long as nineteen hours provided that the electrical changes correctly represent the state of excitability. Although

[12] See pp. 137–145.

[13] For this and other suggestions, I am under great obligation to Dr. Parker.

[14] Waller: 1896, Brain, xix, p. 53.

Herzen claims that under certain conditions of local narcosis the nerve fibre may give an action current without any muscular contraction (Wedenshi and Boruttau both deny this), and Ellinson [15] recently demonstrated by the use of cinchonamine hydrochloride the absence of negative variations without abolishing the excitability of the nerve, yet evidences are now abundant to indicate that the action current is a normal physiological phenomenon in uninjured tissue expressing the simultaneous activity resulting in a corresponding change in the peripheral organ.[16] These facts, therefore, must be taken as showing that as long as a negative variation remains, the nerve is probably excitable; and that the phenomena observed in the isolated nerve could be regarded as identical with that of a normal nerve as far as the passage of a nerve impulse in an isolated nerve fibre is concerned.

CO_2 Production from Resting Nerve

In this study of the metabolism of the resting nerve, particular care was taken to select those fibres which were free from nerve cells. The work of several investigators [17] seems to indicate that tissue oxidation is primarily concerned with the cell nucleus. Inasmuch as the respiration in the central nervous system is certain [18] and the blood supply to fibres is seemingly scanty, the notion persists among certain biologists that a nerve fibre should not respire since it has no nucleus. In order to test the correctness of such an idea, I have studied quantitatively the output of CO_2 from various lengths of nerve which are known to be free from nerve cells.[19] Here is the result:

[15] Ellinson: Journal of physiology, 1911, xlii, p. i.

[16] For further details, see: Gotch and Horsley: Philosophical transactions of the Royal Society, 1891, clxxii, p. 514; Bernstein: Archiv für die gesammte Physiologie, 1898, lxxiii, p. 376; Reid and McDonald: Journal of physiology, 1898–9, xxiii, p. 100; Lewandowsky: Archiv für die gesammte Physiologie, 1898, lxxiii, p. 288; Alcock and Seemann, *ibid.*, 1905, cviii, p. 426.

[17] See Spitzer: Archiv für die gesammte Physiologie, 1897, lxvii, p. 615; M. Nussbaun: Archiv für mikroskopische Anatomie, 1886, xxvi, p. 485; R. S. Lillie: This Journal, 1902, vii, p. 412.

[18] L. Hill: Quoted from Hulliburton's Chemistry of nerve and muscle, p. 79.

[19] In this connection, I wish to express my indebtedness to Prof. H. H. Donaldson for his kind advice.

Non-Medullated Nerve Fibre. — (The nerve of the spider crab, and apparatus 2 for the qualitative, and apparatus 1, for the quantitative, estimations were used.) When I place the nerve of a spider crab in the right chamber and no nerve in the left, and watch for the deposit of barium carbonate, the drop on the right will soon be coated with the white precipitate, but no precipitate whatever is visible with a lens in the left. CO_2 is thus shown to be produced by this resting nerve. Now, by interchanging the nerve from the right to the left, no nerve being in the right, we can convince ourselves of the correctness of this conclusion, by eliminating any technical error which might produce the different results in different chambers. The rate at which the precipitate appears and the quantity of the precipitate, depends on the size of the nerve. In fact, CO_2 production from the resting nerve of the spider crab is found to be proportional to its weight, other things being equal, and is constant: For 10 milligrams per ten minutes it gives 6.7×10^{-7} grams at 15 – 16°c.

The quantitative determination of this amount is made in the following manner:

The claws of the crab are carefully removed, and, by gently cracking them, the long fibre of the nerve trunk is easily isolated. After removing the last drops of the water by a filter paper, the nerve, with the aid of glass chop sticks, is carefully placed on the glass plate,[20] and quickly weighed. The glass plate with the nerve is now hung on the platinum hooks in the respiratory chamber A, and then the chamber sealed with mercury. The analytic chamber is now filled with mercury in the manner described elsewhere,[21] and then the apparatus is washed by CO_2 free air as usual. The time when the barium hydroxide is introduced to the cup in chamber B is recorded, and the stop-cock between the two chambers is closed. When at the end of ten minutes the drop at cut F is perfectly clear, having not a single granule of the precipitate visible to a lens, thus insuring that the air is absolutely free from CO_2 then a known portion of the gas from the respiratory chamber is introduced into the chamber below in which the clear drop of barium hydroxide has been exposed, and it is determined whether or not the amount of the gas taken contains

[20] The weight of this plate is known so that the weight of the nerve can be determined very quickly. See p. 120.

[21] See pp. 139.