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THE RELATION OF RESPIRATION TO RHYTHM IN THE CARDIAC GANGLION OF LIMULUS POLYPHEMUS.*

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A study of the carbon dioxide production by the cardiac ganglion of *Limulus polyphemus* has shown a definite relation between the rate at which the neurogenic heart beats and the intensity of the respiration of the nerve cells which develop the rhythm. This has been demonstrated by the parallelism between the temperature coefficient of rate of heart beat and of CO_2 production,[1,2] and also by the striking diminution in the CO_2 produced by the nerve cells when the inhibitory nerve to the ganglion is stimulated—a fact which correlates the inhibitory action of the ganglion with the respiratory processes of its nerve cells. These facts at once suggest that automatic impulse formation by the cardiac ganglion of *Limulus*, and probably by all automatic nerve centers, may be determined by the velocity of those chemical reactions in which carbon dioxide is produced by the nerve cells.

The following report presents additional evidence in support of this thesis and shows that a variety of agencies which stimulate the cardiac ganglion and increase the rate of heart beat, produce a similar increase in the rate of CO_2 production by the nerve cells of the heart ganglion.

Method.

The method was the same as that used in previous work.[1,2,3] The color change of phenolsulfonephthalein was used to determine the

* Funds granted by the American Association for the Advancement of Science were used to meet the expenses of this investigation.

[1] Garrey, W. E., *J. Gen. Physiol.*, 1920–21, iii, 41.

[2] Garrey, W. E., *J. Gen. Physiol.*, 1920–21, iii, 49.

[3] Garrey, W. E., *J. Gen. Physiol.*, 1920–21, iii, 163.

rate of change in hydrogen ion concentration which resulted from the formation of CO_2 by the excised cardiac ganglion. The ganglionic cord was immersed in 3 cc. of a standard non-buffer balanced saline solution in small Pyrex glass tubes. The initial pH was 7.8 and the time required to reduce the alkalinity to pH 7.4 was used as an index of the rate of CO_2 formation. To facilitate manipulation the ganglion was draped over glass hooks on a non-soluble glass rod which was cemented into the paraffined cork used to stopper the indicator tube. The standard immersion solution was made by adding 2.2 cc. of M/2 $CaCl_2$ to 100 cc. of M/2 NaCl and the desired initial pH, 7.8, was secured by adding the requisite amount of sodium hydroxide.

EXPERIMENTAL RESULTS.

Electrical Stimulation.—Faradic stimulation of the posterior end of the cardiac ganglion always produces acceleration of the beat of the intact heart. The excised immersed ganglion was similarly stimulated by means of platinum electrodes passed through the cork of the indicator tube. The rate of change in the CO_2 production was compared under this treatment with that of the unstimulated ganglion and the results indicate that during stimulation the rate of CO_2 production is enormously increased, being at least doubled or trebled as shown in Table I. This result is unquestionably due to increase in the chemical processes in the nerve cells, for the faradic shocks do not produce the color changes in the solution, even when the electrodes dip directly into the solution or when applied to a narcotized ganglion. The *decrease* in CO_2 production, when the inhibitory nerve to the ganglion is stimulated, also forms a control experiment which supports the conclusion that we are concerned with a true stimulation of the processes of respiration in the nerve cells in question.

Mechanical Stimulation. Stretching.—A further check upon the results of faradization is obtained by stretching the nerve cord. Carlson[4] had found that distension of the *Limulus* heart cavity increased the rate of the heart beats by its mechanical effect upon the cardiac ganglion. That stretching the nerve cord might produce

[4] Carlson, A. J., *Am. J. Physiol.*, 1907, xviii, 149; *Ergebn. Physiol.*, 1909, viii, 423.

distinct chemical changes seemed likely from analogy with a stretched striated muscle—which increases its osmotic pressure,[5,6] and produces excess of both lactic acid[7] and CO_2.[8]

To determine the rate of CO_2 production by the cardiac ganglion when stretched, silk ligatures were attached to either end of the nerve cord and looped over hooks on the glass mounting rod. By means of these threads it was possible to stretch the ganglion repeatedly, increasing its length some 20 per cent of normal, without apparent injury. The rates of CO_2 production before, during, and after the stretching in five experiments are given as averages in

TABLE I.

Faradic Stimulation and Rate of CO_2 Production.

Condition of ganglion.	Time required to change pH 7.8 to pH 7.4.				
	Experiment No.				
	1	2	3	4	5
	sec.	*sec.*	*sec.*	*sec.*	*sec*
Normal.....	192	266	327	840	1620
	221	268	332		
	218		315		
During stimulation.....	81	130	137	263	255
	100	168			
After stimulation.....	238	310	367	907	1620
	229	275	342		
	222				

Table II. That the increase during stretching is not merely a result of increasing surface for diffusion, is indicated by the persistent increase in the rate of CO_2 production after the stretching operation had stopped. It is due to the mechanical stimulation of the respiratory rate; and the results thus run parallel to the increase in the rate of heart beat when the ganglion is similarly stretched.

[5] Cooke, E., *J. Physiol.*, 1898, xxiii, 137.
[6] Garrey, W. E., *J. Biol. Chem.*, 1909, vi, p. x.
[7] Gotschlich, E., *Arch. Physiol.*, 1894, lvi, 355.
[8] Eddy, N. B., and Downs, A. W., *Am. J. Physiol.*, 1921, lvi, 188.

Alcohol.—In concentrations of one-half to one per cent by volume the cardiac ganglion of *Limulus* is markedly stimulated by ethyl alcohol[9] and may double the rate of heart beat. These concentrations of purified absolute alcohol, when added to the standard sodium-calcium chloride mixture do not of themselves modify the color of phenolsulfonephthalein used as pH indicator. In these concentrations, however, there results an easily demonstrable increase in the rate of CO_2 production by the ganglion, a result which is again in concord with the interpretation that the increased rate of heart beat depends upon the increased respiration in the nerve cells of the ganglion. Table III records the results of some of the experiments with ethyl alcohol as the stimulating agent.

TABLE II.

Rate of CO_2 Production by Stretched Ganglion.

Condition of ganglion.	Average time to change pH 7.8 to pH 7.4.				
	Experiment No.				
	1	2	3	4	5
	sec.	*sec.*	*sec.*	*sec.*	*sec.*
Normal	200	228	117	251	375
Stretched	142	170	77	163	263
Immediately after stretching	168	200	206	236	318
15 minutes after stretching	190	208		245	335

Sodium Chloride.—In the isotonic concentration, M/2, a sodium chloride solution is a pronounced stimulus to the automaticity of the cardiac ganglion of *Limulus*. The rate of heart beat is promptly accelerated and the individual beats of the muscle merge until a tetanic condition is induced by the continuous discharge of impulses from the ganglionic cells. Determinations of the rate of CO_2 production by the ganglion immersed in M/2 NaCl showed an increase in the rate of respiration of the nerve cells which harmonizes with expectations based on the increase in rate. The rate of CO_2 production was tremendously increased. The averages found in experiments with six ganglia immersed in isotonic NaCl gave the

[9]Carlson, A. J., *Am. J. Physiol.*, 1906, xvii, 177.

following percentages of the normal rate; *i.e.*, 311 per cent, 218 per cent, 237 per cent, 145 per cent, 260 per cent, 134 per cent. Subsequent to these determinations immersion of the ganglia in the sodium-calcium solution demonstrated the antagonism of these ions in their effects on animal oxidations for the mixture caused a return of the rate of respiration to within 15 per cent of the previous normal in every instance. This fact excludes the possibility that the increase in CO_2 formation in the isotonic NaCl is a lethal phenomenon, in the ordinary sense of that term, or at least that the stages dealt with

TABLE III.

Effects of 1 per cent Ethyl Alcohol on Rate of CO_2 Production.

Condition of ganglion	Time required to change pH 7.8 to pH 7.4. Experiment No. 1	2	3	4	Average time of all experiments.	Average rate of CO_2 production.
	sec.	*sec.*	*sec.*	*sec.*	*sec.*	*per cent*
In Na/Ca solution (normal).....	184	265	315	448	305	100
In Na/Ca + 1 per cent ethyl alcohol.	110	150	145	245	160	190
	90	140	150	265		
	102		165	290		
In Na/Ca solution.....	130	148	230	485	296	103
	147		255	230		
	201	248				
In Na/Ca solution + 1 per cent alcohol	152	166	160	315	198	154

are still reversible (Osterhout)[10]. Loeb and Wasteneys[11] working with concentration of sodium chloride isotonic with sea water found a decrease in oxidation in eggs of *Arbacia* and of *Gonionemus*; this apparent disharmony with the behavior of *Limulus* heart ganglia may be specific for the material used or may be merely an expression of the duration of exposure to NaCl or of the concentrations of the sodium chloride to which the tissue is subjected since Brooks[12] has

[10] Osterhout, W. J. V., *J. Gen. Physiol.*, 1920–21, iii, 15.

[11] Loeb, J., and Wasteneys, H., *Biochem. Z.*, 1910, xxviii, 340; 1911, xxxi, 168.

[12] Brooks, M. M., *J. Gen. Physiol.*, 1919–20, ii, 5.

shown that one can get an increase or a decrease in the respiration of *Bacillus subtilis* depending upon the concentration of NaCl employed.

Adrenalin Chloride.—Addition of one drop of adrenalin chloride solution (1 part to 10,000 parts of solvent) to each cubic centimeter of the standard sodium-calcium solution stimulates the ganglion and increases the heart rate[9]. Experiments with three ganglia immersed in this concentration of the drug gave the average rate of CO_2 production as 204 per cent, 194 per cent, and 166 per cent of the normal.

These experiments amply justify the generalization that those stimuli, which acting on the cardiac ganglion to cause an increase in the rate of the heart beat, likewise produce changes which result in a marked increase in the respiratory processes within the nerve cells. Impulse formation by the ganglion, and CO_2 production are therefore intimately related processes and appear to stand in relation of effect and cause respectively. A complete justification of this postulate requires that we shall be able to establish a quantitative correspondence between the processes of impulse formation, that is rate of heart beat, and the rate of CO_2 formation. Experiments directed to this end could not be conducted in such a way as to record both the rate of heart beat and the rate of CO_2 production on the same preparation simultaneously, but it was possible to secure a satisfactory approximation by taking advantage of the characteristic effects produced by changes of temperature especially the effects of transient exposure to relatively high and low temperatures. These are considered in the following paragraphs.

Effects of Temperature Extremes (Transitory Exposures).

Reference has been made in previous communications[1,2] to the fact that if the ganglion is exposed for a few minutes to a very low temperature (0°C.) and then warmed to 10°C., for example, a new rate of rhythm is established which is faster than was obtained at this temperature before cooling. This increase in rate may amount to 25 per cent or 30 per cent. A similar effect upon the rate of carbon dioxide produced by the ganglion has been noted under like treatment, and eight examples have been referred to in Tables I and II of a former paper.[2] In four experiments more recently carried out the times required for the color change in our indicator at 10°C.

were 408, 532, 618, and 803 seconds, but after exposing the ganglia for five minutes to 0°C. then again warming to 10°C. the time was in each case shortened being respectively 320, 416, 482, and 629 seconds. The increase in the rate of CO_2 production averages 28 per cent in these experiments and is in good quantitative correspondence to the acceleration rate of rhythm as stated above.

When on the other hand ganglia are exposed to the upper extremes of temperature compatible with function, *e.g.*, to 35°C. or 40°C., and are subsequently cooled the rate of rhythm is much *slower* at the lower temperature than before warming process; thus in five experiments in which the rates at 25°C. were determined before and after heating the ratios of the actual rates of beat were 20:17, 20:13, 18:13, 24:20, and 17:8. This corresponds to an average decrease to 70 per cent of the previous rate. Four other experiments in which similar conditions obtained indicated a depression in the rate of CO_2 to a point only 78 per cent of the value before exposure to the high temperature. These values for both extremes of this temperature range again show a very good quantitative correspondence between the effects upon rate of heart beat and rate of CO_2 formation by the ganglia.

The quantitative relationship between the two processes is made much more striking by the graph given in Fig. 1. This gives the curve for a single typical experiment in which the rates of beat are plotted as ordinates against temperature as abscissæ. In this experiment the ganglion was progressively cooled from room temperature to 0°, then warmed to 38°, and again cooled. The direction of the temperature change is indicated by the arrows and the rate by outline characters, triangle, circle, and square, respectively.

A companion graph was constructed for the rate of CO_2 formation. For this purpose eleven experiments were taken in which the ganglia had been treated to the same progressive changes in temperature as in the previous experiment on rate of heart beat. The average time required for CO_2 formation was determined for different temperatures. It was found that if the rate of CO_2 formation was expressed as the reciprocal of the time in seconds and this was multiplied in each case by the constant 1400 a plot of the results gave a curve identical with that shown in Fig. 1. The points obtained by this calculation

have been introduced into Fig. 1 as solid characters. This striking quantitative parallelism between rate of heart beat and rate of development of CO_2 in the cardiac ganglion, which is brought out by this graph, forcefully substantiates the probability that the two

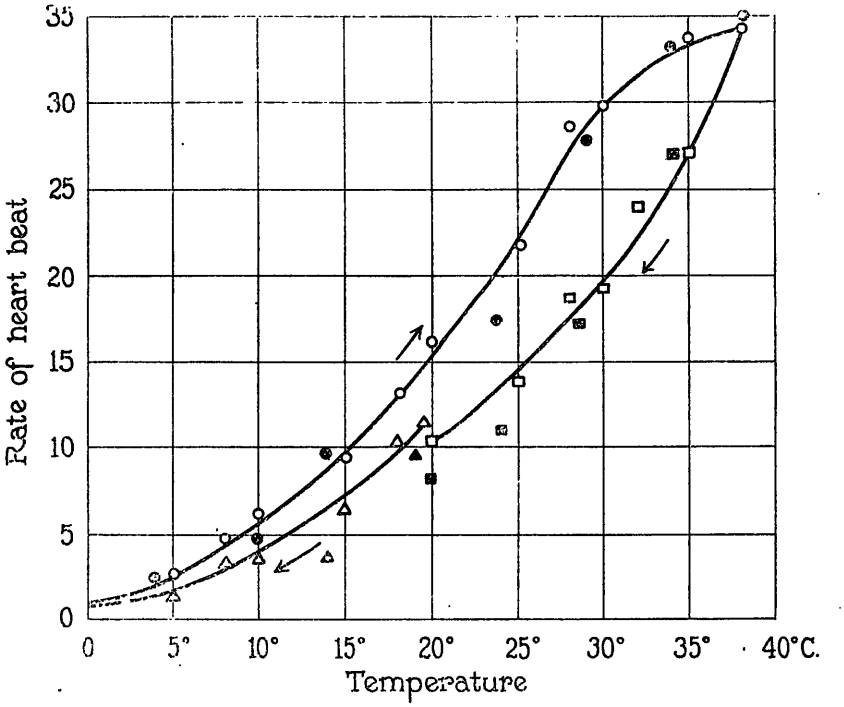

FIG. 1. Curve of rate of heart beat when the ganglion is initially cooled from the normal temperature to 0°C. (△), then progressively warmed to 38°C. (○), and finally cooled to room temperature (□). The solid characters indicate the corresponding average rates of carbon dioxide production by the ganglion.

physiological processes are bound together in the relationship of cause and effect and that it is the velocity of those chemical reactions which give rise to carbon dioxide formation in the cells of the cardiac ganglion which determines the rate of the neurogenic heart of *Limulus polyphemus*.