THE BIOCHEMISTRY OF NERVE TISSUE.

A SURVEY OF THE POSITION.

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In a recent article¹ Dr. W. F. Menzies spoke of the need for a biochemical approach to some of the problems of mental disease; it was evident that he had in mind a real analysis of the function of nerve tissue, on biochemical lines, and not a mere search for "tests" of a chemical nature, that might prove of assistance to diagnosis. An understanding of the chemical dynamics involved in the function of the nervous system would be of very obvious assistance, not only to the psychiatrist, but also to the pharmacologist; indeed, the latter might turn to useful account knowledge which was of too rudimentary a nature to be of very great use to the former.

An increasing amount of work on the biochemistry of nerve tissue is being done, and perhaps it is worth while to review briefly the present position. Such a review will make it clear that the subject is still in its infancy; and the feeling on the part of the public that the laboratory worker should "produce rabbits out of his hat," to which Dr. Menzies refers, and in which, perhaps, the clinician is at times tempted to share, may prove rather inhibitory to workers who are well aware that no immediate "practical" results are likely to crown their efforts. Nevertheless, until the preliminary spade work is accomplished, and some of the fundamental principles are laid bare, it is hardly to be expected that the more intricate phases of the problem can be dealt with successfully.

The problem that naturally first presents itself is that of conduction in the nerve fibre. Since the last century many workers have investigated the electrical changes that accompany nerve activity and their relationship to conduction. Work of this type forms the basis of the current theory of nerve conduction (Lillie²). The classical researches of Keith Lucas and of Adrian, to mention only workers in our own country, are almost too well known to require mention. The rapid development of electricity, and in particular of the thermionic valve, has given a great impetus to work of this kind. In America, Bishop, Erlanger, and Gasser have used a combination of a valve amplifier and a cathode ray oscillograph; Forbes has combined the amplifier and the string galvanometer; and in this country Adrian and Matthews have used valves and the capillary electrometer, and an oscillograph, respectively. By such means these workers have elaborated techniques which allow of observations of a delicacy and an accuracy hitherto quite unattainable. The importance of these researches from the point of view of the analysis of nervous activity cannot be overrated. Undoubtedly they represent one aspect of biochemical phenomena, just as surely as do the measurements of the chemist, but we have not yet the knowledge required to correlate them with those measurements.

From the more obviously chemical point of view, perhaps the most fundamental, though by no means the earliest, observation was that of Downing, Gerard, and Hill,³ who showed that peripheral nerve gives out a minute but measurable amount of heat during rest, and that this heat is increased during stimulation. The heat associated with a single impulse in a frog's nerve at 15° is in the neighbourhood of one-millionth of a calorie per gram of nerve. Like active muscle, active nerve gives out heat in two stages—there is an "initial" and a "recovery" phase; but whereas, in the case of muscle, the ratio between the magnitudes of these two phases is about 1 to 1, in nerve it is about 1 to 9; moreover, in muscle the two phases can be separated, since the "recovery" phase is abolished in the absence of oxygen; but in the case of nerve no such separation is possible; in nitrogen both phases fail together. These facts indicate that the chemical events which underlie the evolution of heat must be different in nature in the case of the two tissues.

Several workers have investigated the gaseous metabolism of nerves. The earliest work was that of Tashiro,⁴ who

estimated the carbon dioxide given off by frog nerve, both during rest and activity. Schwichi Niwe,⁵ using Tashiro's method, showed that this evolution of CO₂ was diminished by cocaine. The accuracy of Tashiro's method has been seriously questioned, and later workers, Parker⁶ and Adam,⁷ have attacked the problem with better methods. They have confirmed the qualitative aspect of Tashiro's work, in that they also observe an evolution of CO₂ at rest, which is increased during stimulation, though they have shown that, qualitatively, his values were inaccurate.

Of very great interest is the work on the oxygen absorption of nerve tissue and its relation to the evolution of CO₂ and of heat. Gerard⁸ observed the oxygen consumption of frog nerve, and found values which agreed very well with those previously obtained for heat production, on the assumption that, both during rest and activity, the source of energy was the oxidation of the ordinary food materials (carbohydrate, protein, or fat). Fenn's⁹ values are in substantial agreement with those of Gerard, and the results of both workers have recently been confirmed, in the main, by Meyerhof.¹⁰ These workers all employed the nerves of cold-blooded animals. Observations of mammalian (rabbit) nerves have, however, been carried out by Gerard¹¹ and Sherif.¹² The results of these two workers show close agreement. Sherif's observations cover both normal nerves and nerves which are exposed to the action of certain drugs.

Taken together, these observations establish the very fundamental point that in the nerve, no less than in tissues such as muscle, we have to do with energy-yielding processes which can be interpreted in the familiar terms of heat, energy, and chemical change, and which therefore can be attacked by the biochemist with hope of ultimate success.

It is natural to seek a clue to the nature of the food-stuffs oxidized by examining the respiratory quotient. Unfortunately, however, the data on this point are still inconclusive. The problem of making simultaneous accurate measurements of oxygen and carbon dioxide on a minute scale is beset with technical difficulties; in point of fact, there is not, as yet, complete agreement between the three workers (Gerard,¹³ Fenn,¹⁴ and Meyerhof¹⁵) on the question. All, however, agree that during activity there is a tendency for the respiratory quotient to rise to, or above, unity—a finding that would naturally be interpreted in the sense that, whatever foodstuffs may be used during rest, activity entails the burning of carbohydrate.

Attempts have been made to observe directly the materials oxidized. Hirschberg and Winterstein's¹⁶ results seem to show that there is a diminution of carbohydrate, protein, and fat in frog nerve during both rest and activity.

Gerard and Meyerhof¹⁷ investigated the formation of lactic acid by frog nerve. They used, for the most part, manometric methods, and concluded that there was a production of lactic acid during survival of the nerve in nitrogen, which was not increased by stimulation. They found reason to believe that carbohydrate furnished the source of this lactic acid production. In oxygen there was no evolution of lactic acid; on the other hand, they could find no unequivocal evidence of the removal of this substance, which, on analogy with the behaviour of muscle, would be expected to take place. Holmes and Gerard¹⁸ have approached the same problem. They employed rabbit nerves, a course which complicated the immediate experimental procedure, since it entailed working at 37°, but which very greatly facilitated the chemical investigations, since adequate amount of tissue could be obtained—a very difficult problem when frog nerves are used. They verified the fact that nerve produced lactic acid when kept in nitrogen, and were able to show a corresponding fall of carbohydrate, to which glycogen and "free sugar" contributed about equally. They showed definitely that there was no production of lactic acid in oxygen, but that there was, on the other hand, a disappearance of carbohydrate. They do not assume that this carbohydrate is oxidized, though they point out that, if it were, it would account for about half of the observed oxygen utilization.

They were able to establish definitely that there is no oxidative removal of lactic acid, a point which serves

quite sharply to differentiate the chemical behaviour of nerve from that of muscle. Finally, they found that these processes, definite enough in the resting nerve, are quite unaltered by stimulation. The importance of this finding is considerable, for clearly it means that we must look elsewhere for the source which provides the energy for the nervous impulse; it is, indeed, almost refreshing to meet a tissue which has apparently rejected glycogen and glucose as a source of energy supply!

Tashiro¹⁹ first showed that peripheral nerve produced ammonia, and that ammonia production was increased by stimulation. These results have been confirmed by Winterstein²⁰ and by Gerard and Meyerhof.¹⁷ These observations may be related to the breakdown of adenylic acid (Embsen²¹). Thudichum²² long ago described the presence of hypoxanthine in brain, and its presence has recently been confirmed by Holmes and Holmes, who isolated it as the silver nitrate (unpublished). They also observed that a pentose was present in brain tissue.²³ Adenylic acid contains a pentose group, adenine and phosphoric acid; and adenine, by oxidative de-amination, would yield hypoxanthine.

This brief survey indicates that at least a beginning has been made in unravelling the chemical factors upon which the propagation of the nervous impulse depends. If, so far, what has been done amounts only to a few scratches on the surface of the problem, at least we possess the very important knowledge that the nervous impulse is accompanied by the evolution of heat and the consumption of oxygen, and that its source of energy is different from that of muscle.

If much remains to be done with regard to the problems of conduction, still more difficult are the tasks presented by the central mechanisms, those of the brain itself. It would seem that there is a fundamental difference between the chemical events occurring in peripheral nerve and those taking place in the brain, for this reason: the oxygen consumption of peripheral nerve, both in rest and activity, is extremely small—of the order of 300 c.mm. per gram per hour for mammalian nerve at rest, and of perhaps 1,000 c.mm. per gram per hour during activity. It is doubtful whether the oxygen consumption of normal, unanaesthetized brain has ever been measured with even approximate accuracy, but the oxygen consumption of anaesthetized brain is of the order of 30 to 50 c.mm. per gram per minute—and anaesthetics are known to inhibit oxidation processes to a very marked degree. Thus anaesthetized brain consumes, at the very least, six times as much oxygen as unanaesthetized resting nerve (Alexander and Cserna,²³ Hou,²⁴ Hou and Siguira,²⁵ Schmidt²⁶). Warburg, Posener, and Negelein²⁷ have shown that brain tissue produces lactic acid very rapidly *in vitro* in the presence of added glucose. Holmes and Holmes^{28, 29, 30} confirmed these findings, and showed, further, that the carbohydrate reserve of brain is very poor; during anaerobic survival very little extra lactic acid is produced unless glucose is added to the preparation. Following up this observation, they produced evidence to show that during life brain is dependent for its lactic acid precursor upon sugar supplied to it by the blood stream, since the amount of lactic acid which can be found after death depends on the level of the blood sugar at the time of killing. In insulin hypoglycaemia the brain lactic acid content may be less than half its normal value; if the animal is killed during hyperglycaemia, however produced, the value may be increased by as much as 200 per cent., depending again upon the precise blood-sugar level. Now hypoglycaemia of a sufficiently severe degree is accompanied by convulsions. On the other hand, one of the causes of hyperglycaemia is the administration of anaesthetics such as ether, chloroform, or urethane. A low brain lactic acid value may therefore accompany increased activity, a high value may coexist with anaesthesia. It is difficult to interpret these findings if it is assumed that lactic acid represents some phase in the chemical process accompanying active impulse conduction, but the difficulty is less acute now we know that it is not this lactic acid mechanism which is directly concerned, at least in the propagation of the impulse. It has been shown that lactic acid is not oxidized by peripheral nerve; brain tissue,

however, oxidizes it very readily (Holmes and Holmes²⁸). It seems very likely, therefore, that this power of oxidation is inherent in the nerve cell, as opposed to the nerve fibre, and it is tempting to speculate whether the oxidative removal of lactic acid may not be connected with the passage of impulses over the synapse.

Ashford and Holmes³¹ have investigated the phosphorus metabolism of brain from the point of view of lactic acid production. They find that, so far as the production of lactic acid from glucose is concerned, phosphorus appears to play no part; on the other hand, the formation of lactic acid from glycogen seems to involve the production of hexose phosphate as an intermediate stage. These investigations bear, perhaps, more on the general questions of carbohydrate metabolism than on the particular problems connected with the brain. They serve, however, to underline the difference between the carbohydrate mechanisms of brain and those of muscle tissue. Incidentally, these experiments have again emphasized the very much more important part played by glucose than by glycogen in the production of lactic acid by brain. Attention has been drawn to this point in earlier papers.^{28, 29} There seems to be no doubt at all that an abundant supply of glucose is absolutely necessary for normal brain function, and that, since little carbohydrate can be stored, the supply must be carried by the blood stream; that apparently neither glucose nor lactic acid provides the energy liberated during impulse conduction in the fibre renders the question all the more intriguing.

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ROYAL MEDICAL BENEVOLENT FUND.

At a recent meeting of the committee of the Royal Medical Benevolent Fund £610 was voted in grants to thirty-five applicants. The following are short notes on two of the cases helped.

A medical man, aged 76, has been bedridden for two years following a stroke; he is now quite childish and unable to look after himself. His two sisters are dependent on him; one is bedridden with cancer; the other, aged about 60, is very frail, and alone looks after these two invalids. She cannot afford a servant or a nurse, and all the household work and the nursing falls upon her. Their income, about £110 a year, is derived from letting rooms in the house. Voted £40.

Daughter, aged 59, of M.D., is crippled with arthritis and unable to use her hands and feet. Her knees are quite fixed, as well as her left arm. She shares a bungalow with an annuitant of this Fund, who is 87 years of age; it is therefore necessary for these two ladies to pay someone to look after them. The grantee's income, £60, is from a sister. The annuitant's income from this Fund and other charities is £130, making a total of £190. Rent amounts to £90, and wages of attendant £39. This leaves £61 for the maintenance of the two ladies. Voted £26 to the grantee, in addition to £20 to the annuitant.

The committee is very restricted in the grants which it is able to make owing to the limited funds available for distribution. Subscriptions and donations are very urgently needed before the end of this year, and should be sent to the Honorary Treasurer, Royal Medical Benevolent Fund, 11, Chandos Street, Cavendish Square, London, W.1.

The Royal Medical Benevolent Fund Guild still receives many applications for clothing, especially for coats and skirts for ladies and girls holding secretarial posts, and suits for working boys. The Guild appeals for second-hand clothes and household articles. The gifts should be sent to the Secretary of the Guild, 58, Great Marlborough Street, W.1.