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THE FRACTIONATE INNERVATION OF THE MYOCARDIUM OF LIMULUS POLYPHEMUS—A DISPROOF OF MYAL CONDUCTION

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FOUR FIGURES

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Quite in contrast to the 'all or none' response of the vertebrate heart, a single effective stimulus applied to the deganglionated myocardium of the heart of Limulus produces only a localized contraction. The extent of the localized area of contraction is increased by increasing the strength of the stimulus and by repetitive stimulation (e.g., faradization), the effectiveness in this latter instance increasing with the rate, strength, and duration of application. The contraction remains localized, however, and never involves the entire myocardium of even a single segment of the heart muscle, thus additional stimuli applied at another locus will augment the contraction. These findings, apparent in the earlier work of Carlson(1), should suffice to prove that conduction in this heart does not take place by direct muscular transmission even within the substance of a single segment. Recent proponents of this idea of muscular conduction (and myogenic rhythm)(2, 3) have ignored this simple basic fact, as well as many other facts of similar portent.

The present communication deals with the analysis of the motor innervation of the myocardium of Limulus and adds substantial and crucial proof that normally conduction does not take place within the myocardium. At first sight this may appear surprising, since the myocardium is described by Meek(4) as a syncytial network of striated muscle fibers. In its physiological responses, however, it does not react as a physiological unit. The author has made a microscopic study of the inner surface of the myocardium when a single fiber is stimulated with a minute pore electrode and has found that the contraction is confined to the fiber stimulated and to the region of the electrode, extending to a greater distance from it with increasing strength of the stimulus; we thus find a graded response in a highly differentiated cross-striated muscle fiber, but it may well be questioned whether this is not due to a simple spread of the effective stimulus with its increasing strength instead of being attributable to any peculiarities of conduction in this tissue.

The tri-neural motor innervation of the anterior segments of this heart affords a unique opportunity to study the insular character of the myocardial responses to motor nerve stimulation. If the entire ganglionated portion of the median nerve 'cord' is removed from the posterior segments (third to ninth) the anterior segments may be manipulated as a simple nerve-muscle preparation, innervated, however, by three motor nerves, a median-dorsal nerve which is the anterior nerve fiber extension of the dorsal ganglion, and two lateral nerves coursing along the right and left lateral angles of the myocardium. The experimental work to be presented shows that each of these nerves innervates a distinct complement of muscle fibers which are not affected by stimulation of the other nerves. In any given region of the heart there is an overlapping motor nerve distribution, such that every part of the myocardium of these anterior segments receives motor fiber innervation from all three nerves, but the elements affected by each nerve are discrete. The quantitative distribution of the motor nerve fibers from each of these sources varies markedly in different parts of the myocardium and is such that particulate fractions (contractile elements, discretely distributed) may be made to contract in the midst of other quiescent muscular elements. This fact precludes the possibility of generalized muscular conduction in this syncytial structure and ipso facto disproves the concept of a normal myogenic rhythm in this heart. The experimental evidence of these assertions follows.

Turning our attention first to the effects on the normal beating heart of decreasing the muscular innervation by cutting the motor nerves as they pass off from the ganglion, one finds observations in the earliest work on this subject(1) indicating a definite decrease in the height of the muscular contractions with progressive section of these nerves. Thus the contractions of the anterior segments can be reduced to complete extinction in three stages by the successive cutting of the two lateral nerves and the median nerve. In like manner, Carlson has shown that successive section of the nerve strands on the dorsum of the heart connecting the ganglion with the lateral nerves causes a progressive weakening of the normal rhythmic contractions. Garrey(5) has confirmed these findings and has also reported the gradual weakening of the contractions by blocking the nerves by progressively tightening a clamp through which they pass. A similar result was produced, without disturbance of rate of rhythm, by progressively removing the ganglion piecemeal, beginning at the posterior end—a procedure which in essence merely sections the motor nerves to the myocardium. The decrease in contraction in these instances might result either from a progressive reduction in the force of contraction of each and every contractile element, or by a decrease in the number of contractile elements participating in the reaction. Experiment proves the latter to be the case, for the author has previously reported a decrease in the contraction of both halves of the anterior segments of the normally beating heart when the median nerve is cut, but it is mainly the homonomous half of the myocardium which is affected when a lateral nerve is cut(5), an overlapping innervation being thus demonstrated. The interpretation of these results is evident, for if one part of a given muscular segment beats strongly, another part weakly, it is certain that the impulses are not being uniformly conducted throughout the myocardium.

Another fact forces to the same conclusion, viz., that the normal beats of the heart are never maximal, an increase in the height of contractions may be induced by faradization of any one of the three motor nerves; if strong stimuli are used, they result in a high grade tetanic plateau with the normal beats superposed upon it. Unpublished experimental work of Garrey and Knowlton shows that, by appropriate weak stimulation of the lateral nerves, it is possible to produce a peripheral augmentation of the rhythmic contractions which may reach a height which is 2 or 3 times the normal without any alteration of the character of the ganglionic impulses. These findings are likewise to be interpreted as indicating that a normal beat involves only a fractional part of the contractile tissue at any given instant, although every part of the myocardium registers contractile deformation. The contracting fractions evidently are discretely disposed throughout the entire muscle, and since they therefore must lie side by side with non-contracting tissue, the idea of muscular conduction is definitely excluded from consideration.

Directing attention now to the effects produced by nerve stimulation and using the deganglionated preparation previously described, it is found that faradic stimuli applied to one of the three motor nerves may produce a tetanic contraction of greater amplitude than that produced by the normal ganglionic discharge over the three motor paths. The normal response, although definitely tetanic in nature, evidently does not call into action all of the contractile tissue with each beat, as was shown in the electrocardiographic studies of this heart made by Garrey(6). If any one of the motor nerves is faradized and its maximal contractile response is thus elicited, it is found that by stimulating a second motor nerve there is a sharp increase in height of contraction to a new maximal level. If one now proceeds to stimulate the third nerve, there results a similar summation of contraction to a third level; thus by three stages a height of contraction may be reached which approximates three times that due to the maximal effect produced by stimulation of one nerve alone. The tracing reproduced in figure

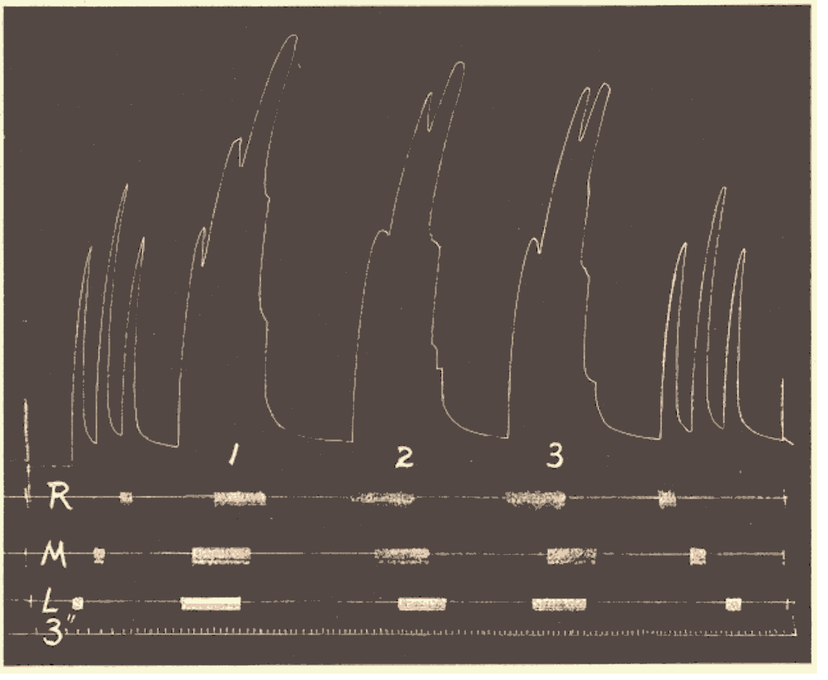

Fig. 1 A tracing of the transverse shortening of the second segment of the deganglionated Limulus heart due to the faradization of the three motor nerves. Signals indicate the stimulation of each nerve, $M =$ the median, $R =$ the right lateral, and $L =$ the left lateral. The individual effects of each nerve are shown at the beginning and end of the trace. Summation of the effects of stimulation and release from stimulation of the three nerves, in different sequence, is shown at *1*, *2*, *3*.

1 illustrates these effects. The separate effects of stimulating the individual nerves is shown at the beginning and end of the trace. It will be noted that the effects produced are independent of the order of sequence of stimulation of the three nerves, and that release from stimulation of any nerve at any time causes a sharp drop in the contraction level.

Due allowance must be made for fatigue effects in any quantitative evaluation of these results. The experiment indicates clearly that each motor nerve has its own complement of contractile response in muscle tissue which is unaffected by stimulation of the other nerves.

The preceding results directed our attention to a study of the distribution of the effective innervation of the myocardium by each of the three motor pathways. Two earlier observations had indicated an homonomous distribution of lateral nerves, one by the author(5), who showed that section of a lateral nerve produced a marked weakening of the myocardium of the corresponding half of the heart, another recorded earlier by Carlson(7), who states that "the action of each lateral nerve is mainly, if not solely, confined to its own side of the heart." Further investigation of this localized innervation has been conducted on a preparation consisting of the anterior segments of the heart with their three motor nerves, after detaching these structures from the rhythmogenic ganglion and the remaining cardiac segments. This preparation was carefully pinned along its median line through both dorsal and ventral walls to a paraffin block; two writing levers were attached to the second segment so that each recorded the contractions of one lateral half of the corresponding muscle. The three nerves were then separately stimulated and the resulting contractions recorded. It was found that the median nerve caused a contraction of both halves of the heart and that its quantitative effects on the two sides were indistinguishable. A sample graph of this result is reproduced in figure 2. The tracings also show that faradic stimulation causes increased height of contraction with progressive increase in the strength (*a*) and duration (*b*) of the stimulation. In both of the records reproduced in figure 3 it will be noted that stimulation of each lateral nerve induced a predominant contraction of the homolateral half of the musculature, but there is also evidence of a moderate overlapping innervation into the musculature of the contralateral half, likewise shown in both tracings. In the lower tracing, taken at a more rapid speed of the drum, the difference in the mechanical latent period and contour of the curves from the two halves of the heart is evidence of the difference in the intensity of the response on the

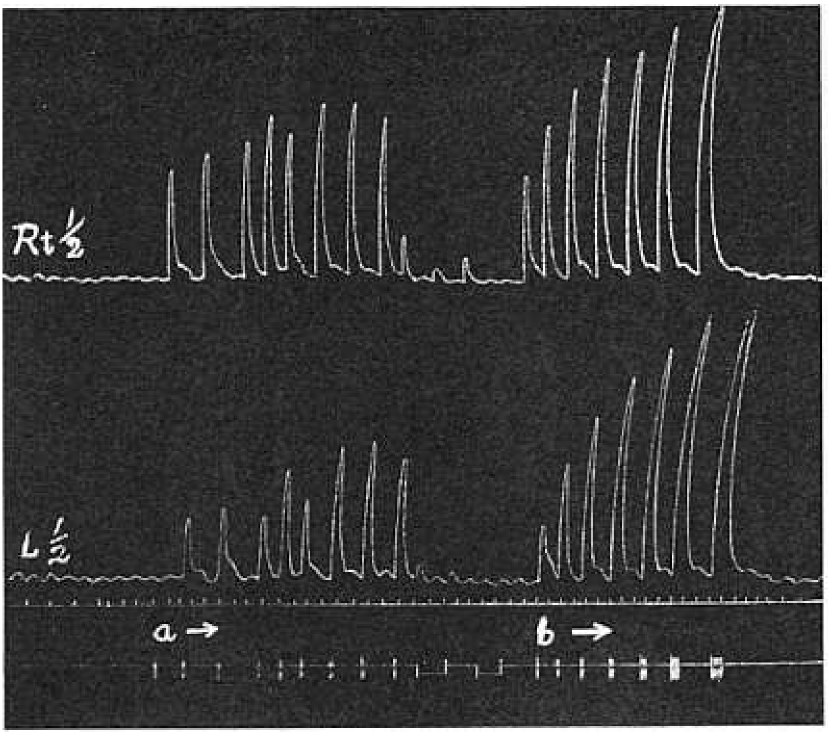

Fig. 2 Records of the contractions of the lateral halves, Rt. ½ and L ½, of the heart (second segment) in response to faradization of the median nerve. Both halves respond equally; *a*) shows increasing strength of stimuli in the direction of the arrow; *b*) shows the effect of increasing the duration of moderate stimuli as one reads with the arrow. Most, but not all, of the ganglion had been removed, resulting in a very feeble rhythmic beat.

two sides of the heart. These records are characteristic of all our experimental results.

A variation of the above experiment on the normally beating heart gave complete corroboration of the above findings. In these experiments when one of the lateral nerves was stimulated by mild faradic shocks, the neurogenic beats of the homonomous half of the heart were superposed on a raised tetanic base and were usually much amplified in height, while those of the contralateral half were altered to

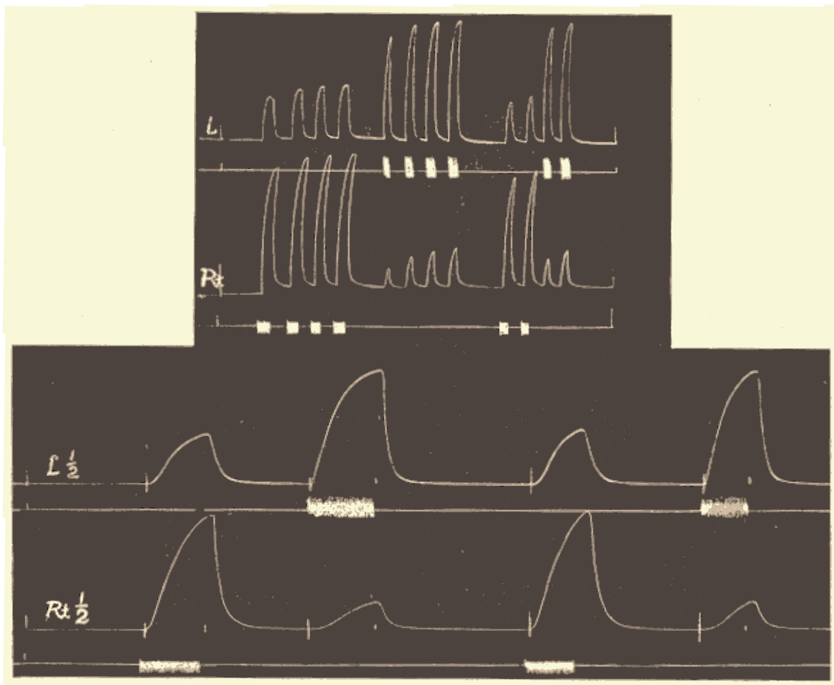

Fig. 3 In the upper smaller section of this graph, tracings are taken from the left (*L*) and right (*Rt*) lateral halves of the second segment of a deganglionated Limulus heart. Stimulation of the lateral nerves, indicated by the signal magnet tracing under the respective myograms, caused a higher contraction on the homolateral side, although the lesser effect on the contralateral side is definite. The lower tracing is taken at a more rapid speed and shows a difference in the mechanical latent period and height and contour of the records of muscle contraction on the two sides.

a far less extent. The result is significant, since the persistence of rhythmic contractions indicates clearly that the stimulated nerve does not innervate all of the contractile elements on the same side as would be the case if muscular conduction were effective. During the nerve stimulation there is a residuum of untetanized muscular elements which can still respond to the rhythmic ganglionic discharge, which indicates again that the innervation by any given nerve is fractionate and discrete.

Further light is thrown on the character and distribution of the myocardial innervation by virtue of the ease with which, in many hearts, transient fatique is induced by faradic stimulation of any one of the three motor nerves to the anterior muscle segments. This fatigue can be induced only in those muscle elements which respond to a given nerve when it is stimulated. If, for example, the median nerve is stimulated, uniform contraction is recorded in both halves of the heart; if the stimulus is continued to complete fatigue, we are in a position to determine whether this nerve affected all muscular elements in the heart either through their direct innervation or by virtue of muscular conduction. The median nerve in this case causes fatigue in both halves of the heart, but the fatigue involves only a fractionate and specific part of the muscular tissue, for it developed that, after producing the fatigue and then stimulating either lateral nerve, the procedure is followed by the contraction which is to be expected within the areas of distribution of that nerve as indicated above. Thus fatigue through any motor nerve has not the slightest effect on the contractile response to stimulation of any other motor nerve innervating the same region, for each in turn confines its effect to its own fractionate complement of the muscle, which could not be the case if the contractile process in one group of muscular fibers was transmitted throughout the myocardium. Experimental tracings illustrating these fatigue effects are reproduced in figure 4. The tracings show that fatigue can be produced by any one of the three motor nerves and that the contractile responses induced by stimulation of the other two nerves can be obtained at any stage of the fatigue curves. The legends under the various figures give added information for their interpretation.

In conclusion, it may be stated that the myocardium of Limulus responds to the stimulation of its motor nerves exactly as would a vertebrate skeletal muscle with a similar polyneural innervation. Other aspects of this general question of conduction and rhythmogenic properties of the Limulus heart have been summarized in a previous communication(8).

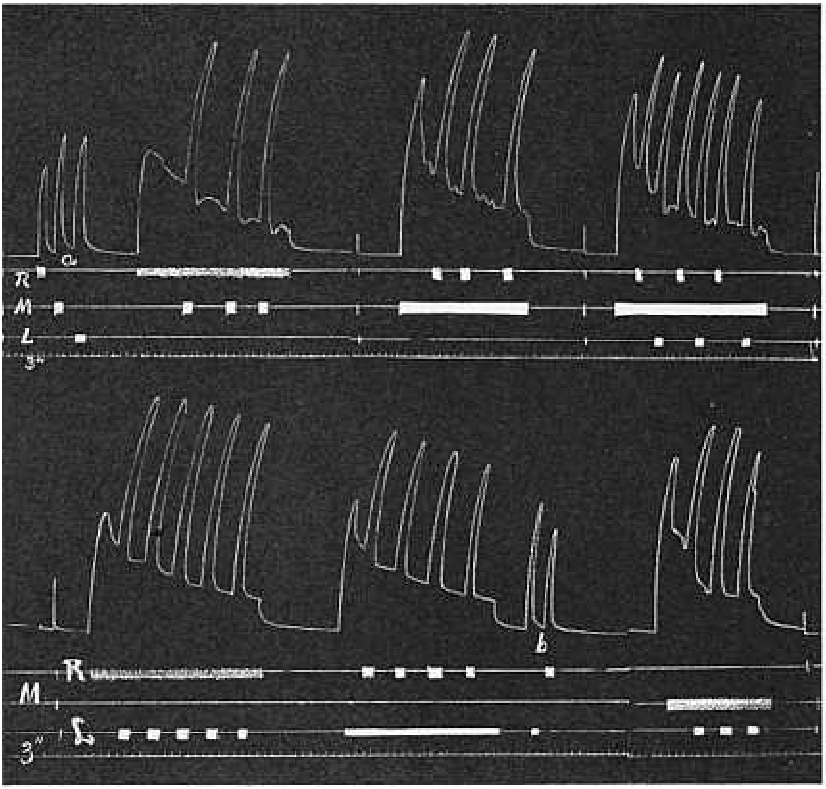

Fig. 4 Six groups of contractions are shown, in each of which fatigue is induced in the contractile tissue innervated by one of the three motor nerves. As fatigue develops, another nerve is stimulated and is found to produce its maximal effect irrespective of the fatigue produced by the prolonged stimulation through the other nerve, indicating that different contractile elements are innervated by each nerve. Stimulation is signalized on the lines marked *R* (right lateral nerve), *M* (median nerve), and *L* (left lateral nerve). The upper right-hand group shows tetanus and fatigue produced by stimulation of the median nerve; alternating faradization of the right and left lateral nerves caused the superposed contractions which are maximal for the strength of stimulus used as are the test stimulations at *a* and *b*. Time in 3-second intervals.

SUMMARY

The experimental results presented by graphs may be summarized by stating that section and stimulation of the motor nerves innervating the myocardium of Limulus show that each nerve affects only its own complement of muscle fibers in which all the effects of nerve stimulation can be induced without altering in the slightest degree the myocardial responses due to the stimulation of other motor nerve fibers. The overlapping distribution of the motor nerve endings proves that the innervation of the muscle elements is fractionate and discrete. Contracting fibers exist in the midst of uncontracted elements of the syncytial structure. The proof is conclusive that there is no muscular conduction in the normal adult Limulus heart, and consequently there can be no myogenic rhythm.

LITERATURE CITED

1 Carlson, A. J. 1905 Further evidence of the nervous origin of the heart-beat in Limulus. Am. J. Physiol., vol. 12, p. 471.

2 Hoshino, N. 1925 Ueber die myogene Automatie des Limulusherzens. Pflüger's Arch., Bd. 208, S. 245.

3 Dubuisson, M. 1931 Contribution à l'étude de la physiologie du muscle cardiaque des invertébrés. VI. La contraction du coeur de Limulus polyphemus nait-elle en un point determine de cet organe et se propage-t-elle aux autres segments par l'intermédiaire des nerfs cardiaques? Arch. Intern. de Physiol., T. 33, p. 217.

4 Meek, W. J. 1909 Structure of Limulus heart muscle. J. Morph., vol. 20, p. 403.

5 Garrey, W. E. 1912 Compression of the cardiac nerves of Limulus, and some analogies which apply to the mechanisms of heart block. Am. J. Physiol., vol. 30, p. 283.

6 ——— 1932 The electrocardiogram of the heart of Limulus polyphemus. J. Cell. and Comp. Physiol., vol. 1, p. 209.

7 Carlson, A. J. 1904 Nervous origin of heart-beat in Limulus. Am. J. Physiol., vol. 12, p. 73.

8 Garrey, W. E. 1932 Some aspects of the physiology of the heart of Limulus polyphemus. The Collecting Net, vol. 7, no. 6, p. 137.