

DYNAMICS OF NERVE CELLS.

II. THE TEMPERATURE COEFFICIENTS OF CARBON DIOXIDE PRODUCTION BY THE HEART GANGLION OF *LIMULUS POLYPHEMUS*.*

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That the rate of the heart beat of *Limulus polyphemus* is determined by the rate of chemical reactions within the nerve cells of the cardiac ganglion is shown by the magnitude of the temperature coefficient (Q_{10}) as determined by the author.¹ The differences in the temperature coefficient, the form of the rate curve, and the effects of extremes of temperature suggested the probability that the velocity of the chemical reaction was modified by a consecutive physical process with a different temperature coefficient. The formation of CO_2 in the nerve cells and its diffusion from them are suggested as processes analogous to those underlying the determination of the rate of impulse formation.

The following report shows that changes in temperature affect carbon dioxide formation by the ganglion cells in a manner which is quantitatively similar to the effect on the rate of the rhythmic contractions, thus establishing a basis for a hypothetical explanation to account for the different magnitude of the temperature coefficient at different ranges of temperature, and also for the change in the rate curve as affected by extremes of heat and cold.

* The experimental work was done at the Marine Biological Laboratory, Woods Hole, Massachusetts. The author wishes to express his appreciation of the courtesy of the Director and staff of the Laboratory for placing the facilities for this work at his disposal.

¹ Garrey, W. E., *J. Gen. Physiol.*, 1920-21, iii, 41.

Method.

The rate of formation of even the small amount of carbon dioxide evolved from ganglia, each weighing only 12 to 30 mg., is not difficult if one uses the indicator method of Sørensen² as modified for tissue work by Osterhout and his collaborators.³ The method of Tashiro,⁴ who demonstrated that the *Limulus* ganglion produces CO₂, did not admit of rapid variation of the temperature and was not adaptable to our purpose.

The indicator method was used with the technique and precautions described by Moore.⁵ The velocity of the reaction at different temperatures is determined by finding the time required to change the hydrogen ion concentration of an immersion fluid from pH 7.7 to 7.4 using phenolsulfonephthalein as the indicator.⁶

Buffer must be avoided in the immersion fluid which was a balanced saline solution of the following composition: 100 parts of M/2 NaCl, 1.5 parts of M/2 KCl, and 2.2 parts of M/2 CaCl₂, to 1 liter of which 15 cc. of 0.01 per cent phenolsulfonephthalein were added. The desired initial alkalinity (pH 7.7) was obtained by adding the requisite amount of NaOH.

The color comparisons were made in "Pyrex" glass tintometer tubes of about 4 cc. capacity, all of the same diameter, and filled with solution to a 3 cc. graduation mark. The tubes were stoppered with corks impregnated before each experiment with neutral, boiling paraffin or with rubber stoppers boiled in distilled water, paraffin-coated, and assuredly free from acid. The tubes and fluid were brought to the desired temperature before the ganglion was introduced and then kept at a constant temperature in large vessels of water. At the low temperatures it sometimes became necessary to adjust the tempera-

² Sørensen, S. P. L., *Biochem. Z.*, 1909, xxi, 131; *Ergebn. Physiol.*, 1912, xii, 393.

³ Osterhout, W. J. V., *J. Gen. Physiol.*, 1918-19, i, 17, 171. Gustafson, F. G., *J. Gen. Physiol.*, 1918-19, i, 181. Brooks, M. M., *J. Gen. Physiol.*, 1918-19, i, 193. Thomas, H. S., *J. Gen. Physiol.*, 1918-19, i, 203. Irwin, M., *J. Gen. Physiol.*, 1918-19, i, 209.

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

⁵ Moore, A. R., *J. Gen. Physiol.*, 1918-19, i, 613.

⁶ The author is indebted to Prof. A. R. Moore who cooperated in the initial experiments of this investigation.

ture by the addition of cold water or ice to the large containers but the variations in temperature were slight and negligible.

With scrupulous care to avoid injury, the ganglion was dissected from the heart and freed of all adventitious tissue, rapidly weighed, and immersed in the fluid (pH 7.7) to wash away all possible trace of acid and buffer. It was then placed in the tintometer tube at the desired temperature and the time required to assume the tint (pH 7.4) was determined with a stop-watch.

Results.

The results obtained in five experiments are presented in Table I. The relative velocity of CO₂ production is the reciprocal of the time

TABLE I.
Rate of Carbon Dioxide Production by Ganglion.

Temperature.	A Weight = 18 mg.		B Weight = 22 mg.		C Weight = 26 mg.		D Weight = 25 mg.		E Weight = 16 mg.	
	Time.	Q_{10}	Time.	Q_{10}	Time.	Q_{10}	Time.	Q_{10}	Time.	Q_{10}
°C.	sec.		sec.		sec.		sec.		sec.	
24	214		165		115		135		68	
14	393	1.8	345	2.1	319	1.9	445	3.3	282	4.0
4	1,800	4.5	1,245	3.6	778	2.4	1,920	4.3	1,480	5.2
14	247	7.4	290	4.3	290	2.6	645	3.0	360	4.1
24	75	3.3	111	2.6	144	2.0	180	3.5	108	3.3
34	37	2.0	65	1.7			110	1.6	59	1.8

in seconds required to change the tint of 3 cc. of solution from pH 7.7 to 7.4. The figures given are averages, usually of three readings, which in the present instance did not vary more than 3 per cent from the mean.

The results shown in Experiments A, B, and C (Table I) are typical. The temperature coefficient (Q_{10}) is equal to or greater than 2, in all except the highest range of temperature. The magnitude of the coefficient is of the same order as that previously found by Garrey¹ for the rate of the heart beat. Another similarity to the effects of temperature on rate of rhythm is to be found in the fact that it is usual that transient exposure to temperatures as low as 4°C. so affects

the nerve cells that, after the temperature is again raised, the activities are pitched at a higher level than that maintained at this temperature prior to cooling. But while this is usual it is not an invariable result of cooling as is shown by the two experiments summarized in D and E (Table I).

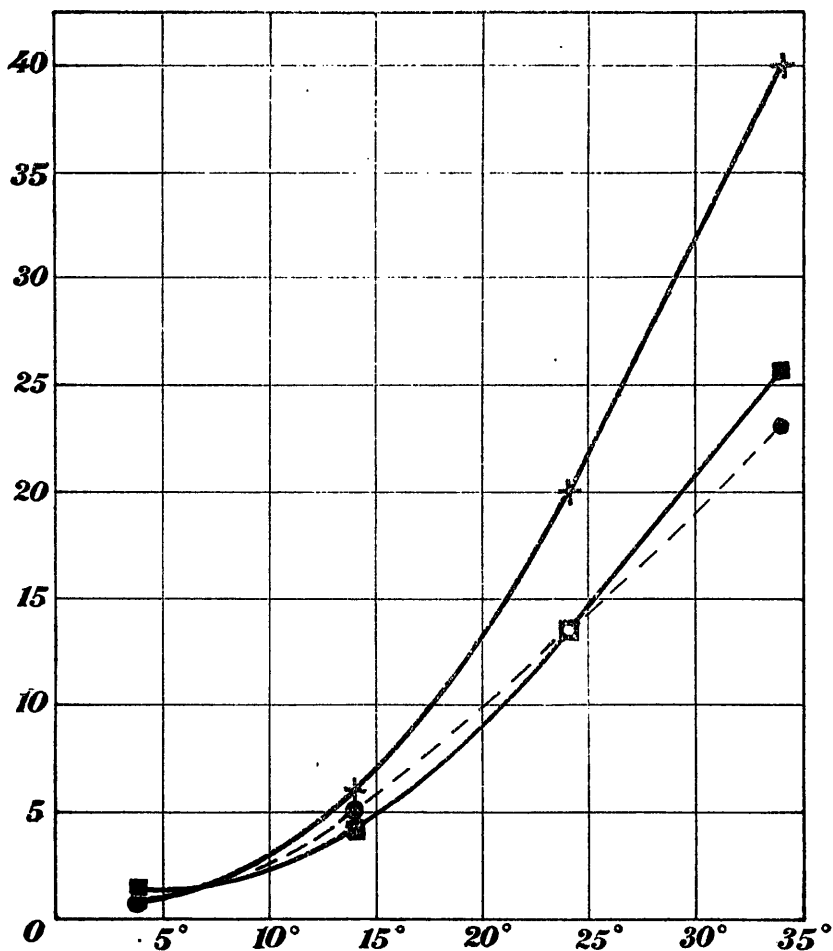


FIG. 1. Curves of the relative velocities of CO_2 production by *Limulus* heart ganglion at different temperatures. The data from Experiments A, B, and E, Table I. The reciprocal of time in seconds is multiplied by 1,500.

The relative velocities with which the nerve cells form CO_2 at different temperatures are also shown graphically in Fig. 1. The curves are obtained from data in Experiments A, B, and E of Table I and are constructed by plotting the reciprocal of time in seconds multiplied by a constant (1,500). These curves of chemical reaction velocities, like those of rate of heart beat, are exponential and not linear functions of the temperature.

Certain exceptions to the rule that the temperature coefficients of heart rate were large at low temperatures and decreased with a rise of temperature have been reported by the author. We submit below data which indicate an exception which likewise applies to the formation of CO_2 by the ganglion. The ganglion in this case was the largest obtainable weighing 32 mg. from a *Limulus* weighing $8\frac{1}{2}$ pounds.

Temperature.	Tint matched after.	Q_{10}
°C.	sec.	
27	155	
37	72	2.1
27	175	2.4
37	79	2.2
27	160	2.0
37	76	2.1
27	153	2.0
17	300	2.0
27	153	2.0

The above data show the usual temperature coefficient (Q_{10}) equal to about 2 for the range 17–27°C. but the coefficient for the upper range, 27–37°C., is uniformly larger than that usually found in our other experiments. It is also seen that after the first treatment of the ganglion at 37°C. and then cooling to 27°, the rate of CO_2 development is somewhat slower than at 27° prior to the heating. This is usual but it should also be noted that at the end of the experiment the rate at which the carbon dioxide was developed was identical to the initial rate at 27°C. It is evident from this result that even frequent subsection of the ganglion to 37° did not cause injury but produced effects which were entirely reversible in character. The constancy of the coefficients (Q_{10}) at different temperature levels in

this case is suggestive of the results found by Loeb and Wasteneys⁷ for oxidation processes in *Arbacia* eggs between 3 and 30°C.

It might be supposed from the results recorded in the preceding pages that CO₂ production is not proportional to the ganglionic mass since the rate at which carbon dioxide is produced is often very different for ganglia of equal weight, but it is also true that the rates at which hearts of equal size beat likewise show normally such variations. These facts merely mean that the rate of metabolic activity of different ganglia is different, for if we make the comparison with tissue from a single ganglion we find that the CO₂ development is proportional to the mass. In the following experiment showing this, the whole ganglion weighed 21 mg. Its rate of CO₂ development

TABLE II.

Relation of Ganglionic Mass to Rate of CO₂ Production.

Temperature.	Whole ganglion = 21 mg.		Anterior part = 10 mg.		Posterior part = 11 mg.	
	Time.	Q ₁₀	Time.	Q ₁₀	Time.	Q ₁₀
°C.	<i>sec.</i>		<i>sec.</i>		<i>sec.</i>	
24	75		145		140	
14	160	2.1	340	2.3	325	2.3
4	510	3.1	1,200	3.5	1,095	3.3
14	150	3.4	330	3.6	275	4.0
24	60	2.5	150	2.2	120	2.3
34	35	1.8				

at different temperatures was determined and the ganglion then divided so that the posterior portion weighed 10 mg. and the anterior part 11 mg. After division, the parts were kept for a short time in neutral saline solution to avoid any complication from the acid which might have developed due to the injury; the rate of CO₂ development by each portion was then determined for the temperatures to which the whole ganglion had previously been subjected. The results are given in Table II and show conclusively that in a given ganglion the carbon dioxide production is proportional to the mass of ganglionic tissue.

⁷ Loeb, J., and Wasteneys, H., *Biochem. Z.*, 1911, xxxvi, 345.

That the cardiac ganglia of different *Limuli* should show individual differences in the rate of carbon dioxide development is not surprising but the physiological significance of the metabolic variations is not so apparent. In our first paper¹ attention was directed to the fact that there were marked individual variations in the rate of the cardiac rhythm of different *Limuli* but that all showed temperature coefficients indicating the chemical character of the causative reaction. The present investigation was undertaken to determine whether carbon dioxide development was affected by temperature like the heart rate, and while it has been demonstrated that this is the case it has yet to be shown that, given two ganglia of equal weight but with different heart rates, the rate of carbon dioxide development is proportional

TABLE III.
Relation of Cardiac Rate to Development of CO₂.

Temperature.	A Weight of ganglion = 18.4 mg.		B Weight of ganglion = 17.7 mg.	
	Heart rate per min.	CO ₂ development. Time.	Heart rate per min.	CO ₂ development. Time.
°C.		<i>sec.</i>		<i>sec.</i>
22	16	105	8	185
12	7	240	3	495
Q ₁₀	2.3	2.3	2.6	2.7

to the rate of the rhythm. To settle this point two *Limuli* of the same size were selected which showed heart rates of 8 and 16 per minute respectively at room temperature (22°C.). The hearts were excised and the rate of each at 12° was determined, after which the ganglia were dissected from the hearts and the rate of carbon dioxide development was then determined. The results, compared with the previous rates, are given in Table III.

The results of this experiment (Table III) leave no doubt that the rate of CO₂ development by the ganglia and the rate of cardiac rhythm run parallel courses.

All the experimental results herein reported thus show that the temperature coefficients of carbon dioxide production by the ganglia are of a magnitude entirely commensurate with those previously re-

ported by Garrey for the rate of neurogenic rhythm of *Limulus* hearts. The data strongly support the view that the chemical process within the nerve cells which is responsible for the rate of the heart beat is one which is associated with the development and rate of diffusion of carbon dioxide.

SUMMARY.

1. It is possible to determine by the colorimetric method the rate of production of carbon dioxide by the cardiac ganglion of *Limulus*.
2. Carbon dioxide formation in the cardiac ganglion was found to run parallel to the rate of heart beat for different temperatures.
3. The conclusion seems justified that the rate of cardiac rhythm of *Limulus* depends upon a chemical reaction in the nerve cells of the cardiac ganglion and that this reaction is associated with the production of carbon dioxide since the rate of beat and the rate of CO₂ production are similarly affected by changes in temperature.