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SOME ASPECTS OF THE PHYSIOLOGY OF THE HEART OF LIMULUS POLYPHEMUS

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We are all familiar with Pasteur's dictum that "chance favors only the prepared mind." All physiologists have had their chance to investigate the fascinating heart of Limulus since its anatomical description by Milne-Edwards in 1873 and the more extensive studies by Patten and Redenbaugh in 1899. There were no reports of physiological studies until 1904 when Prof. A. J. Carlson undertook the investigation of this heart and published his papers—now physiological classics—as a culmination of a series of studies on the invertebrate heart. His was the prepared mind. Subsequently many physiologists in this country and abroad have extended this work, but always to confirm his experimental findings, proving that the rhythm of this heart is neurogenic, that the impulses arise in one or all of the ganglion cells of the median dorsal ganglion (and plexus) of this heart, that they are conducted by nerve fibers and cause muscular contractions in the same way that contractions of skeletal muscles are caused by motor nerve impulses from the central nervous system of vertebrates. Carlson reasoned by analogy that the vertebrate heart was likewise neurogenic, but all evidence now seems to point to the conclusion that his deductions were erroneous and that the vertebrate heart is purely myogenic. The differences in the physiological responses of these two classes of heart far outweigh the similarities and definitely label the vertebrate heart "myogenic," the Limulus heart "neurogenic." A recently launched attempt of Dubuisson, following the lead of Hoshino to show that the Limulus heart is myogenic has been wrecked on the rocks of faulty technique, insensitive methods and inadequate controls. The following review will emphasize the extent of the wreckage.

Both the vertebrate heart and that of Limulus are automatically rhythmic; they beat when excised from the body. The rhythm of the vertebrate heart originates in the basal part of the heart, in the mammalian heart in a definite collection of modified muscle cells called the "sinus node". In Limulus the rhythmic impulses originate in the elongated median dorsal ganglion, the ganglion cells of which are distributed chiefly from the third to the eighth cardiac segments. Removal of the ganglion brings the heart to rest. In rare instances weak contractions may still persist after this operation and may be demonstrated more clearly by distending the heart and thus increasing its excitability. The origin of these impulses can be demonstrated to be due to ganglion cells in the outlying dorsal nerve plexus. They may be located by systematically hunting for them with the end of a heated test tube; when found they respond to heat with an accelerated rhythm which affects the rate of response of the muscle which may be located several segments away. The rhythm disappears when the nerve cells are destroyed or their efferent nerve fibers cut. Impulse formation by the ganglion is incontrovertibly proven by the demonstration of rhythmic electrical changes in the excised ganglion, the action potentials of which have been recorded by Heinbecker and thoroughly studied by Rijlant in this laboratory by means of the kathode ray oscillograph.

Conduction and coordination in the vertebrate heart are effected by conduction from muscle cell to muscle cell, and the organization is such that if one fiber contracts, the entire muscular structure likewise responds according to the all or none law. The Limulus heart is very different; conduction is effected only by nerve fibers. While anatomically the heart muscle of Limulus is described as a syncytium, its physiological response shows that it is really made up of independent contractile elements.

If the muscle is directly stimulated by an electric shock, the contractile response is limited to the area stimulated and does not spread through the muscle The contraction is greater the stronger the stimulus and repeated stimuli likewise induce greater contractions than single shocks Unlike the vertebrate heart tetanus can be induced by repeated stimuli, even as few as ten per second sufficing to this end. We thus see that three characteristics of the vertebrate heart fail in the muscle of Limulus heart, viz., conduction, the "all or none" response and failure of tetanic response. The same results can be obtained by stimulating the motor nerve fibers which form the conducting bridge between ganglion cells and muscle fibers. If one progressively removes the ganglion piece meal, beginning at the posterior end, while recording the contractions of the anterior (non-ganglionated) muscle segments, there is a progressive weakening of the contractions. The operation progressively severs the nerves connecting the ganglion cell with the muscle, thus extinguishing some of the ganglionic impulses and paralyzing some of the contractile elements. I have shown that this progressive paralysis of the muscle may be induced in three stages by cutting the median dorsal nerve and the two lateral nerves

which are the only motor nerves to the anterior muscular segments. Stimulation of these three nerves likewise demonstrates a partial and fractionate innervation of the musculature by each. Stimulation of each lateral nerve causes a contraction affecting predominately the ipselateral half of the muscular ring of each segment. The median nerve innervates both halves of the heart. A maximum contraction can be secured only by stimulating all three of these nerves and only by the use of rapid repetitive stimuli, thus inducing multi-wave and multi-fiber summation. By bringing these three nerves into action in succession the height of the tetanic contractions may be superimposed in three successive stages and must be due to the independent contraction of three separate groups of muscle fibers. Any one of these groups may be completely fatigued without affecting in any way the responses of the other groups. Normal contraction due to the rhythmic discharge of the ganglion is never maximal and may be significantly increased by stimulation of any one of the motor paths, the rhythmic contractions being then superimposed on the tetanic base thus established.

These hitherto unpublished results are crucial proof that the Limulus heart beat is not and cannot be myogenic as Dubuisson has claimed; and they dispose of all analogies to the contraction of the vertebrate heart which does not manifest any responses comparable to those of the Limulus heart muscle.

Stimulation of the ganglion at any point with a single stimulus, electrical or mechanical, induces a discharge of motor impulses from the entire length of the ganglion; it induces an extra systole which involves the musculature of every segment. This reaction necessitates an intimate connection of every part of the ganglion with every other part of it and shows that the ganglion at one point or another is connected by nerves with every part of the heart. The refractory period of the ganglion is very short and such extra systoles may be summed with the contraction induced by the preceding normal contraction. The normal autogenous impulse which follows such an extra systole does so at an interval slightly greater than the normal interval. This response is characteristic of only one locus in the vertebrate heart, viz., the "pace maker"; it likewise proves the ganglion to be the "pace maker" of the Limulus heart.

If we turn now to the consideration of the "pace maker" function of the ganglion, the experiments just considered indicate the possibility of impulse formation in any part of this extended cord-like structure. This is easily demonstrable by the localized application of heat to the ganglion; for example, touching the ganglion anywhere between the third and eighth segment with the bottom of a warm test tube will always accelerate the rhythm; furthermore, this result may be secured by heating a very restricted region one or two millimeters in length by means of a loop of resistance wire carrying current enough to induce the desired heating effect; stretching by means of a thread passed under the ganglion at any point will accomplish the same result. By these means we have been able to demonstrate the rhythmogenic power of every part of the ganglion and to develop a "pace maker" at any desired point. The rhythm of the entire structure is determined by that part of the ganglion having the greatest rhythmicity. Heating or treating deganglionated muscle in this way never develops rhythmic properties in it.

At this point we may ask: where is the normal "pace maker" located in the ganglion? By dividing the heart into smaller pieces by transection at different levels, Carlson demonstrated a slightly greater rate of contraction of the fifth and sixth segments. Edwards by optical means found that the fifth segment beat slightly in advance of those either anterior or posterior to it, and Rijlant, with the kathode ray oscillograph, found a like spread of the action currents in the ganglion and anterior portion of the median nerve. Both found that the conduction proceeds at the rate of about seventy-five centimeters per second. Thus the whole heart does not beat synchronously as Dubuisson claims, but there is a successive involvement of the muscle farther away from the fifth or sixth segment. The time required for this process, however, is less than one-tenth of a second, and since the actual contraction lasts for more than a second at laboratory temperatures it follows that for most of the time of systole all segments are contracting, as anyone can easily see, but only methods of precision and a skilled technique can detect and measure the velocity of a nerve impulse.

A further analysis of the ganglionic discharge can be made by a study of the electrical action potentials of the muscle nerve and ganglion. Since single induction shocks or the make or break stimulus of the constant current produces only a minimal contraction when applied to either muscle or motor nerve, but the ganglionic discharge whether normal or extra-systolic causes a sustained contraction like that produced by repetitive stimulation of the muscle or nerve, Carlson concluded, rightly, that the normal contractions are brief tetanic responses. Piper had demonstrated the oscillatory potential variations in skeletal muscles of vertebrates when activated from the central nervous system, thus demonstrating the tetanic nature of voluntary and reflex responses. Hoffmann in 1911 showed similar oscillations during the contraction of the muscle of the Limulus

heart and attributed them to the tetanic nature of the responses. I have recently published electrograms which entirely substantiate this conclusion. The failure of Dubuisson and of Dubuisson and Monier to detect these oscillations is due to their failure to appreciate the fact that the salt solution of the body tissues and fluids, equal to a 3% solution of NaCl, offers little resistance to the passage of an electric current and effectively short-circuits the lead-off electrodes, thus making the detection of slight potential variations impossible. Insulation of the tissues is necessary to success with the string galvanometer. With Rijilant's kathode ray oscillograph (1931) practically identical electrograms may be obtained on simultaneous records of a motor nerve and the corresponding part of the cardiac musculature. Since the isolated ganglion and nerve give corresponding potential changes, we have here crucial proof of the relation of cause and effect in the two processes, i. e., proof that the heart is neurogenic and that the contraction partakes of the nature of a neurogenous tetanus. The string galvanometer follows the muscular changes quite faithfully and with amplification will indicate the nerve changes. The examples thrown on the screen show that there is a sharp initial potential change followed by a succession of major oscillations at the rate of about ten per second at room temperature, about twelve of them for each contraction. Superimposed upon these and markedly distorting their regular form are minor waves. These indicate the asynchronous contraction of the contractile elements and constitute further evidence of the fractionate character of the muscular innervation already discussed. The precise form of the electrogram is variable depending upon the position of the lead-off electrodes and the sequential relation of the physiological processes under each. The initial deflections may be made monophasic or diphasic at will.

The long duration of the tetanic discharge is matter for thought and speculation. One may conceive the ganglion to be made up of a series of cell groups which initiate the major oscillations, the minor oscillations being caused by another type of cell more discretely disposed, but we still are faced with an interesting problem. Since the conduction rate would involve the whole ganglion within one-tenth of a second or less why does the ganglionic discharge and muscular contraction continue for more than a second? It may be that once the discharge is started the ganglion cell continues in action for this length of time; on the other hand, there may be a reactivation of the pace maker cells by those subsequently involved through recurrent pathways and the establishment thus of a succession of circulating impulses within the ganglion. The idea has intriguing possibilities in the explanation of many processes in the central nervous system of vertebrates—it awaits the test of some ingenious investigator.

Let us turn now to the consideration of the processes which underlie the development of the rhythm. An indirect attack may be made by a study of the effects of different temperatures. Subjecting the muscle alone, for example, the denervated heart or the anterior segments which contain no effective rhythmogenic nerve cells, to different temperatures never develops a rhythm in the former instance or alters the rhythm in the latter; there is no myogenic rhythm. The procedure merely alters the excitability and force of contraction whether in response to artificial stimulation or the normal ganglionic impulses. The optimal temperature for the muscle is around ten or twelve degrees, Centigrade; the muscle enters reversibly into heat paralysis at about 32°. The ganglion, on the other hand, shows a progressive increase in rate of impulse formation up to 40° C. or higher and is correspondingly slowed by cold, not ceasing its action even at —2° when the fluid about it is in a frozen state. In plotting the rate against temperature I have found that an S-shaped curve is obtained. When the temperature coefficients (Q_{10}) are calculated, they prove to be uniformly greater than 2 in the normal range of temperatures, very large, even 12 at low temperatures, gradually decreasing in the higher ranges of temperature. Such temperature coefficients are highly presumptive evidence that the underlying process is chemical in nature as one would expect, and I naturally turned my attention to oxidation processes as the energy source of the dynamic variations. Carbon dioxide, an end product of oxidation, is evolved from the ganglion as Tashiro had shown. The rate of its development at different temperatures was tested by the change in the hydrogen ion concentration of a non-buffered, isotonic, balanced salt solution, and I found that the curve was identical with that of rate changes. The two phenomena showed identical temperature coefficients. Thus was established a quantitative correlation between the two processes which pointed to the relationship of cause and effect. All agencies tested showed that acceleration was accompanied by increased evolution of carbon dioxide, while depression of the ganglionic rate of impulse formation depressed the production of carbon dioxide. A similar relationship holds for the utilization of oxygen as shown by Miss Dann and Miss Gardner although the quantitative aspects of this work are still open for investigation.

The antithesis of stimulation viz., inhibition, can be investigated and fits in admirably with the chemical phases of this study. The ganglion can be inhibited either by the stimulation of afferent inhibitory nerves or by direct stimulation, for in

a study with Professor Knowlton it was found that while slow rates of stimulation of the ganglion cause a response (extra systole) to each stimulus, increasing the rates to about twenty per second causes a gradual lapse to complete inhibition. This is a condition in which the ganglion is relatively or absolutely inexcitable—the muscles are simply "arrested," *not inhibited;* they remain normally excitable to artificial stimulation. Time will not allow further consideration of the interesting inhibitory phenomena beyond the statement that carbon dioxide production and oxygen consumption by the ganglion fall far below the normal; the chemical processes which we conceive to be at the seat of normal impulse formation are suppressed; whether there is a development of a humoral inhibitory substance remains an open question. I cannot close without alluding to the fact that the ganglion of Limulus can continue to function for a long time in an atmosphere of hydrogen or nitrogen (Newman) and after treatment with cyanides. The ganglion can then function anaerobically and we picture to ourselves some chemical mechanism possibly like that in the anaerobic activity of muscle and nerve in which lactic acid and carbon dioxide are formed, with the concomitant changes in hexose phosphate and creatine phosphate, the oxygen being needed in the recovery processes. Such speculation opens an interesting field for investigation which promises results in the interpretation of the dynamics of the nervous system of higher forms.

All of the evidence presented in this brief review point clearly to the neurogenic nature of the beat of the Limulus heart. The characteristic properties are all those of nerve cells with nerve conduction to muscle which in all its reactions is like the skeletal muscle of higher forms and in no way like that of the vertebrate heart.

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