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THE PACE MAKER OF THE CARDIAC GANGLION OF LIMULUS POLYPHEMUS

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The ganglion on the dorsal surface of the heart of Limulus polyphemus originates the impulses which cause the contractions of the cardiac muscle (1). In large specimens the portion of the dorsal nerve cord which contains the ganglion cells may be 10 or even 12 cm. in length and one naturally wonders where in the extent of this long structure the impulse actually arises. Does it arise in a specific locus? Can it arise at different places? Does the whole ganglion function with each contraction, and if so what is the coördinating mechanism? These questions have been dealt with experimentally, treating the ganglion as if it were comparable and analogous to the pace maker of the vertebrate heart.

Section and excision: a. If a transverse section be made across the entire tubular heart at the level of the sixth segment, counted from the anterior end, the two pieces thus separated each contain about one half of the length of the ganglionated portion of the nerve cord. After the initial mechanical stimulation due to the operation the pieces settle down each to a perfect rhythm which measurement of tracings shows to be identical with the original rhythm, and each of the two pieces thus procured may be again cut into two sections and again each of the pieces will finally settle down to the original rhythm. The removal of the ganglion, i.e., the ganglionic cord and all outlying extensions which frequently are present, from any piece at once and forever stops all contractions of the muscle of that piece, proving the neurogenic origin of the beat as has been noted by Carlson. Two facts are noticeable: *a.* Although the rate of each piece remains the same as that of the original whole heart, the pieces no longer beat synchronously, but are out of phase; for example, while one piece is contracting another will be relaxing. The result is identical with that which Dr. Ida Hyde described for the movements of the gill plates of Limulus, which occur at a definite rate and in definite sequence, but if the ventral nerve cord is cut between the ganglia controlling each of the five gill plates the contractions of each gill plate continue at the same rate as before the operation but different ganglia are out of phase. These experiments indicate that the

inherent rhythm of the ganglion cells of all parts of the nerve cord of the Limulus heart is very approximately identical and that a coördinating mechanism exists such that all acting cells discharge their impulses with each contraction, i.e., that the ganglion normally acts as a coördinated whole in its discharge of impulses. *b.* The second noticeable result is the weakening of the height of contraction of any given segment as the pieces are decreased in size. That this is a result of decreasing the amount of ganglionic tissue connected with the contracting muscle is shown by another type of experiment in which records were taken from the second muscular segment. All ganglion cells are caudad to this segmental level, and the impulses which cause this second muscular segment to contract are con-

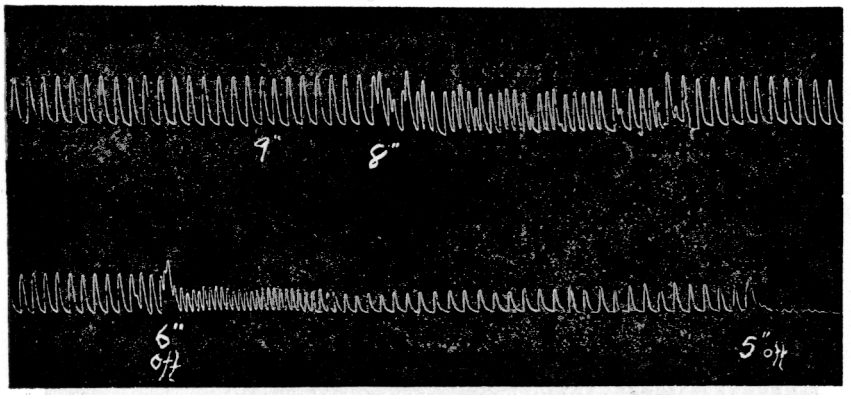

Fig. 1. The effects of progressive removal of the ganglion from Limulus heart: with progressive decrease in height of contraction. Return to normal rate in spite of the reduction in the amount of ganglionic tissue. The number represents the segment removed. Between the upper and lower tracing the ganglionic cord above segment 7 was removed. Note the temporary acceleration of rate due to cutting the ganglion at each level. Normal rate 20 beats per minute.

ducted forward from the ganglion by the median and two lateral nerves. If now one removes small bits of the ganglion piece-meal, working progressively from the posterior end forward, it is found that although the rate remains unchanged, except for the temporary acceleration due to cutting, the height of contraction of the anterior muscular segments decreases pari passu (fig. 1). Only one interpretation is admissible, viz., that all parts of the ganglion, although not necessarily all the ganglion cells, take part in the development of every impulse and that every part of the ganglion is connected with the cardiac musculature independently of the segmental arrangement. This conclusion is compatible with the anatomical description by Patten (2), i.e., with branching nerve fibers within the nerve cord and with their distribution by means of the median nerve and by lateral

branches to the lateral marginal nerve strands. Removal of pieces of the ganglion is tantamount to cutting off the nerve fibers from it to the muscle and Carlson (loc. cit.), Garrey (3) also, already have shown that cutting these nerve fibers to the muscle decreases the height of the contractions. The result is comparable to the cutting of the motor nerve fibers innervating a skeletal muscle, thus decreasing the number of contracting muscle fibers.

Having thus established the fact that under normal conditions the ganglion acts as a coördinated whole in the development of the impulses and that the inherent rhythmicity of all parts of the ganglion is practically identical, experiments were directed to the analysis of the conditions

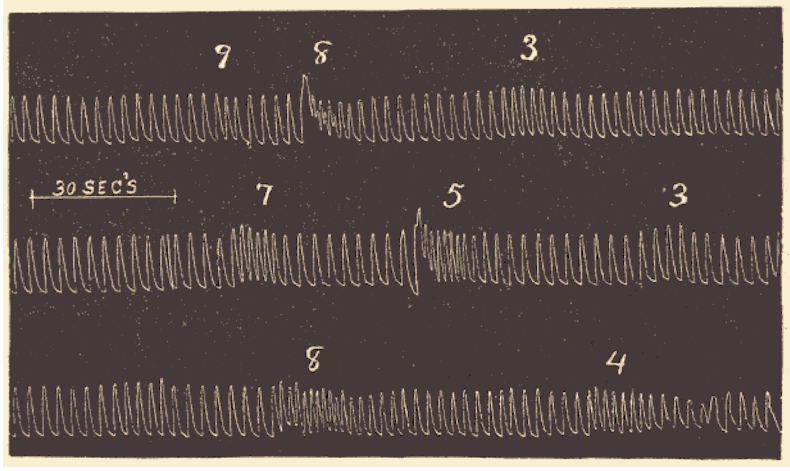

Fig. 2. Effects of applying a warm test tube to the ganglion at the segmental level indicated by the figures above the respective tracings. There is temporary accelerated coördinated rhythm, with return to normal rate after warming has ceased.

affecting individual parts of the ganglion. In this connection attention should be drawn to the fact that in the experiments described above cutting the nerve cord acts as a mechanical stimulus the response to which is an acceleration of the rate in which all segments participate coördinately and which may continue for several seconds or even minutes.

No matter at what segmental level the ganglion is cut, the sharply localized stimulus causes a reaction on the part of the ganglion as a whole. Evidently any small group of cells when stimulated can act as a pace maker and impress their rate upon other cells of the rhythm producing ganglion, as is likewise true of the rhythm producing foci of the vertebrate heart (Gaskell, 4).

Localized heating. Gaskell applied heat to the sinus of the cold blooded

heart and produced acceleration of rate; this was an effect solely upon the pace maker, for heating the ventricles caused no change in rate. In like manner MacWilliam (5) localized the pace maker of the mammalian heart by cooling and warming, and Garrey (6) has shown that it is possible by cooling one part of the turtle's sinus and warming another to shift the pace maker to the warmed region. Since warming the Limulus heart ganglion accelerates the rhythm (Carlson, loc. cit.; Garrey, 7), this method was resorted to in the production of localized changes which it was thought might localize the pace maker in definite and circumscribed parts of the ganglionic cord. In its crudest form the experiment was conducted as follows: a myogram was taken, any segmental level sufficing, although the second segmental anterior to the level of the ganglion cells was usually selected so that it was possible to work with the ganglion without disturbing the muscle record. The dry bottom of a small test tube containing heated

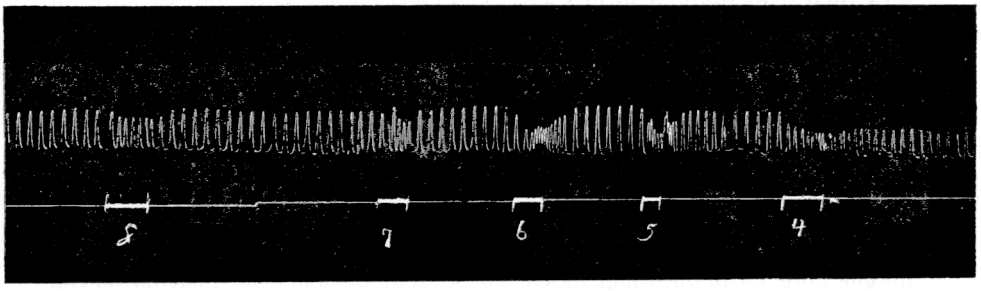

Fig. 3. The effects of heating one millimeter length of the ganglion of Limulus heart using an electro-thermal loop. Signals mark the duration of heating and the figures the segmental level.

sea water was applied at different levels to the ganglion, less than 0.5 cm. of the length of the ganglion was thus warmed, the remainder of the ganglion which in every case was from ten to twenty times the length heated, was kept at the original temperature. In every instance, and no matter at what level between the third and eighth segments the heat was applied, there was sharp acceleration of the rate while the localized high temperature was maintained (fig. 2). There being no ganglion cells anterior (cephalad) to the third segment no effects could be expected by heating this part of the nerve cord; the experiment thus serves as a means of determining the presence of ganglion cells and determining their anatomical location.

A smaller and more sharply circumscribed part of the ganglion was heated by the use of a tiny loop of fine resistance wire placed either under or against the nerve cord, at any level, and heated by closing a galvanic current. In this way a length not exceeding 2 mm. of the ganglionic cord was warmed and again in each instance there was an accelerated rhythm.

From these experiments we are justified in concluding that any small group of cells, no matter at what level in the ganglion they are situated, may become the pace maker and force the discharge of impulses from cells in all parts of the ganglion at the rate of those most active, i.e., of the heated cells (fig. 3).

Localized cooling. If a cold test tube (2°C.) is applied to a local spot on the ganglion no change in rate is observable. This result was expected as a deduction from the experiments described above. As long as any part of the ganglion remains at the original temperature which is above that of the new artificial experimental conditions then the part at the original higher temperature will determine the rate of the whole heart. Even if three-fourths or more of the ganglion is thus cooled there will be no change in rate. In fact when the whole ganglion is kept cold by covering it lightly with the bottom of a beaker or side of a test tube containing iced sea water and then heat applied to any localized spot on the ganglion, the rate of impulse formation is determined by the small region thus heated and is quite independent of the cold condition of the remainder of the ganglion, although exceptionally, owing to failure of normal conduction and therefore of coördination in the ganglion, the heated and cooled regions act independently and the muscle responds incoördinately and irregularly, the rapid rhythm being superimposed on the slow rhythm. These reactions in general are entirely comparable to the effects which one can induce in the cold blooded vertebrate heart in which the exact site of the pace maker may be determined or shifted at will by localized heating of the sinus venosus.

Localized stretching. Another factor which normally may come into play in determining cardiac rate in this animal is the degree of intracardiac tension. Carlson has demonstrated that when the intracardiac pressure is increased there is an increased rate, and ultimate incoördination if the pressure is excessive. We have noted in our experiments that when the anterior end of the animal was raised, changing the heart from the horizontal to the vertical position, the increased intracardiac pressure and consequent distention of the posterior segments of the heart completely altered the character of its contraction. Normally with the heart in the horizontal position the posterior segments beat approximately at the same time and as a unit (cf. Edwards, Pond, loc. cit.), but under the new experimental conditions in the vertical position the posterior segments beat first and the contractions progressed forward involving the anterior segments successively. This suggested the probability that there was a localization of the pace maker in the posterior end of the cord developed by stretching it. If a small hook or fine silk thread was passed under the ganglion, traction and the consequent slight stretching could be confined to a very localized region; this served to form a localized stimulus and to

establish a pace maker at the point stretched and as in other experiments, the coördinated impulses at the faster rate were developed by the ganglion, which in all these cases responded as a unit to the fastest rhythm of the experimental pace maker. Continued traction applied as above described, to the posterior end of the ganglion duplicated the effect of change to the vertical position and caused the posterior segments to beat before those

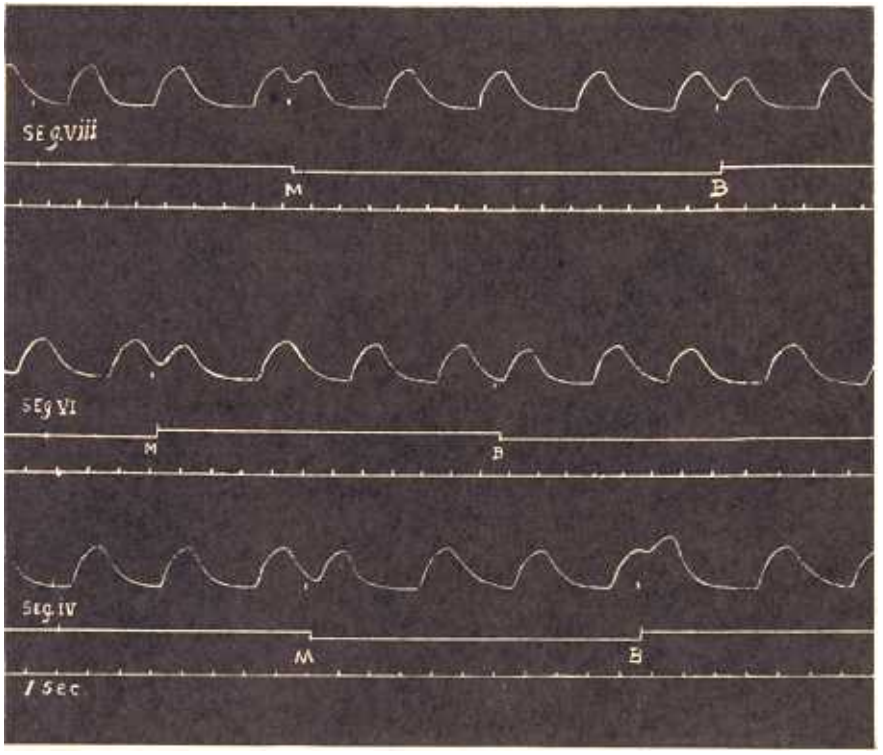

Fig. 4. Extra systoles produced by single induction shocks applied to the Limulus heart ganglion at any segmental level (indicated for each tracing). The extra systoles may be induced at any phase of contraction. There is no compensatory pause. The ganglion acts like the pace maker of the vertebrate heart.

anterior to it and there was a progressive involvement of segments from the posterior to the anterior end of the heart.

Localized electrical stimulation. A single induction shock applied at any point on the ganglion between the third and posterior segments (using electrodes with the two pints not over a millimeter apart) will produce an extra systole involving the entire musculature of the heart, thus demonstrating an evident coördinated involvement of cells throughout the entire extent of the ganglion. The heart thus responds with an extra systole

in exactly the same way the vertebrate heart does when the pace maker, i.e., the sinus venosus of the cold blooded heart, is stimulated, i.e., there is no compensatory pause and the normal rhythm is resumed after an interval only slightly exceeding the normal intersystolic interval (fig. 4). Analytic measurements of tracings showing the effects of extra systoles have been made by Prof. A. Samojloff (8), and his results appear elsewhere in this issue of THIS JOURNAL.

The effects of faradic stimulation are complicated by a number of factors which will not be considered in this communication beyond the general statement that if the stimulus be of selected intensity its application to any point in the course of the ganglion will accelerate the rhythmic discharge of the entire ganglion and force a rhythm determined by a localized pace maker.

It is inconceivable that all the multitude of ganglion cells throughout the course of the entire ganglion should always be in exactly the same physiological state at all times; therefore, the fact that they do discharge their impulses coördinately can only be interpreted as meaning that fortituous circumstances determine which cells will set the pace. It is conceivable, in fact it is highly probable, that this locus shifts from one part of the ganglion to another with inherent or local variations in physiological state. Carlson has noted that under certain conditions the posterior segments contracted before the others. That this is true when the pace maker is forced to the caudal end of the ganglion either by stimulation or by change to the vertical position has been noted above. Other reports indicate that the beat may begin in the middle segments (Carlson, 9). To settle this question Edwards (10) made accurate time measurements of the relative sequence of contractions in different segments of the Limulus heart and Pond (11) has also made a similar study. It is clear from results of these workers that the anterior segments beat later than the middle segments,—they are farther away from the rhythm producing ganglionated part of the cord and it takes time for the impulses to reach them. One may also conclude from their experiments that segments in the middle or posterior part of the heart which receive lateral branches from approximately common points on the ganglion beat together. Beyond these facts there is no evidence that there is an invariable sequence in contraction of the various segments. This may be interpreted as meaning that the pace maker has no fixed location, but may be located at different levels in different hearts, or may even shift in a given heart from one level to another where conditions, either natural, or experimentally induced, cause variations in the rhythmicity of a circumscribed group of cells.

SUMMARY

The ganglionated portion of the dorsal nerve cord on the heart of Limulus polyphemus, which is known to initiate the cardiac rhythm, is in every way comparable to the pace maker of the vertebrate heart.

The actual initiation of impulses may, however, be restricted to a very small group of nerve cells, and it is shown that it is possible by experimental methods to shift this pace maker group to any level of the ganglionated cord. In each such case a coördinating mechanism forces the rest of the ganglion to assume the rate of impulse formation impressed upon it by the most active cells. Normally this pace maker group has no fixed location and is determined by fortuitous circumstances.

BIBLIOGRAPHY

(1) CARLSON, A. J. Ergebn. d. Physiol., 1909, viii, 371. (cf. Literature)
(2) PATTEN, W. Evolution of vertebrates, etc. Philadelphia, 1912.
(3) GARREY, W. E. This Journal, 1912, xxx, 283.
(4) GASKELL, W. H. Phil. Trans. (London), 1882, 993.
(5) MACWILLIAM, J. A. Journ. Physiol., 1888, ix, 167.
(6) GARREY, W. E. This Journal, 1911, xxviii, 330.
(7) GARREY, W. E. Journ. Gen. Physiol., 1920, iii, 41, 49, 149.
(8) SAMOJLOFF, A. This Journal, 1930 (in press).
(9) CARLSON, A. J. This Journal, 1904, xii, 471.
(10) EDWARDS, D. J. This Journal, 1920, lii, 276.
(11) POND, S. E. Journ. Gen. Physiol., 1921, iii, 807.