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THE ELECTROCARDIOGRAM OF THE HEART OF LIMULUS POLYPHEMUS

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SIX FIGURES

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That the impulses giving rise to the contraction of the heart of Limulus polyphemus arise in ganglion cells, situated chiefly (but not exclusively) in the ganglionic cord on the dorsum of the heart, was proved by Carlson(1). He demonstrated that it is only by repetitive stimulation of the myocardium that a sustained contraction in any measure comparable to a normal contraction can be elicited; showing in this, and in other ways, that the beat is really a tetanic response to a volley of nerve impulses in a manner quite comparable to the tetanic contractions of the skeletal muscles of higher forms during voluntary contraction or in response to the discharge of an automatic center—like the respiratory center, for example. In a thoroughly scientific manner Hoffmann, in 1911(2), studied the Limulus heart, using the string galvanometer. He demonstrated the oscillatory character of the potential changes in the contracting muscle, obtaining electrograms similar to those which Piper(3) has obtained on vertebrate muscles during tetanic reflex and voluntary contraction. Hoffmann's work substantiated the conclusions of Carlson and many succeeding workers that the heart beat of this form was a neurogenic tetanus, a view which is correct in its general formulation, although, as will be shown later, the tetanus is of a rather complex character. The electrograms reproduced by Nukada(4) show oscillations, but he chose to ignore them in his interpretations of the nature of the heart beat of Limulus longispina. More recently, Hoshino(5), Dubuisson(6), and Dubuisson and Monier(7) have published reports which call into question the seemingly established neurogenic conception of the heart beat of Limulus. They deny the oscillatory character of the electromyogram and attribute to it a form similar to that of the electrocardiogram of the vertebrate heart. It is obvious to one familiar with this field of work that these recent results are obtained by faulty technique and insensitive methods of recording and that, on the whole, Hoffmann's observations were correct, as were the conclusions he drew from them.

The present report is the outcome of an experience with electrographic registration beginning in 1912 and continued annually since then at Woods Hole. The investigations dealt with numerous problems with divergent interests and hence with many variations as to conditions, kind of electrodes, and mode and place of their application. The author's records were all made with a string galvanometer. Many attempts were made to increase the sensitiveness of the recording apparatus by amplification, but with indifferent results, and owing to 'pick-up' and distortion, they were abandoned pending further technical developments in the recording field. Consistent results were obtained without amplification, and the oscillatory character of electromotive changes during contraction of the muscle could be demonstrated with every heart. The contention of Dubuisson that the wavy form of the electromyogram is an artefact due to movement of the tissue on the electrodes is wholly gratuitous and quite untenable. The waves can be obtained with liquid electrodes, in which movement produces no string deflection; they are obtained during the systolic phase of contraction, but only infrequently during the phase of relaxation when the movement is quite as marked, the individual waves are altered by processes of ganglionic inhibition or excision; in fact, movement such as that due to the contraction of the vertebrate heart produces no wavy oscillations and one may therefore dismiss the criticism on these grounds alone. The recent finding of rhythmic oscillatory potential changes in the Limulus ganglion and in the motor nerve fibers connected with it when these structures are wholly detached from the myocardium establishes a crucial proof of the neurogenic origin of the beat of the Limulus heart. These neurogenous oscillations were first observed by Heinbecker(8), using the kathode-ray oscillograph, and Rijlant(9) has obtained a continuous record of these potential changes in the nerves and ganglion; he finds that the oscillatory waves occur with each ganglionic discharge of impulses and continue throughout the discharge for about 1.2 seconds, although they may be shorter or longer, depending upon temperature conditions. Rijlant's graphs were obtained with d'Ardenne tubes and with high amplification, using a balanced amplifier. His methods mark a distinct forward step in facilitating the easy continuous registration and measurement of minute action currents. During the summer of 1931 at Woods Hole, Professor Rijlant generously extended to the author the opportunity to work jointly with him, using this apparatus. It is a privilege to reproduce in figure 1 one of the electrograms of the Limulus heart made in the course of this work.

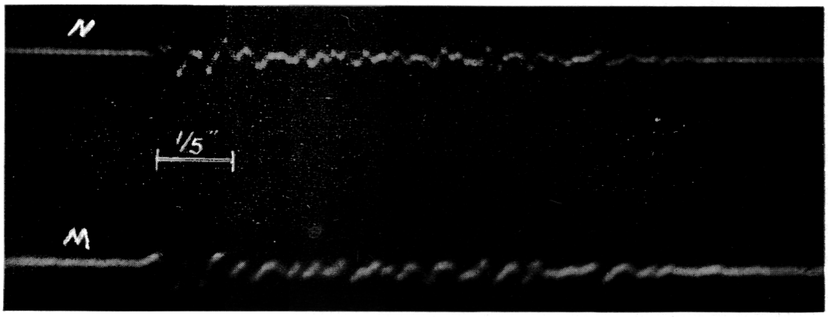

Fig. 1 Electrograms of the action currents in the isolated nerve (upper line *N*) and in the corresponding anterior muscle segments (lower line *M*) of the Limulus heart. Records from two d'Ardenne tubes, by Rijlant.

In order to record simultaneously the action potentials of both nerve and muscle, Rijlant arranged two d'Ardenne tubes, one connected by silver-silver chloride electrodes with the dorsal median nerve which had been dissected free from the underlying muscle of the two anterior segments and lifted some 2 cm. away from the myocardium it had previously innervated; this tube recorded the action currents of this motor nerve in the upper line of the graph (fig. 1). The other tube was connected with similar electrodes lying on the muscle and recorded its potential changes on the lower line of the graph; with each ganglionic impulse the muscle contracted, due to its unsevered innervation through the two lateral nerves. The oscillatory, and therefore tetanic, character of the action potentials during systolic contraction as recorded by the lower line is quite similar to that which the string galvanometer records, although doubtless much more accurate in its delineation. What the string galvanometer has not yet been able to show, however, is brought out strikingly in this figure, viz.: that the action potentials in the motor nerve, isolated from the muscle but connected with the ganglion, are remarkably similar in time, duration, and contour to those in the contracting muscle.

Very naturally there are minor differences, which, however, can be readily accounted for by the multiple and overlapping innervation of the syncytial myocardium and by the physiological properties of the muscle which limit its ability to respond to all the nerve impulses impinging upon it. The string galvanometer is able to respond readily to all but the finer potential changes, the minor oscillations, of the muscle contraction. The author therefore feels justified in presenting a series of observations made with the string galvanometer, in the belief that such electrocardiograms represent a relatively accurate picture of the action currents of the contracting muscle of the Limulus heart.

If one wishes to make an electrographic study of the normal Limulus heart in situ, he is at once confronted with the necessity of introducing the lead-off electrode through openings in the insulating carapace, and introducing them into the tissues and body fluids, which are themselves excellent conductors (3 per cent NaCl), in the midst of which the heart is located. The electrodes are thus effectively short-circuited and only the grossest potential changes can be recorded; by this procedure the arrangement defeats its own objective, for it irons out all minor potential changes—a fact which doubtless accounts for the failures of Dubuisson and other workers mentioned above. One may or may not obtain evidence of an oscillatory variation in heart potentials in such a study, depending upon the position of the electrodes and adjustment of the sensitivity of the string and the external resistance. Figure 2 shows four examples of the variation in the type of electrogram obtained by four different adjustments; there is evidence of an oscillatory variation during cardiac systole in all except the first (a) in the series. The lowest electro-

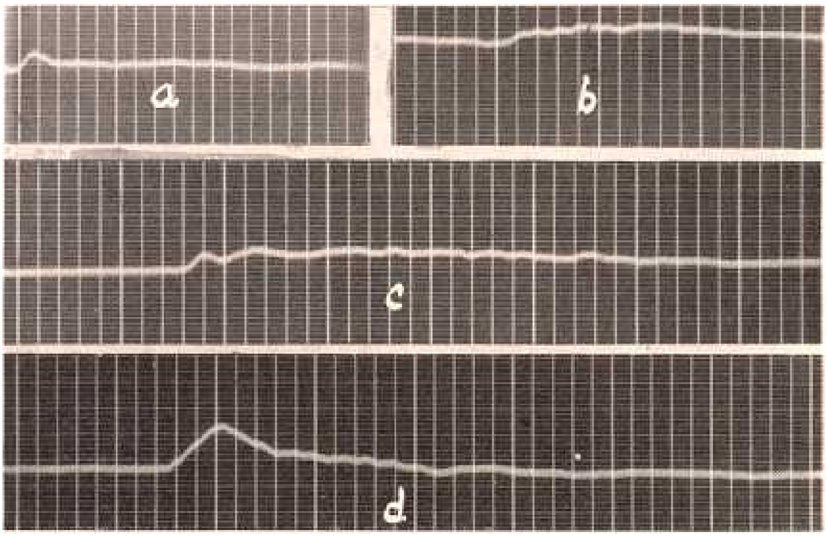

Fig. 2 String-galvanometer electrograms of the action currents taken from the Limulus heart in situ. Electrodes introduced through openings in the carapace. Four records illustrating differences due to adjustments of electrodes and the poor results due to the short-circuiting of the electrodes. Time in one twenty-fifth and one-fifth seconds.

gram (d) of the series was made after removing the carapace above the two anterior segments of the heart and placing a cotton terminal of the anterior unpolarizable electrode directly on the heart muscle to which it became attached by means of a blood clot. This arrangement accounts for the monophasic character of the deflection recorded by the galvanometer; the short circuit through fluid-impregnated tissues, however, was still a hindrance to obtaining a good electrogram. In order to avoid this technical obstacle the suspensory ligaments to the two anterior segments were cut and the muscle raised enough to introduce under these two segments a thin flexible insulating sheet of mica, all other conditions remaining constant. This procedure removed the short circuit from the two segments in question with the result seen in figure 3 (a). Here one sees a strong initial

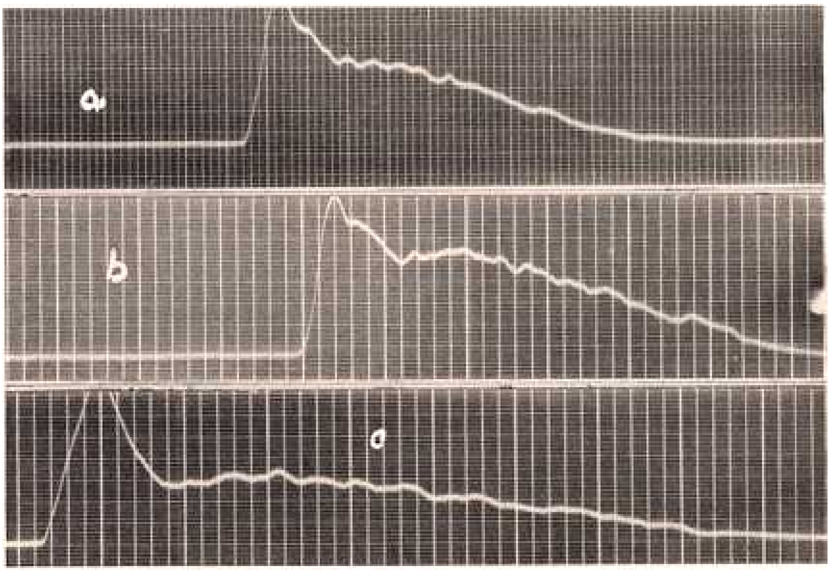

Fig. 3 Monophasic electrocardiograms: a) Obtained by insulating the anterior segments in situ; b) from the excised heart at 20°C. and, c) when the ganglion is cooled to 12°C., with the same set-up as in b. Time in upper tracing at double rate one-fiftieth and one-tenth seconds; in the others, one twenty-fifth and one-fifth seconds.

monophasic action current developed in the muscle segments during this systole, and the oscillatory character of the sustained contraction is clearly elicited and revealed in the succeeding portions of the electrogram. In order to show that this electrocardiogram can be duplicated in its general features on the excised heart, it was removed from the body; it was suspended by means of the electrode attached to the anterior segments and the posterior segments were immersed in sea-water into which the other electrode was placed. The whole ganglionated portion was thus submerged, leaving only the two anterior segments outside the fluid. The arrangement is thus quite comparable to that last mentioned above. The electrogram obtained is shown in figure 3 (b). It varies only in the somewhat more definite detail of the minor oscillations and is slightly longer in duration, due to the slightly lower temperature of the sea water. Lower temperatures lengthen all processes of the ganglionic discharge. This is brought out forcibly in figure 3 (c), in which instance the temperature of the same preparation was lowered from 20°C. to 12°C. The period of negativity at 20° during which oscillations of this heart continued was 1.2 seconds, and at 12°C. they continued 2.2 seconds, i.e., the physiological processes causing systole were retarded to about half their previous rate by the cold solution. This retardation is also noticeable in the duration of each of the major oscillations shown on the electrogram. Concerning the oscillations, it may be stated that the electrograms generally show a series of eleven to fourteen major deflections with minor deflections superposed on them. This number seems to be quite constant for a given heart and is the same in all systoles. The number is independent of the temperature or of rate of beat. The delimitation of these major waves is a somewhat arbitrary procedure, owing to irregularities produced by the superposition of minor waves, and is therefore open to some criticism, but the validity of the classification seems justified in view of the fact that there is uniformly a progressive spreading and slowing of these waves in the late phase of the electrograms.

It further develops that by properly graded faradic stimulation of the ganglion at a rate and intensity just short of that which produces complete inhibition, the minor irregularities are suppressed and the oscillations may occur with great regularity during the entire systole. This change from the normal irregular type of electrogram, figure 4 (d), to the regular one due to such stimulation is shown in figure 4 (a), in which during systole twelve such 'major' oscillations occur after the wide initial systolic deflection. These waves recur at the rate of eight per second. Due to the stimulation of the ganglion, the muscle in this instance was thrown into a state of slight tone and in the intersystolic periods—in fact, even when the automatic systoles were completely inhibited—these major oscillations appeared as is seen in figure 4 (b and c). The registration of muscle potential does not of course establish the neurogenic origin of these waves, but in view of the fact that similar oscillations are to be found in Rijlant's oscillograms recorded from the motor nerve fibers of the heart (fig. 1), it seems certain that this is the case.

The relative constancy in number and rate is significant in the physiology of the ganglion. It means that the cells are functionally associated and that each so affects the other that unity of response is assured. It is this characteristic which is responsible for the fact that a single stimulus applied anywhere along the ganglion elicits a coordinate discharge of impulses resulting in an extra systole which involves the whole heart (Samojloff(10); Garrey(11)), and thus accounts for the fact that the location of the ganglionic pacemaker may be shifted to any point along the ganglion (Garrey(11)). Inhibition of the beat may likewise be induced by appropriate stimulation of any part of the ganglion—a condition which is associated with a reduced CO_2 production (Garrey(12)) and a corresponding decreased oxygen consumption by the whole ganglion (Dann and Gardner(13)). The author has also found that this inhibition is not confined to the immediate cells stimulated, but if properly graded, involves all ganglionic cells, so that extra stimuli applied at any other locus

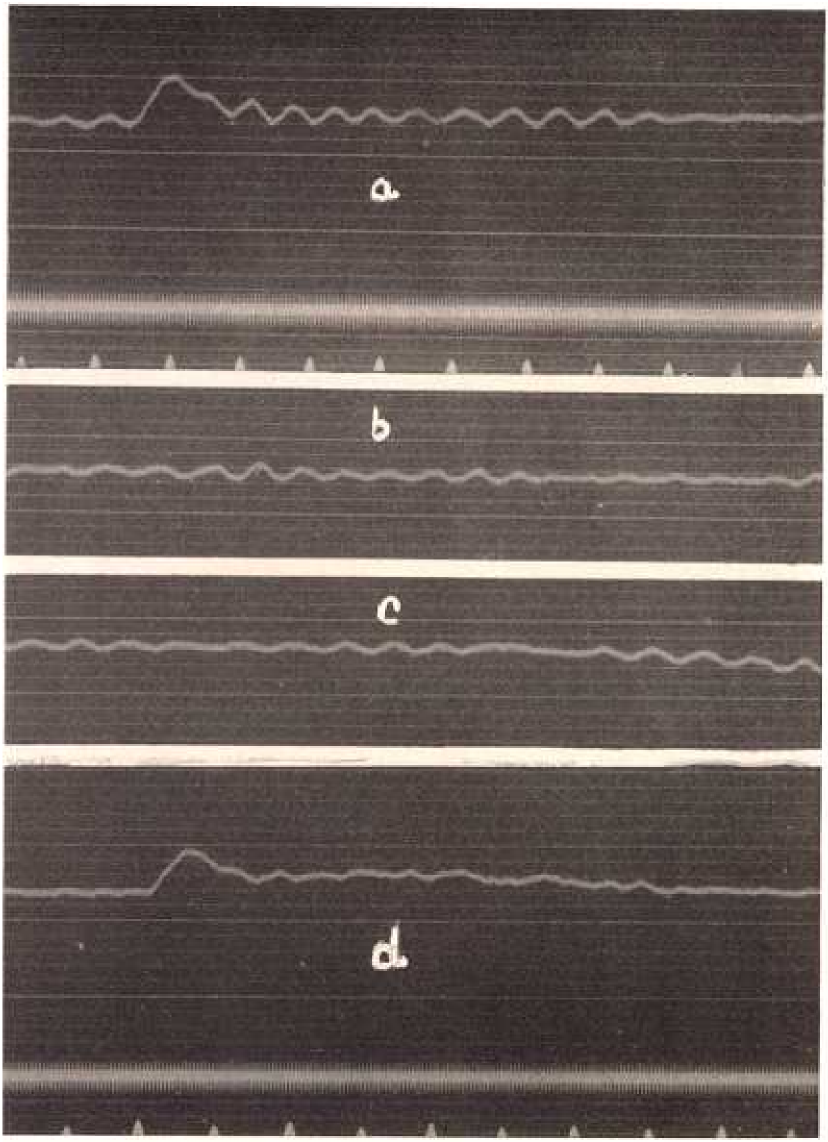

Fig. 4 Monophasic electrocardiogram of the anterior segment. The top record (a) shows the effect of mild faradic stimulation which caused mild-grade inhibition and suppression of the irregular minor potential waves, thus emphasizing the regularity of the major oscillations at the time of systole. The second and third records (b, c) show intersystolic 'tone' waves. The lower tracing (d) shows the minor oscillations and irregularity in a normal systole. Time, one-fifth second and one one-hundredth second; time record removed from the two middle records to save space.

fail to produce extra systolic responses. The consideration of these fundamentals so evident in the elementary and relatively simple ganglion of the Limulus and their application to the groups of cells which constitute the rhythmic (and other) centers in the mammalian nervous system should render the task of investigating and interpreting their action much less difficult.

If one turns now to the consideration of the irregularities in the oscillations of the electromyogram of the Limulus heart, one notes first of all that the nerve and ganglion show more than one type of potential wave (Heinbecker(8); Rijlant(9)). The minor waves of different potential and high frequency are superposed on the major waves of muscle potential and have a consequent distorting effect upon the record. Their analysis must be left to those having facilities for their investigation, since the string galvanometer, without adequate amplification, is incapable of giving a true record of the events. There are other factors, however, to be taken into account. The myocardium of the Limulus is peculiar in that its innervation is derived from at least three sets of motor nerves, a dorsal median nerve and two lateral (marginal) nerves, the fibers of which originate in many different nerve cells. Furthermore, the muscle, although classed as a syncytium, responds to a local stimulus with localized contraction which does not spread to other parts even of a single segment. The innervation of this non-conducting musculature is thus peculiar in that it is of a fractionate character, affecting only insular masses of the tissue, as will be shown later in another communication. The innervated areas overlap, however, and impulses traveling over different nerves affect the muscle asynchronously and produce both multiwave summation and multifiber summation, these effects being algebraically summed in fractionate areas. We are thus dealing in each systole with a tetaniform contraction, but the tetanus is of a singularly complicated origin with correspondingly complicated and irregular potential changes. From these considerations it is easy to comprehend why successive electrograms are not identical in form; in fact, it is surprising that they show even the similarities they do show.

One may well ask, after the above considerations, what the form of the electrocardiogram of the Limulus heart really is like, and one may answer by saying that there is no typical form, since this will vary, depending upon the mode and site of attachment of the electrodes, upon the location of the ganglionic pacemaker, and upon the sequence of events in the different segments. Some conditions which give an initial monophasic variation of the whole electrocardiogram have been considered above (compare fig. 3). Injury under one electrode with the other resting on adjacent normal contracting muscles is another condition for typical monophasic electrograms with the oscillatory waves likewise recorded from one segment. If the electrodes are applied at the anterior and posterior ends so that the whole cardiac tube is included between the leads, the irregularities due to each segment's contraction will be impressed upon the picture, and due to differences in their time relations the initial phase of the electrogram is usually diphasic in character; figure 5 (a) is of this form with a sharply diphasic initial phase, while during the remainder of the systole the electrodes showed a nearly equipotential state with only slight oscillatory variations.

Another heart recorded the electromyogram shown in figure 5 (b). This likewise is diphasic in its initial part, but the time relations of these phases differ markedly from those in figure 5 (a), as do the secondary oscillatory characteristics of the record. It was a simple matter to readjust the electrodes on other segments of this heart and thereby to completely reverse the direction not only of the initial potential deflections, but of the entire record, as seen in figure 5 (c), which in its general contour is a mirror image of figure 5 (b). The experiment just considered emphasizes the fact that there is a sequential and easily discernible difference in the time of contraction of the different segments of the Limulus heart, as has been stated by Carlson(1), Edwards(14), Pond (15), and Garrey(11). Rijlant has confirmed these findings and has made an accurate quantitative study of the rate of conduction in the ganglion along the nerves and of the delay at the myoneural junction(9), although his extended report is still to appear. Our own findings have shown that the segment which contracts first is the one immediately adjacent to that point in the ganglion which acts as a pacemaker. While usually this point is in the region of the fifth or sixth segment, it may shift normally to other loci and may be experimentally determined by localized warming of any desired part of the ganglion. Even so slight a shift as that due to such warming will alter the sequence of events in the different segments and alter the form of the electrogram. The

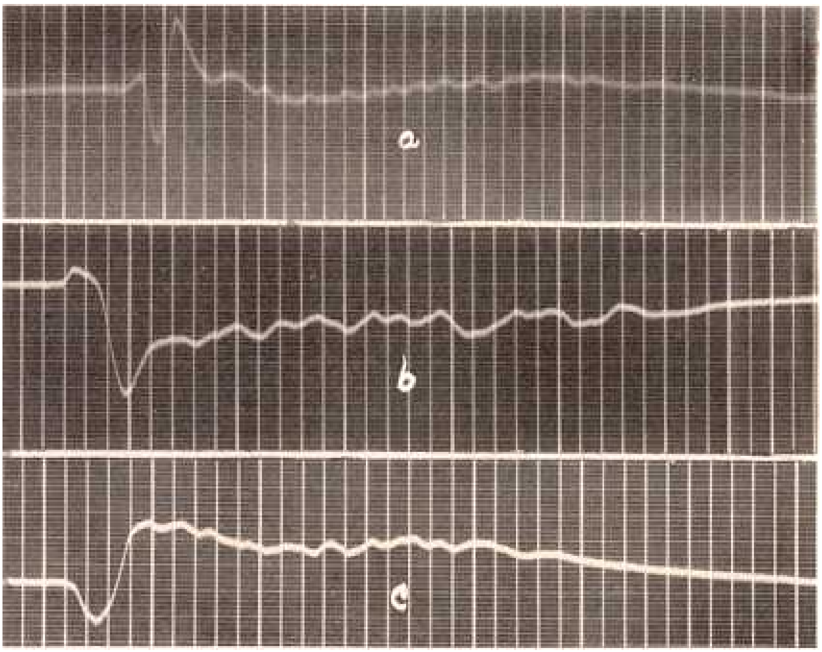

Fig. 5 Upper electrogram (a) shows sharp diphasic initial deflection; electrodes on anterior and posterior segments. Middle record (b) from another heart with a similar, slower, diphasic initial potential change; the lower (c), taken from the same heart as (b), shows a complete reversal of the picture, due merely to changing the position of the electrodes relative to the segment contracting first. Time, one twenty-fifth second and one-fifth second.

differences in the form of the records reproduced in the figure already referred to will illustrate this point. A series of these electrocardiograms is shown in figure 6 (a, b, c). The lead-off electrodes were attached at the anterior and posterior ends of the heart, respectively; a is the response when all segments were contracting; b shows the effect of crushing the heart transversely at the posterior end of the second segment, thus throwing the two anterior segments out of function. Tracing c shows the effect of a similar procedure which eliminated the three posterior segments. There could be no more striking demonstration of the fact that the total form

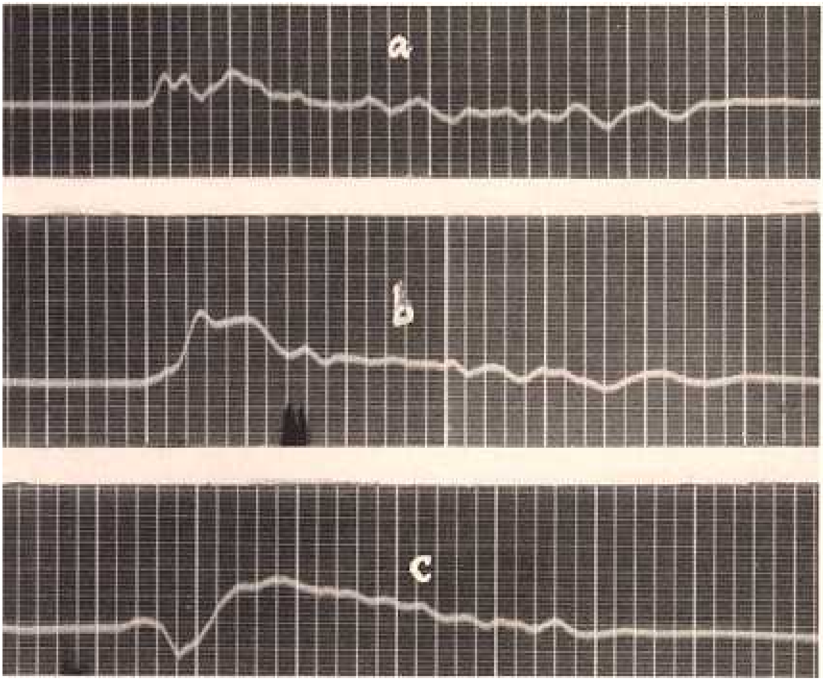

Fig. 6 Three electrograms from the same heart; the entire heart included between the leads placed at the anterior and posterior ends. In the upper (a) record all segments are contracting. In the middle record (b) the two anterior segments are quiet, due to a transverse clamp; in the lower record (c) the three posterior segments are also clamped off and the beat of only the third and sixth segments, inclusive, is recorded. Time as in previous figures.

of the electrocardiogram and the direction of the initial deflection are a result of the summation of the action currents in every contracting segment; it further demonstrates that the electrogram is independent of mechanical movements of the heart or the electrodes, for in c both points of lead were quiescent and stationary.

RÉSUMÉ

1. The electromyogram of the Limulus heart is due to action potentials developed in the muscle as a reaction to rhythmic nerve impulses from the intrinsic ganglion cells.
2. The potentials developed are oscillatory in character and correspond to similar oscillations in the motor nerve fibers.
3. The contraction is a complicated tetanus due to both multiwave and multifiber summation and to fractionate contractions.
4. The number of major oscillations is relatively constant for each heart beat, but marked irregularity of individual waves is the rule. These irregularities are due to the summation of minor waves upon major waves, to the fractionate character of the innervation, and to the algebraic summation of the events in the different segments.
5. The form of any given electrocardiogram depends upon the mode and place of application of the leads, upon the number of segments recording, and upon the sequence of their contractions. This sequence may be altered by experimentally altering the locus of the pacemaker in the ganglion.

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