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THE NERVOUS ORIGIN OF THE HEART-BEAT IN LIMULUS, AND THE NERVOUS NATURE OF CO-ORDINATION OR CONDUCTION IN THE HEART.

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THE NERVOUS ORIGIN OF THE HEART-BEAT IN LIMULUS, AND THE NERVOUS NATURE OF CO-ORDINATION OR CONDUCTION IN THE HEART.

BY A. J. CARLSON.[1]

[*From the Marine Biological Laboratory, Woods Hole, Mass.*]

IN taking up the study of the physiology of the heart-muscle and the heart-nerves in Limulus in order to compare the heart of arachnids with that of crustaceans, I was rewarded by finding that in this animal the relation of the cardiac ganglia and the cardiac nerves to the heart-muscle is such that the questions of the origin of the heart-beat and the nature of the process of conduction in the heart can be settled once and for all by simple and conclusive experiments. It can now be stated as a fact that in Limulus the origin of the heart-beat is nervous, not muscular, and that conduction of the impulse or the co-ordination of the different parts of the heart takes place through the nerves, not through the muscular tissue.

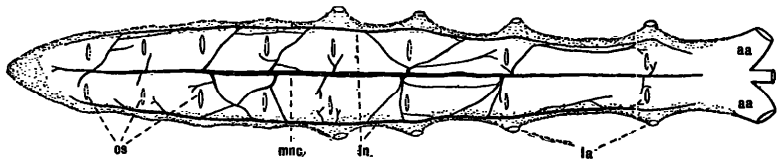

FIGURE 1. — The heart and the heart-nerves of Limulus, dorsal view. The heart is figured one-half the natural size of a large specimen. *aa*, anterior artery; *la* lateral arteries; *ln*, lateral nerves; *mnc*, median nerve-cord; *os*, ostia.

The structure and innervation of the Limulus heart are shown in Fig. 1. In large specimens the length of the heart is from fifteen to twenty centimetres. When empty and collapsed it measures about two and one-half centimetres from side to side at its widest portion. The heart is plainly segmental in its make-

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up, as indicated by the eight pairs of ostia (*os*) leading into the cavity of the heart on the dorsal side. These ostia are probably located between the segments, in which case the heart is composed of nine segments. Four pairs of arteries (*l a*) are given off laterally from the corresponding four anterior segments. The main arteries (*a a*) (two, lateral and one, median) take their origin from the anterior end of the heart. No arteries are given off from the posterior end. The heart is held in position in the pericardial sinus by systems of suspensory elastic tissue fibres essentially the same as in the crab or the lobster, the suspensory ligaments being connected with the heart by means of elastic tissue fibres which run longitudinally on the surface of the heart. Below this layer of longitudinal elastic fibres is, according to Patten and Redenbaugh,[1] a homogeneous membrane (basement membrane) to which the strands of the heart-muscle are attached. The muscle bands are arranged circularly, branching and anastomosing the one with the other. The walls of the heart are thickest at the lateral angles. The muscle is of the ordinary transversely striated type like that in the heart of all arthropods (with the exception of Peripatus). According to Patten and Redenbaugh, there is no endothelium lining the cavity of the heart, and the blood circulates freely between the muscular strands making up the walls.

The cardiac nervous complex, confined mainly to the dorsal and lateral sides of the heart, is represented in Fig. 1. It is composed of three longitudinal nerve-trunks, one (*m n c*) in the dorso-median line, and one (*l n*) at each lateral angle, and an almost segmental system of anastomoses between the median and the lateral nerves. The median nerve is in reality a nerve-cord or elongated ganglion, as it is composed of longitudinal nerve-fibres and ganglion cells. The ganglion cells are of the bipolar type (Patten and Redenbaugh). Ganglion cells also extend for some distance into the main lateral branches of the nerve-cord. Patten and Redenbaugh state that there are no ganglion cells in the two lateral nerves. The median nerve-cord is thickest in the fourth, fifth, and sixth segments, diminishing in size both anteriorally and posteriorally. Ganglion cells are distributed throughout its whole length. The lateral nerves are also of largest size in the middle region of the heart. The median nerve is relatively large and of grayish white color, which makes it readily distinguished from the adjoining connective tissue in the living specimen. The lateral nerves are much smaller and branch considerably,

[1] PATTEN and REDENBAUGH: Journal of morphology, 1899, xvi, p. 91.

and are, in addition, of nearly the same color and transparency as the surrounding connective tissue ; nevertheless they can be isolated in the living heart without the aid of a lens, and without injury to the heart-muscle. Both the lateral nerves and the dorso-median nerve-cord are separated from the heart-muscle by the ectocardium or basement membrane of Patten and Redenbaugh, and can therefore, by careful dissection, be isolated for experimental purposes without the slightest injury to the heart.

The main branches from the median nerve-cord to the walls of heart and the lateral nerves are not as definitely segmental in their points of origin as would appear from the drawings of Milne-Edwards.[1] Some individual variations appear, but on the whole a pair of nerves is given off at the level of each pair of ostia, those given off in the middle region of the heart being the stoutest. These lateral branches bifurcate and anastomose extensively on the dorsal side of the heart, one or more branches joining the lateral nerves. The smaller branches are especially abundant about the ostia. In addition to these main lateral branches the median nerve-cord also gives off numerous tiny branches which penetrate the walls immediately ventral and lateral to the nerve-cord, and therefore cannot be followed for any distance. The connection of the central with the cardiac nervous system is made at various levels of the median nerve-cord. This will be considered in another place.

The vascular system is partly lacunar ; exposing the heart either from the ventral or the dorsal side leaves it, therefore, empty of blood, but, owing to the suspensory ligaments, the heart does not collapse. In the absence of blood in the pericardial space, air is sucked in through the ostia and forced through the arteries. When the heart is exposed from the dorsal side, by removing the digestive tract and part of the reproductive gland, care being taken not to sever the connections of the ventral or central with the cardiac nervous system, the rhythm of the heart is at first irregular and relatively slow, usually not exceeding twelve to sixteen beats per minute, and sometimes falling as low as eight to ten per minute. The slowness and irregularity of the rhythm is not due to the heart being empty of blood, because the irregularities disappear when the connections between the ventral and the cardiac nervous systems are severed, and they do not appear when the heart is exposed from the dorsal side, an operation which necessarily severs these connections. The rhythm of the heart ex-

[1] MILNE-EDWARDS: Annales des sciences naturelles, 1873, xvii, ser. 5.

posed from the dorsal side is regular from the first, the rate of pulsation varying in different individuals from eighteen to twenty-eight, the usual being about twenty beats per minute. If the hearts are protected from evaporation, this rhythm is kept up with perfect regularity for from twelve to fifteen hours, provided the animal is in good condition when prepared. Hearts from specimens in poor condition cease to pulsate much sooner.

The first thing about the heart-beat that arrests the attention of the observer is the apparently simultaneous contraction of all the parts of the heart. We have here a tubular or segmental heart half a foot in length, yet in the fresh specimens no difference in the beginning of contraction or relaxation of the foremost and the hindmost segments can be made out with the unaided eye. This is in striking contrast with the exceedingly slow propagation of the contraction wave in the tubular heart of the tunicates. The contraction of the Limulus heart must either begin practically at the same time in all the segments, or else the conduction of the contraction from segment to segment is much more rapid than the conduction even in the vertebrate heart. When the empty heart has been beating for several hours, or till nearly exhausted, and the rate of pulsation is in consequence much reduced, it can be made out even with the unaided eye that the contractions start in the posterior third of the heart and travel anteriorly. This is evidently also the condition in the fresh and vigorous heart. The processes which effect co-ordination in this heart are therefore conducted at a very rapid rate from one end of the heart to the other. Is it a conduction in the nerve-fibres or in the heart-muscle itself? Two simple experiments decide the question. Lesion of the median nerve-cord and the two lateral nerves in any segment of the heart destroys the co-ordination of the two ends of the heart on either side of the lesion; and, conversely, cross-section of the heart in any segment, leaving the longitudinal nerves intact, does not interfere with the co-ordination, the portions of the heart on either side of the cross-section keeping in perfect unison. As the nerves are separated from the heart-muscle by the basement membrane, every muscle-fibre can be severed in the cross-section without injury to the nerves. Whatever co-ordination or conduction that is effected between the two ends of the heart after such a lesion must, therefore, be brought about by means of the nerves alone. The abolition of co-ordination by sectioning the longitudinal nerves is immediate and permanent. Both ends of the heart continue to beat, but

with independent rhythm, the contraction not passing the region of the lesion either in the postero-anterior or in the reverse direction. There is no exception to these reactions. The experiments are so simple that any beginner in physiology can perform them. The only conclusion to be drawn from these reactions is that *conduction in the heart of this animal takes place in the nervous and does not take place in the muscular tissue.*

The lateral nerves are not essential to the co-ordination or conduction. The whole heart and the two lateral nerves may be severed, leaving only the median nerve-cord intact, yet the co-ordination of the two ends of the heart is maintained. The lateral nerves can, however, effect co-ordination to some extent, especially when a lesion of the median nerve-cord is made in the middle and posterior regions of the heart, leaving the heart walls and the lateral nerves intact. But in this case the co-ordination may also be partly due to the presence of anastomosing branches from the nerve-cord passing parallel to it to the next segment, as well as obliquely to the lateral nerves (see Fig. 1). The segmental distribution of the neurones in the nerve-cord will be considered in another connection.

From the fact that in this animal the conduction or co-ordination is concerned with nervous and not with muscular elements we may not conclude that the condition is the same in all hearts, invertebrate as well as vertebrate. Engelmann's classical "zigzag experiment" on the amphibian heart argues so strongly in favor of the view that the conduction takes place through the muscle substance that I believe the majority of physiologists to-day accept that theory. But it seems to me that the purely muscular nature of conduction is not yet an established fact for the heart of any animal, for even the great amount of work done to determine the nature of the processes of co-ordination in the vertebrate heart has not yielded a demonstration of the purely muscular nature of conduction approaching the decisiveness of the proof of the purely nervous nature of the conduction in the heart of Limulus.

Having found that the co-ordination of the heart is effected solely by means of the nervous elements, it was but a step to extirpate the median nerve-cord and the lateral nerves in order to determine whether without these the heart beats at all. It has already been stated that the complete removal of the median nerve-cord, together with the main lateral branches, as well as the lateral nerves, can be accomplished without the least injury to the heart, so that any effects

following the extirpation of these nervous elements cannot be ascribed to injury to the heart-muscle. The results of this line of experiments are just as conclusive as those proving the nervous nature of the co-ordination. A heart or part of a heart that will beat with perfect rhythm for from twelve to fifteen hours, when the median nerve-cord is left intact, ceases to beat immediately and permanently on extirpation of the nerve-cord. The heart or part of the heart from which the nerve-cord has been removed may be made to contract by mechanical or electrical stimulation, but the contraction always ceases with the cessation of the stimulation. I have never observed a spontaneous contraction in a heart or part of the heart deprived of the nerve-cord. The presence of the lateral nerves is not sufficient to maintain the rhythm in the absence of the median nerve-cord. And removing these nerves, leaving the median nerve-cord intact, greatly diminishes the strength and regularity of the contraction in the different segments, but the rhythm and co-ordination are still maintained. *The heart-beat in Limulus is, therefore, of purely nervous origin, the result of rhythmic nervous impulses sent out from the median nerve-cord.*

Thanks to the great length of the heart, this experiment can be varied in several ways, always yielding the same results. The heart may be cut up into four parts of approximately two segments each, each portion continuing to beat with its own independent rhythm. Sectioning or any mechanical handling of the median nerve-cord always produces acceleration, and sometimes temporary inco-ordination of the rhythm. In fatigued hearts, the initial acceleration may be followed by quiescence lasting for several minutes. The rate of pulsation is almost invariably greatest in the two portions involving the posterior end of the heart. Now the nerve-cord may be removed from either or both of these portions, and the rhythm ceases at once and for all, while the control portions, with the nerve-cord intact, continue to beat for hours. A single segment in any portion of the heart will beat rhythmically, provided the nerve-cord is intact. I have even seen localized rhythmical contractions in segments from which the main part of the nerve-cord had been removed, but one or two of the large side branches left attached to the dorsal wall. Removing these branches destroyed the rhythm.

While it is true that any segment of the heart will beat rhythmically, provided the median nerve-cord is intact, such small portions of the heart do not maintain the rhythm for as long a time as the in-

tact heart, or longer portions of the heart, and frequently no rhythm at all is exhibited by portions of the heart reduced to only one segment. This is particularly the case with the anterior heart-segments. Commencing with the fifth segment, the posterior end of the heart can be divided transversely into relatively smaller portions, and still maintain the rhythm for a considerable time.

The dorso-median nerve-cord on the heart of Limulus is, therefore, an elongated ganglion whose rhythmical activity is the direct cause of the heart rhythm. All the nerve cells having to do with the direct production of the heart-beat appear to be located in this ganglion, which fact allows this crucial experiment, so far afforded by the cardiac apparatus of no other animal. The rhythmic activity of this ganglion is not dependent on the ventral nervous system. Nor is it conditioned on the maintenance of the circulation, except so far as the irrigation and nutrition of the tissues. The heart of Limulus allows the determination in what way, if any, the rhythmic discharges of the nerve cells are conditioned by afferent impulses from the heart. This question will be considered in another paper.

The nerves that pass from the median nerve-cord to the heart-muscle are of the ordinary motor type. Stimulation of these nerves (the two lateral nerves and their ramifications) produces, not a rhythmical series of beats in the resting and an acceleration of the rhythm in the pulsating heart, but a tetanus closely resembling that produced in skeletal muscle on the stimulation of a motor nerve. Stimulation of the lateral nerves produces contraction in the muscle hours after the rhythm has ceased from exhaustion or extirpation of the ganglion. The action of each lateral nerve is mainly, if not solely, confined to its own side of the heart.

The heart can be inhibited by stimulation of the ventral or central nervous system, as well as by stimulation of the median nerve-cord on the heart. The effect on the heart of stimulation of the median nerve-cord with the interrupted current depends on the strength of the current and the point of the cord stimulated. For example, a strength of the interrupted current which fails to affect the heart when applied to the nerve-cord in the first segment, when applied to the nerve-cord in the fifth or sixth segment inhibits the heart posteriorly and accelerates the rhythm or causes tetanus in the part of the heart anterior to the region stimulated. This will be considered in detail in connection with the physiology of inhibition of the Limulus heart.

The mechanism of the cardiac rhythm in Limulus is, then, in every essential similar to the mechanism of respiration, both in invertebrates and vertebrates, the contraction of the muscle being brought about by rhythmical discharges from automatic nerve-centres. And yet the cardiac rhythm in Limulus presents such perfect resemblance to that of the crustacean and molluscan, and even that of the vertebrate heart, that were the ganglion cells and the nerves scattered all through the substance of the heart, instead of being concentrated into one median nerve-cord and two lateral nerves lying external to the heart-muscle, so that they can be handled experimentally apart from the muscle, no one could have suspected, much less proved, that the heart of Limulus differed from any other heart as regards the origin of the heart-beat and the mechanism of co-ordination. And does the heart of Limulus form an exception, or must we, on the basis of the clearly demonstrable conditions in Limulus, infer that similar mechanisms are operative in hearts which have not admitted of conclusive demonstrations of the neurogen or the myogen theory? I believe that the nervous origin of the heart-beat can be demonstrated in other arthropods. The segmental heart of Peripatus and the myriapods is provided with a median nerve on the dorsal side of the heart, similar to that in Limulus, and I expect that this nerve (or nerve-cord) bears a similar relation to the heart-rhythm. In the summer of 1903 I made some experiments on the heart of the large tarantula of southern California, but satisfactory results were not obtained, partly because of scarcity of material, but especially because of the delicate structure of the heart and the difficulty of isolating its nervous connection for the purpose of experiments. But suppositions are not to be accepted in lieu of demonstration; the orientation afforded by the heart of Limulus makes a renewed investigation of the hearts of these forms more certain of success.

It is not within the scope of this paper to review the arguments for and against the theory of the purely muscular origin of the beat of the vertebrate heart. The rhythm of the embryonic heart, prior to the appearance of nervous elements in it, appears to me the most conclusive fact in favor of that view. Nevertheless, the presence of ganglion cells in all hearts, vertebrate as well as invertebrate, in which a thorough search for them has been made, renders a mechanism of the cardiac rhythm and co-ordination similar to that in Limulus at least possible.