THE RESPIRATORY RATE OF THE SCIATIC NERVE OF THE FROG IN REST AND ACTIVITY.

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It has been accepted generally that nerve fibers have extremely low metabolic activity and that their oxidation processes in no way increase as the result of excitation. Such conclusions are based on the facts that (1) tetanization does not increase heat production in the nerve,[1] and (2) the nerve cannot be fatigued by stimulation. Furthermore, the well known fact that the nerve trunks are poorly supplied with blood vessels weighs against any assumption of the dependence of physiological function upon a high degree of metabolic activity.

On the other hand, it has been shown by Waller[2] that the intensity of the reponse of nerve fibers to excitation is increased by the application of carbon dioxide, and that nerves show the staircase phenomenon when tetanized. From this the conclusion has been drawn that carbon dioxide is produced by the nerve itself as a result of activity, and that this carbon dioxide acts further to increase the sensitivity of the tissue, thus resulting in the staircase effect. It should be pointed out that there are many substances which increase the sensitivity of nerve fiber, for example, salts like the sulfates, oxalates, or citrates. In fact Waller does not insist on the fact of carbon dioxide production by nerve, but says that the staircase effect may be due to some other substances.[3]

Recently, Tashiro[4] attempted directly to estimate the amount of carbon dioxide given off by the excised nerves of frogs and crabs.

[1] Hill, A. V., *J. Physiol.*, 1911–12, xliii, 433.

[2] Waller, A. D., *Proc. Roy. Soc. London*, 1895–96, lix, 308.

[3] Waller, A. D., Physiology, the servant of medicine, London, 1910, 43.

[4] Tashiro, S., A chemical sign of life, Chicago, 1917.

An elaborate apparatus, to which the inventors have given the name "biometer," was used to collect the carbon dioxide produced by the tissue and to bring it into contact with a drop of barium hydroxide solution. The formation of the precipitate of barium carbonate was observed under the microscope. The time required for the formation of an amount of carbon dioxide sufficient to cover the drop with a film of barium carbonate was used as a measure of the rate of carbon dioxide production. With this method, Tashiro has estimated the frog's sciatic nerve to give off carbon dioxide at the rate of 5.5 $\times 10^{-7}$ gm. per 10 mg. of tissue per 10 minutes. He compares this result with the data obtained by other workers for the rate of carbon dioxide output of the entire animal and of muscle tissue. From such a comparison he concludes that nerves have not only a carbon dioxide metabolism equal to that of other tissues but greater.[5]

With a view to getting at the facts regarding carbon dioxide production in nervous tissue, the writer has employed the indicator method developed by Haas[6] in Osterhout's laboratory and subsequently used by Osterhout and his pupils.[7] In practice a quantity of the indicator, phenolsulfonephthalein, sufficient to give the desired tint was added to Ringer's solution. A liter of this solution in a glass-stoppered bottle was brought to the required reaction by a few drops of 0.1 N NaOH. For the measurements, small tubes of Pyrex glass calibrated to hold 3 cc. of solution were used. These were closed with paraffined corks. Both corks and tubes were boiled repeatedly in neutral water, and before being used were washed in alcohol, then in ether, and finally the corks were saturated with hot neutral paraffin. The tubes were next tested for several hours by filling with the tinted solution, corking, and inverting. Those in which any color change occurred were discarded. This procedure was carried out before each experiment. In the experiments, the rate of carbon dioxide production was determined by measuring the time necessary for the tint to change from pH 7.8 to 7.4. The

[5] Mathews, A. P., Physiological chemistry. A text-book and manual for students, New York, 1915, 590.

[6] Haas, A. R., *Science*, 1916, xliv, 105.

[7] Osterhout, W. J. V., Gustafson, F. G., Brooks, M. M., Thomas, H. S., Irwin, M., *J. Gen. Physiol.*, 1918, i, 171–209.

end-point was determined by matching the experimental tube with another containing a solution having a tint corresponding to pH 7.4. The matching was done by diffuse daylight or by the light of a daylight lamp, and the time taken with a stop-watch. The experimental error is not over 10 per cent. Obviously the method measures only the rate and not the absolute amounts of carbon dioxide produced. But since the metabolism of nervous tissue is of the greatest significance when compared with that of other tissues, and since the problem of stimulation is entirely a question of comparative rates, any attempt to determine absolute values is, for us, beside the point.

Some of the earlier workers[8] in attempting to determine the reaction of nerve substance to indicators had found the cut surface of the gray matter in the brain and cord to be acid to litmus and other indicators. I have shown that the brain, cord, and sciatic nerves of frogs upon being cut or crushed give an acid reaction with phenolsulfonephthalein.[9] If the sciatic nerve of a frog is kept for half an hour in alkaline (pH = 8.0) Ringer's solution saturated with the indicator, the nerve takes up some of the stain and appears pink, except at the cut end where it is yellow; *viz.*, acid. Microscopic examination shows that the cut end of each fiber is yellow. If the stained nerve is crushed on a glass plate with a glass rod, the entire tissue turns yellow. The same result follows if the nerve is allowed to dry in the air or in CO_2-free air, while neutral cotton tinted with the indicator and treated in the same way shows no change in reaction. This acid of injury is not due to stimulation, since it is strictly localized at the points of cutting or crushing.

It can be shown that all the acid given off by nervous tissue and the greater part of that given off by muscle is carbon dioxide. For this purpose the tissue is put into a tube of tinted Ringer's solution previously freed from carbon dioxide by aspirating with CO_2-free air. When a certain amount of acid has been formed, the tissue is removed and the solution again aspirated with CO_2-free air. This treatment removes all the acid formed by nervous tissue, as shown

[8] Pflüger, E., *Arch. ges. Physiol.*, 1875, x, 312.

[9] Moore, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1917–18, xv, 18.

by the recovery of the tint corresponding to the initial pH value of the solution. By the use of Hopkins' thiophene test I have found that brain tissue produces small amounts of lactic acid as the result of cooking. But any lactic acid produced by injury to nervous tissue in my experiments presumably reacted with the carbonates of the tissue causing carbon dioxide to be given off.

It is apparent that the first problem in determining tissue respiration is to eliminate the acid of injury from the measurements. This proved to be a simpler matter than at first seemed possible, because it was found that the acid is given off only during a very short period after injury. This can be shown by determining the rate of acid

TABLE I.

Effect of Crushing on Rate of Acid Production.

Sciatic nerve.			
Condition.	Reaction time.		Rate.*
		sec.	
Resting.		1,620	0.06
Crushed.	Successive measurements.	420	0.24
		390	0.26
		790	0.13
		1,500	0.07

* Rate $= \frac{100}{\text{seconds}}$. Temperature 18–19°C.

output of a sciatic nerve, before and after crushing. The rate increases at once as a result of the acid produced by the injury but falls rapidly to the original rate. This indicates the momentary character of the production of the acid of injury and proves its rapid elimination.[10] Table I illustrates this point.

It is therefore possible by making a number of successive readings for each point, and by discarding the first readings, to obtain a fairly accurate estimation of the respiratory rate of the tissue by this method. In case the periods of time were not long, *i.e.* less than 3 minutes, five successive readings were taken, and an average of the

[10] Moore, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1918–19, xvi, 35.

last two used as the true value, figures being given to the nearest 5 seconds. When the time extended over 5 minutes it was possible to make only one or two readings, the time being given to the nearest half minute.

The first point to be settled is: Does a nerve trunk give off carbon dioxide at a rate equal to that shown by muscle and by central nervous system tissue? For the purpose of answering this, equal weights of sciatic nerve, sartorius muscle, and brain were taken from the same frog. Measurements of the rate of acid production were made as soon as the tissues were removed from the body and at intervals of an hour subsequently. It was found that if the tissues were kept in a small quantity of Ringer's solution during the time intervening between the measurements the acid production increased again after 2 hours, coincident with the rigor and opacity of the muscle. If, however, the pieces of tissue were kept in a large quantity of neutral Ringer's solution during the time interval, there was a falling off in the rate each hour for about 4 hours. Table II and the corresponding graphs (Figs. 1 and 2) show the relative respiratory rates of the three types of tissue and the decrease in rate with time. A large number of experiments of this character were made, and in all cases it was found that central nervous system tissue and muscle showed a high rate, while sciatic nerves always gave a rate of much lower order; *viz.*, 10 to 30 per cent of that of the other tissues. Just what part of this carbon dioxide is due to the metabolism of the axis cylinder and what to the connective tissue cells of the nerve trunk remains to be determined.

The question of greatest interest in connection with the metabolism of nervous tissue is: Does the functional activity of the nerve cause an increase in the production of carbon dioxide? Tashiro has answered this in the affirmative, and states that stimulation of a nerve more than doubles the rate of carbon dioxide production. With a view to getting additional evidence on this point, I stimulated sciatic nerves with induction shocks while the acid measurements were being made.[11] For this purpose fine copper wires were led into the tube beside the cork. The ends of the wires were bent

[11] Moore, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1918–19, xvi, 66.

hook-shape and the nerves wound around them. The hooks reached to within 1 to 2 mm. of the surface of the solution and the suspended tissue dipped into it. Stimulation was made with a Harvard inductorium, giving maximal shocks. Readings were first made with

TABLE II.*

A, Weight of Each Tissue 140 mg.

	Medulla.		Muscle.		Sciatic.		Sciatic / Medulla (Ratio of rates).	Sciatic / Muscle (Ratio of rates).
	Time.	Rate.	Time.	Rate.	Time.	Rate.		
hr.	*sec.*		*sec.*		*sec.*			
0	30	3.3	70	1.43	270	0.37	0.11	0.26
1	60	1.7	90	1.11	540	0.185	0.11	0.17
2	100	1.0	120	0.83	1,000	0.10	0.10	0.12
3	120	0.83	190	0.52	1,200	0.07	0.08	0.13
4	145	0.69	220	0.45	1,200	0.07	0.10	0.15
Average							0.10	0.17

B, Weight of Each Tissue 50 mg.

	Optic lobes.		Muscle.		Sciatic.		Sciatic / Optic lobes (Ratio of rates).	Sciatic / Muscle (Ratio of rates).
	Time.	Rate.	Time.	Rate.	Time.	Rate.		
hr.	*sec.*		*sec.*		*sec.*			
0.0	50	2.0	60	1.7	240	0.41	0.20	0.24
0.5	65	1.54	55	1.8	300	0.33	0.21	0.18
1.0	130	0.77	120	0.83	600	0.17	0.22	0.20
2.5	190	0.52	225	0.44	1,020	0.10	0.19	0.23
3.5	220	0.45	270	0.37	1,440	0.07	0.16	0.19
Average							0.196	0.208

* In the first column are given the intervals of time between the measurements, by hours. The first column under each tissue shows the reaction time in seconds at the end of each time interval. The second column contains the corresponding rates of reaction. Rate $= \frac{100}{\text{seconds}}$. Temperature 18–19°C.

the nerve at rest, then during stimulation, and lastly at rest again. The frequent inversion of the tube kept the tissues bathed with the solution and cooled. With this method it was found impossible constantly or noticeably to increase the rate of carbon dioxide out-

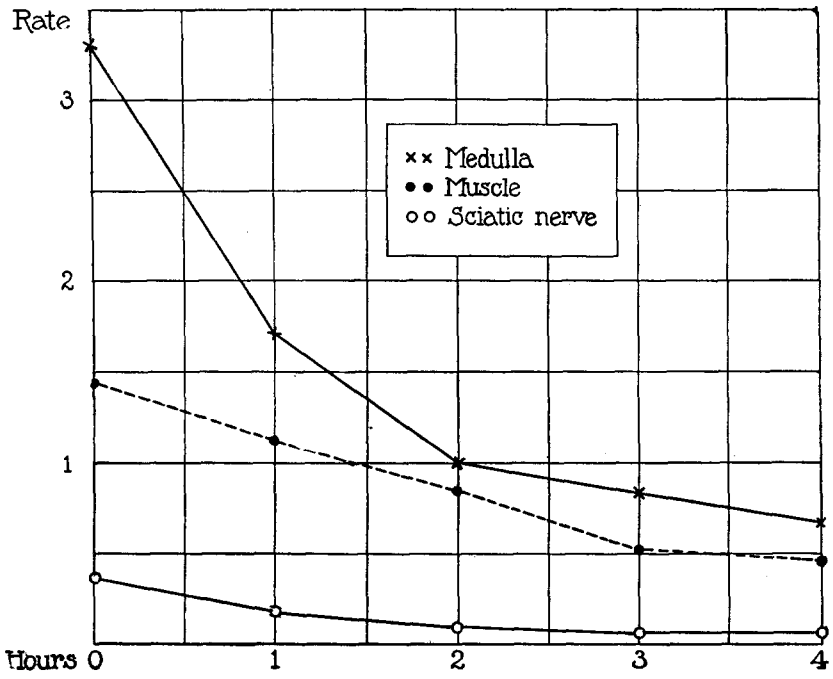

FIG. 1. Constructed from data of Table II A.

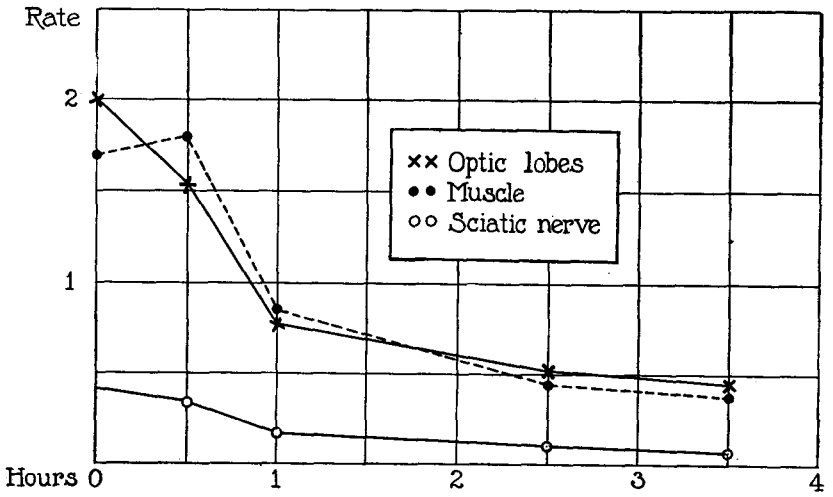

FIG. 2. Constructed from data of Table II B.

put by stimulation, although the nerve in some cases was tetanized for 30 minutes. In Table III are shown the figures for four experiments. A series of controls was made with sartorius muscle. The

TABLE III.

Effects of Stimulation on the Rate of Carbon Dioxide Production in Nerve and Muscle. Individual Animal Denoted by Letters on the Left. Temperature 17–19°C.

Sciatic nerves.

	Condition.	Reaction time.	Rate.
		sec.	
A	Resting	480	0.21
	Stimulated	600	0.17
	Resting	810	0.12
B	Resting	1,800	0.055
	Stimulated	2,100	0.048
C	Resting	1,020	0.098
	Stimulated	930	0.107
	Resting	1,080	0.092
D	Resting	600	0.17
	Stimulated	1,500	0.067
	Resting	1,620	0.062

Sartorius muscle.

Condition.	Reaction time.	Rate.
	sec.	
Resting	85	1.2
Stimulated	95	1.05
Resting	**60**	**1.7**
"	80	1.25
"	95	1.05
Stimulated	100	1.0
Resting	**80**	**1.25**
"	**70**	**1.4**
"	95	1.05

muscle was put on the electrode hooks and allowed to contract isotonically during stimulation. Measurements made during contraction showed no increase, and usually a slight decrease in respira-

tory rate, since the surface was reduced by contraction. Immediately upon relaxation the rate increased from 30 to 100 per cent of that of the resting muscle (see figures under "sartorius muscle" Table III). The method used is therefore adequate to show changes in the metabolic activity of muscle resulting from contraction. Since the same method fails to reveal changes in carbon dioxide production in the nerve as the result of stimulation, it is necessary to conclude that the functional activity of nerve fiber does not depend upon processes resulting in the production of carbon dioxide.

SUMMARY.

1. With the indicator method of Haas, the rates of carbon dioxide production have been measured in the case of the sciatic nerve, various parts of the brain, and the sartorius muscle of the frog. The rate of respiration of the sciatic nerve is from 10 to 30 per cent of that of the other tissues, varying somewhat with the individual.

2. Stimulation of the sciatic nerve with induction shocks sufficient to induce tetanus of the muscle does not increase the output of carbon dioxide from the sciatic nerve, even if continued as long as 30 minutes. Sartorius muscle used as a control showed a marked increase in carbon dioxide production upon relaxation after contraction resulting from such stimulation.

3. These facts indicate that the nerve impulse does not depend upon processes leading to the production of carbon dioxide.