CARBON DIOXIDE FROM THE NERVE CORD OF THE LOBSTER.

By G. H. PARKER.

(*From the Zoological Laboratory, Harvard University, Cambridge.*)

(Accepted for publication, April 14, 1925.)

I. INTRODUCTION.

Since it has been shown in a previous paper (Parker, 1925) that CO₂ is given out in measurable quantities by the lateral-line nerve of the dogfish and that this gas increases in amount when the nerve from which it comes is stimulated, it is natural to inquire if like occurrences take place in other types of nervous tissue. The lateral-line nerve is a purely sensory nerve and the pieces used in the tests just referred to were entirely devoid of ganglion cells. This nerve therefore represented a transmitting organ uncomplicated by any central connections or activities. In the present study a portion of the central nervous system, the nerve cord of the lobster (*Homarus americanus* Milne-Edwards), has been selected because this part includes not only nerve fibers but also ganglion cells and fibrillar material, all the components of a complicated central structure. The portion of the nerve cord chosen was the section including the last three abdominal ganglia. This section could be easily dissected out as a single strand and could be handled with convenience in experimentation.

The apparatus used in this study and the general technique employed have been described in the paper, already referred to, on the lateral-line nerve. In the present study as in the previous one the rates of CO₂ production have been expressed in milligrams of CO₂ per gram of nervous material per minute. This form of expression allows easy comparisons with the results of earlier work. Quantitative studies on the CO₂ from ganglionated organs have already been reported by Tashiro and Adams (1914) and by Moore (1922).

II. OBSERVATIONS.

The nerve cord of the lobster is extremely delicate as compared with the lateral-line nerve of the dogfish. Tests quickly demonstrated that after its removal from the animal it could not be relied upon as a satisfactory preparation for much more than two hours. Hence all results reported in this paper are based upon determinations made within an hour or so of the time when the cord was excised. The relatively rapid decline in the vitality of the cord of the lobster is apparently duplicated by that of the crawfish where Moore (1922) found it well to restrict his period of observation to the first half hour after dissection. This condition is in strong contrast to that of nerves from cold-blooded vertebrates which may be successfully worked upon for hours after their removal from the animal.

When the last three ganglia of the nerve cord of the lobster are put in a closed type of respiratory chamber and tested for CO_2 , the discharge of this gas can be shown to be relatively high at the outset and to fall off rapidly till by the end of an hour it becomes fairly low. The change of rate seen in this fall is well illustrated in Fig. 1 where are plotted the rates of CO_2 discharge from three preparations of the ventral cord. In cord I the rate was at the beginning 0.2330 mg. CO_2 per gram of cord per minute. It then fell successively to 0.1882, 0.1378 and finally to 0.0915 mg. Cord II began at 0.1858 mg. and ended at 0.0619 mg., and cord III at 0.1678 mg. and ended at 0.0618 mg. An inspection of the plottings in Fig. 1 shows that it is not possible to divide the CO_2 production of the lobster cord into the two periods, one of initial gush and the other of steady outflow, as could be done with the lateral-line nerve of the dogfish (Parker, 1925). In the lobster cord the discharge of CO_2 is on a steadily decreasing rate which never passes over into a period of uniformity as with dogfish nerve. The process resembles a prolonged gush which reaches from beginning to end. At the outset of the discharge the average rate for the three cords tested was 0.19553 mg. CO_2 per gram of cord per minute and at the end 0.07173 mg., a falling off to nearly one-third the original rate. In consequence of this condition it is impossible to give any average rate that may be taken to represent the rate of a quiescent cord, for such a quiescent condition does not exist in the prepared cord. All that can be stated is that during the first hour after the

preparation of the cord the average rate of output of CO_2 in round figures declines from 0.20 mg. at the beginning to 0.07 mg. at the end. Taking all in all this is a higher rate than that shown by the lateral-line nerve of the dogfish which averaged 0.0095 mg. (Parker, 1925).

Tashiro and Adams (1914) have measured by means of the biometer the rate of CO_2 production of a piece of nerve and of the heart ganglion of *Limulus* and have found the nerve to discharge 2.6×10^{-7} and the

FIG. 1. Plottings of the respiratory rates in milligrams of CO_2 per gram of cord per minute for three nerve-cord preparations from the lobster, *Homarus americanus*. The readings from Cord I are represented by dots inclosed within circles; from Cord II by dots within squares; and from Cord III by dots within triangles. Temperature about 23°C .

ganglion 2.3 to 4.7×10^{-7} grams CO_2 per centigram of tissue per 10 minutes. Transposing these determinations into the form of expression used in this paper, the ganglion may be said to discharge from 0.0023 to 0.0047 mg. CO_2 per gram of ganglion per minute. These rates are about one-thirtieth of those which I have found for the lobster cord. The occasion of this discrepancy is not easily discovered. I do not believe it to be due to a real difference in the nature of the two tissues tested. It is in my opinion rather a matter of technique. As

I have already observed (Parker, 1925), the biometer method of Tashiro though very sensitive from a qualitative standpoint, is probably not reliable for quantitative work. At least this is my experience with it and in this my results agree with those of Adam (1921). I therefore believe that the determinations of Tashiro and Adams just quoted are less likely to prove accurate than those given in this paper.

The nerve cord of the lobster differs from the lateral-line nerve of the dogfish not only in the rapidity with which its rate of discharge declines but also in its susceptibility to mechanical disturbance. This is easily demonstrated by handling. When a nerve cord is prepared and tested in the usual way, its CO_2 output can be considerably increased if during the trial the cord is merely lifted from the rod of the respiratory apparatus and returned to it. Thus in a cord whose rate on three successive readings was 0.0793, 0.0705, and 0.0693, mere handling of the kind described induced an immediate rise of rate to 0.1168 mg. About a quarter of an hour after this the rate was found to have dropped again to 0.0764, 0.0681, and finally 0.0644. The increase of rate on handling was repeatedly observed and led to special care in the technique of this research which was quite unnecessary in that on the dogfish nerve.

As might have been expected, cutting the nerve cord of the lobster also excited an increased output of CO_2 . A nerve cord that was discharging CO_2 at rates of 0.0727 and 0.0670 mg. on being cut across in eight places showed immediately an increased rate of 0.1049 mg. which quickly subsided, however, to 0.0954, 0.0795, and finally 0.0700 mg. Thus both handling and cutting strikingly increase for a brief period the CO_2 discharged from the nerve cord of the lobster and indicate its relatively high sensitiveness to mechanical stimulation. This delicacy is quite in line with what is known of such nerve cord preparations. Only with the greatest care can they be kept alive for the one or two hours necessary for their study.

Is the CO_2 output of the nerve cord of the lobster influenced by stimulation? Since this cord, unlike the lateral-line nerve of the dogfish, does not show a period of uniform discharge of CO_2 , somewhat different methods of testing it for an increased CO_2 output on stimulation were resorted to than those used with the dogfish nerve. In

Table I are given the successive rates of two cords alternately stimulated and unstimulated.

TABLE I.

Rates of CO₂ Production in Milligrams of CO₂ per Gram of Cord per Minute of Two Nerve Cords from the Lobster, Homarus americanus, Alternately Stimulated and Unstimulated. Temperature about 23°C.

Cord No.	Condition of cord.						
	St.	Unst.	St.	Unst.	St.	Unst.	St.
1	0.0911	0.0762	0.0770	0.0609	0.0698	0.0473	
2		0.0668	0.0782	0.0470	0.0673	0.0532	0.0612

In cord 1 the records begin with the stimulated condition at 0.0911 mg. and after five tests end with a record from the unstimulated state at 0.0473 mg. In cord 2 the relations are reversed in that the series begins with an unstimulated state and ends with a stimulated one. In both series, as can be seen by inspecting the table, there is an almost steady decline in the rate in such a direction that overweight is given to the kind of rate, stimulated or unstimulated, with which a particular series begins. Hence in cord 1 the rates for the stimulated states must be too high in comparison with those for the unstimulated states, and consequently averages from this series alone would be misleading. In cord 2 on the other hand the rates for the unstimulated state are presumably too high and, though the averages from this cord alone would be as misleading as those from cord 1, the general averages from both cords together ought to yield results of an unbiased kind. Averaging all the records in Table I for the stimulated condition yields 0.07410 mg. and for the unstimulated state 0.05857 mg. The difference between these two amounts, 0.01553 mg. represents the increase of rate in consequence of stimulation, an increase of 26.5 per cent of that for the quiescent cord. This amount is roughly 1.6 times that of the percentage increase (15.8 per cent) in stimulated lateral-line nerve (Parker, 1925).

This increase of rate in the nerve cord must represent CO₂ from a strictly nervous source, for it is the excess CO₂ produced on stimula-

tion. Such cannot be said of the CO_2 given out by the quiescent cord. As Bayliss (1915, p. 379) pointed out for nerve, the CO_2 of the quiescent state may come from connective tissue, from nerve proper, or from both and, until the proportion of these two possible contributions is ascertained, the percentage increase on a strictly nervous basis cannot be calculated.

Tashiro and Adams (1914) and Moore (1922) report for the ganglionated organs studied by them no significant difference in the CO_2 output on stimulation. The heart ganglion of *Limulus*, according to Tashiro and Adams, gave out CO_2 at about the same rate as a nerve from that animal and showed no increase on stimulation. The resting nerve cord of *Cambarus*, according to Moore, discharged CO_2 at a rate which for comparison was called 100. On stimulation this fell to 89 per cent and on final quiescence to 86 per cent. So far as the results of Tashiro and Adams are concerned, I have already (Parker, 1925) stated the grounds for my belief that the biometer, though it may be very sensitive for qualitative determinations, is not reliable for quantitative work. These authors have in my opinion shown beyond a doubt that the heart ganglion of *Limulus* discharges CO_2 , but I believe that the method which they have used is not one adapted to demonstrate a change of rate in this discharge in consequence of stimulation.

Moore, working with a method that gave relative, not absolute, results showed in the nerve cord of *Cambarus* a decline of rate such as that recorded in this paper but since he was unable to extend his observations beyond a series of three readings he failed to find the significance of the downward steps which when inspected in as long a series as that shown in nerve I (Fig. 1) demonstrate a real increase of CO_2 on stimulation. Hence I cannot agree with the negative results of Tashiro and Adams and of Moore, but I conclude on the basis of the observations recorded in this paper that stimulated central nervous organs do show an increased rate of CO_2 production. Bayliss (1924, p. 379), in commenting on the results of these workers, expressed his doubts on the conclusion that stimulated ganglionated masses do not discharge CO_2 at a higher rate than nerve fibers and this doubt is entirely justified by what is reported in the present contribution.

III. SUMMARY.

1. The nerve cord of the lobster (*Homarus americanus* Milne-Edwards) is very delicate and can be used as a living preparation for only a few hours after its removal from the animal.
2. During the first hour or so after removal it discharges CO₂ at a steadily decreasing rate beginning at about 0.20 mg. CO₂ per gram of cord per minute and ending at about 0.07 mg.
3. This discharge exhibits a steady decrease in rate and is not divisible into a period of gush and a period of uniform outflow as with the lateral-line nerve of the dogfish. It terminates in a very few hours with the complete death of the cord.
4. Both handling and cutting the cord temporarily increase the rate of CO₂ output.
5. The stimulated cord discharges CO₂ at a rate about 26 per cent higher than that of the quiescent cord, an increase of about 1.6 times that of the increase observed in the lateral-line nerve of the dogfish under similar circumstances.

LITERATURE.

- Adam, N. K. 1921. Note on the Oxygen Consumption of Amphibian Muscle and Nerve. *Biochem. J.*, **15**, 358.
- Bayliss, W. M. 1915. *Principles of General Physiology*. London.
- Bayliss, W. M. 1924. *Principles of General Physiology*. 4th edition, London.
- Moore, A. R. 1922. The Respiratory Rate of the Nerve Cord of *Cambarus clarkii*. *Proc. Soc. Exp. Biol. Med.*, **19**, 335.
- Parker, G. H. 1925. The Production of Carbon Dioxide by Nerve. *J. Gen. Physiol.*, **7**, 641.
- Riggs, L. K. 1919. Action of Salts upon the Metabolism of Nerves. *J. Biol. Chem.*, **39**, 385.
- Tashiro, S. and H. S. Adams. 1914. Comparison of the Carbon-dioxide Output of Nerve Fibers and Ganglia in *Limulus*. *J. Biol. Chem.*, **18**, 329.