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THE ACTION OF INHIBITORY NERVES ON CARBON DIOXIDE PRODUCTION IN THE HEART GANGLION OF LIMULUS.

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While stimulation of nerves generally results in increased activity, the vagus has the opposite effect of diminishing the activity of the heart. The mechanism of this inhibition has not yet been explained. The author's recent experiments on the heart ganglion of *Limulus polyphemus*[1] have shown that stimulation of the inhibitory nerves of this heart results in a decrease in the production of CO_2 in the ganglion, while direct stimulation of the ganglion by electrical or mechanical or chemical means has the opposite result, namely an increase in CO_2 production in the heart ganglion, as will be shown in a later paper. This leaves no doubt that the action of the inhibitory nerves upon automatic ganglia is due to a decrease in those chemical processes in the ganglion which result in the formation of CO_2.

Methods.

The rate of CO_2 production was determined by the method previously described by the author.[2] Briefly, the ganglion, with its inhibitory nerve is dissected from the heart and immersed in 3 cc. of a

[1] The special suitability of the heart ganglion of *Limulus* arises from the fact that the heart may be isolated from the animal but beats for a day or more, likewise the ganglion may be dissected free from the heart and if connected with one segment alone will continue to deliver its impulses to the muscle for hours in sea water or in the solution used in this investigation. We are thus able to make our tests without concern about questions of changes of blood pressure, of vasomotor action, of chemical composition of the medium, or of the secondary action of other nerve supply which so complicate our experiments on the vertebrate nerve centers.

[2] Garrey, W. E., *J. Gen. Physiol.*, 1920–21, iii, 41, 49.

physiological saline solution consisting of 100 cc. of M/2 NaCl plus 2 cc. of M/2 $CaCl_2$ and containing phenolsulfonephthalein to indicate the hydrogen ion concentration.

We note the time required to produce the color change which indicates a change in the hydrogen ion concentration from the initial value pH 7.8 to 7.4. This gives a normal standard of the rate of development of CO_2 which can be compared with the rate at which the ganglion produces the same change while the inhibitory nerves are being stimulated.

Owing to the many failures to secure preparations of the ganglion with the inhibitory nerve uninjured and functioning, the following technique was adopted. Upon opening the dorsal carapace and pericardium the inhibitory nerves were identified by faradic stimulation; they were then secured distally with a fine silk ligature. The pericardium was removed from the whole heart and the cardiac ganglion, from its posterior end to the middle of the second segment, was dissected from the heart. This left functional connection of the ganglion with the first muscular segment which therefore continued to beat. The ganglion, free from all extraneous tissue, was now looped over tiny hooks on a slender glass rod which was cemented into the stopper of the indicator tube and the inhibitory nerves connected with it were laid across fine platinum wire electrodes passing through and cemented into the stopper. The nerves were again stimulated to determine whether they still inhibited the ganglion and stopped the beats of the first muscular segment. If the dissection has been successful it is only necessary to snip the nerve cord anterior to the point at which the inhibitory nerves enter to obtain the desired preparation of the ganglion mounted and ready for introduction into the tubes containing the solution with the indicator (phenolsulfonephthalein). We now determine the rate of the desired color change.[3]

[3] In a simpler preparation the ganglion alone was used and stimulation applied to its anterior end. This usually produces inhibition but stronger faradization may stimulate, so that the analysis of the results of the respiratory changes was sometimes problematical, therefore we have confined our published results to those obtained by the method described in the text.

Results.

The results of several typical experiments are summarized in Table I. The normal values for the time required to produce the change in pH are obtained before and after stimulating the inhibitory nerves and compared with the corresponding time while the stimulation is maintained. Differences in the time normally required by the different preparations to produce the change are due in part to differences in the size of the ganglia and in part to inherent differences in the intensity of the processes of CO_2 production. The results obtained by these experiments deal essentially with relative rates of CO_2 production, no attempt having been made to determine absolute values for the amount of CO_2 produced.

TABLE I.

Rate of CO_2 Production, before, during, and after Stimulation of Inhibitory Nerves.

	CO_2 production expressed in time required to produce color change.				
Experiment No.	1	2	3	4	5
	sec.	*sec.*	*sec.*	*sec.*	*sec.*
Normally. a	98	262	130	179	72
Normally. b	113	250	130	184	—
Normally. c	102	—	—	—	—
During inhibition	258	848	268	289	154
	—	—	242	—	—
After inhibition. a	197	546	154	201	78
After inhibition. b	124	314	—	—	75
After inhibition. c	120	286	126	180	—

Analysis of the experiments leaves no doubt that inhibition of the automatic cardiac ganglion is a process involving a well marked depression of the CO_2 production in the ganglia (20 to 60 per cent of normal). The fact that the carbon dioxide production is only retarded and not completely suppressed is probably due to incomplete inhibition or to escape from inhibition toward the end of the period during which the determination is being made, since it has always been noted that the rate at which the color change progressed was more rapid toward the end of a determination when the ganglion was presumably gradually escaping from inhibition.

In preparations showing a low initial rate of CO_2 production it is easy to depress this rate by inhibition for a much longer time than with vigorous preparations. In one experiment (not tabulated) no color change was noted at the end of 30 minutes of inhibitory nerve stimulation, although subsequently the normal rate of 5 minutes was restored. These observations are in complete harmony with what we know about inhibition of the contraction rate in this form.[4]

TABLE II.

Effect of Strength of Inhibiting Stimuli on Time For CO_2 Production.

Experiment No.	Normal (average).	Weak inhibition. Harvard coil. (13 to 10 cm.)	Strong inhibition. Harvard coil. (7 to 4 cm.)
	sec.	*sec.*	*sec.*
1	131	197	276
2	201	237	309
3	167	216	388
4	259	402	734
5	172	327	498
6	78	112	286
7	118	196	243

Since strong stimuli applied to the inhibitory nerves of the heart produce a greater slowing of the rate of heart beat than weak stimuli, experiments were made to see whether they produce a similar effect upon the rate of CO_2 production in the ganglia. Some typical experiments are given in Table II.

The results leave no doubt that the stronger inhibiting stimulation, which is known to produce a slower heart beat, also causes a greater depression of the rate of the chemical processes in the ganglia which give rise to the formation of CO_2.

If we view the entire process of inhibition we may represent the experimental results graphically as has been done in Fig. 1 which is constructed for four of the experiments in Table I. The graph shows

[4] Carlson, A. J., *Am. J. Physiol.*, 1905, xiii, 217.

that the drop in the rate of CO_2 production follows abruptly upon the incidence of inhibition but that the return to the normal after inhibition is a much more gradual process, indicating a persistence of the inhibitory state after the cessation of stimulation of the inhibitory nerves. The recovery to the normal rate of CO_2 production is slower the greater the degree of depression during inhibition.

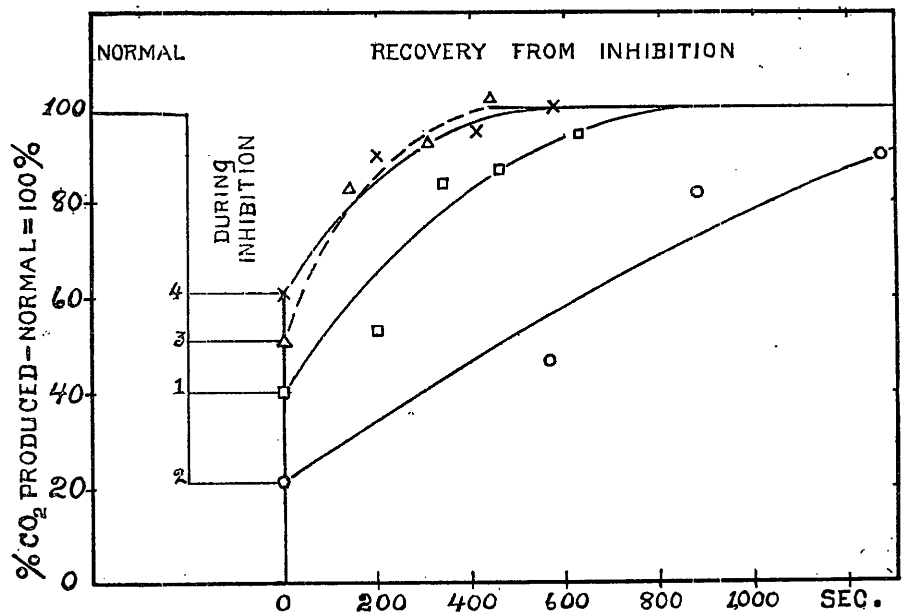

FIG. 1. Curves showing the percentage rate of CO_2 production compared with normal = 100 per cent. The numbers 1, 2, 3, and 4 correspond to the experiment numbers in Table I. The horizontal levels opposite these numbers show the average depression of CO_2 production during the period of inhibition which in each case continued for the time required to make the determination (*cf.* Table I). The readings for the curves of recovery are designated for each experiment by the character at the end of the inhibition period. Recovery time in seconds.

It is significant that the recovery process involves a return merely to the previous norm and that there is no subsequent increase in the rate of CO_2 production, no acceleration or after-augmentation, so that experimental evidence lends no support to the view that inhibition as exemplified in these nerve cells is accompanied by any constructive or anabolic processes. The latter processes are typical of arrest,[5] but mere cessation of action, such as occurs in blocking of impulses, is not to be confused with true inhibition which must be conceived as synonymous with depression of the chemical processes concerned in CO_2 production in the nerve cells. When this conception of inhibition is applied to other types of cells as well as to nerve cells, it will do much to clear up conflicting and confusing ideas concerning the true nature of the inhibitory mechanism.

SUMMARY AND CONCLUSIONS.

It has been shown in this paper that stimulation of the inhibitory nerves of the neurogenic heart of *Limulus*, which correspond to the vagus nerves of the vertebrate heart, results in a marked diminution of CO_2 production in the heart ganglion, while stimulation of the ganglion, leading to increased activity of the heart, leads also to increased CO_2 production by the ganglion. This shows that inhibition of the automaticity of this ganglion by the action of its inhibitory nerves consists, not in a process of blocking, but in a diminution of those chemical reactions in the ganglion cells which give rise to the production of CO_2.

[5] *Arrest* may be quite independent of inhibition and due to secondary causes such as blocking or removal of stimulating impulses. This is exemplified upon stimulation of the vagus fibers to the turtle heart; the auricles are inhibited (true depression), the ventricles merely arrested, and the latter only show the after-augmentation.