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PERIPHERAL NEUROMUSCULAR AUGMENTATION IN THE HEART OF LIMULUS POLYPHEMUS

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SIX FIGURES

When one of the three motor nerves, which convey rhythmic impulses from the cardiac ganglion of the Limulus heart to the anterior muscle segments, is stimulated by mild faradic shocks or the repetitive condenser discharges of a Campbell stimulator ('29) there results a significant augmentation of the contraction of the innervated segments. At first one gains the impression as did Carlson ('05 a) that one is dealing with an augmentation of the ganglionic discharge effected by nerve fibers afferent to the ganglion. A more detailed study revealed the fact that the mechanism for the exhibition of this phenomenon is purely peripheral. While unique in certain aspects, it is not wholly unrelated to the Samojloff-Wedensky Einzelreiztetanus (Samojloff, '30). This communication presents the evidence that the phenomenon is purely a peripheral myoneural reaction and is independent of ganglionic involvement. Some conclusions to be drawn from the experiments are presented and discussed.

The various procedures resorted to will be more readily appreciated by reference to figure 1 which is a diagram of the Limulus heart about two-thirds the size of the specimens used. It indicates the segmented nature of the myocardium, the situation of the ganglion, its efferent median nerve to the anterior muscular segments and the two lateral (marginal) nerves likewise carrying motor fibers to the myocardium.

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Stimulation of a lateral nerve. At the outset it was noted that the repetitive stimulation of a lateral nerve caused augmenentation but that only rarely was there any acceleration of the rhythm. When such acceleration did supervene it was found to be due to escaped current which directly stimulated the ganglion. If this was avoided augmentation was the sole consequence as will be noted in all the tracings reproduced in this communication. It will be shown later that the ganglionic discharge may be weakened (inhibition) without extinguishing the augmentation.

Another feature which eliminates the ganglion from consideration as a factor in the augmentation is the fact that the

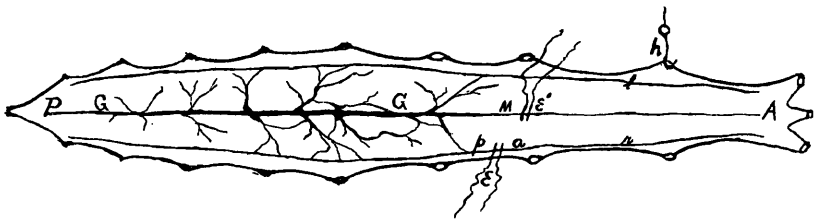

Fig. 1 Diagram showing the cardiac ganglion and distribution of nerves to the myocardium of Limulus (from photograph). A. and P., anterior and posterior ends respectively; GG, the ganglion; l, r, and m = left lateral, right lateral and median nerve, respectively; ϵ and ϵ', electrodes on the right lateral and median nerves respectively; a and p, loci of nerve sections anterior and posterior to stimulating electrode ϵ.

contraction is not increased commensurately in the different segments of the myocardium; for example, if the stimuli are applied to the lateral nerve at about the posterior level of the second segment it is found that the contractions of the anterior segments are markedly augmented, those immediately posterior less so, and those most posterior may be wholly unaffected or mildly augmented with optimal stimulation rate and strength. A segmental or localized differential grading of the strength of impulse formation (the only possible ganglionic explanation of such variation in muscular response) is quite untenable, being thrown out of count by the experiments recorded below.

If, in the experiment just described, the stimulating electrodes be shifted along the lateral nerve toward the posterior segments it will be found that there is an increase in the augmenting effect upon these segments and usually a corresponding decrease in the augmentation of the anterior myocardium. It developed that the degree of increase in the contraction of a given segment is a function of the number of its innervating motor fibers involved in the stimulation; thus in shifting the electrodes posteriorly more of the fibers coursing posteriorly are involved and fewer of those carrying impulses to the anterior segments.

In confirmation of the above statements are the results of two procedures to which we have resorted. In the first place, we stimulated a lateral nerve (at ε, fig. 1) and thus produced augmentation both anterior and posterior to the position of the electrodes. Subsequent section of the lateral nerve posterior to the electrodes (at p, fig. 1) at once obliterated the posterior augmentation leaving that of the anterior segments unaffected and still responsive to augmentation as is illustrated in figure 2. In like manner section anterior to the electrodes (at a, fig. 1) eliminated the augmentation of the anterior segments only. The effects were due therefore solely to the involvement of motor fibers in the reaction. Had the ganglion been implicated the entire myocardium would have shown the effects of these operative procedures. Furthermore attention is directed to the fact that by section posterior to the electrodes or as in many cases by an L-shaped cut across the nerve then parallel to its course, all afferents to the ganglion, assuming that there might be such afferents, would be severed from their connection with it and we are forced to the conclusion that the augmentation of the rhythmic contractions is due to a peripheral effect. It is quite immaterial whether the stimulated lateral nerve is intact and conducting normal rhythmic impulses or has been severed and conducts only the artificial faradic stimuli. In the latter instance the stimuli affect and augment the rhythmic effects of ganglionic impulses which are conducted to the myocardium over the

other intact motor paths, viz., the median nerve and the other lateral nerve.

Concerning the second experimental approach relating to the number of nerve fibers it may be stated without elaboration that while augmentation is being induced by stimulation of one intact lateral nerve the application of very weak, just liminal, stimuli to the other lateral nerve results at once in a significant increase in the height of the recorded beat. This effect while due in large measure to the added augmentator action of the repetitive stimulation of a larger number of

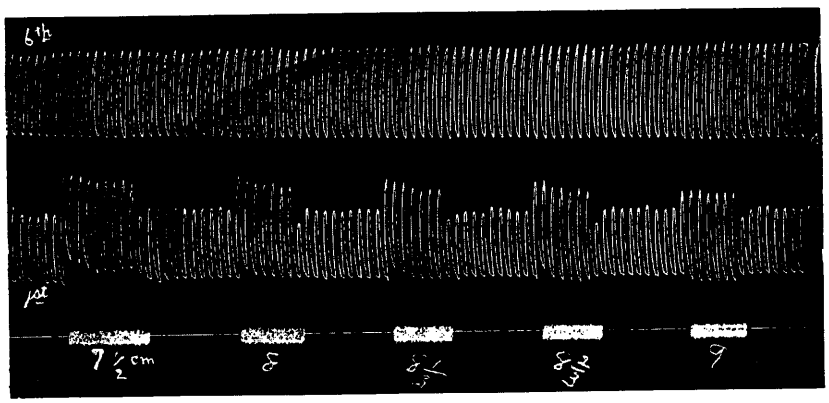

Fig. 2 Myograms from Limulus heart showing augmentation of contraction of the anterior segment (lower tracing) and its absence in the sixth segment (upper tracing) due to section of a lateral motor nerve just posterior to the point of its stimulation. Signals indicate five strengths of faradic stimulation.

motor nerve fibers is due also to the involvement of additional parts of the myocardium which is a result of the fractionate distribution of the motor nerves (Garrey, '32).

Stimulation of the median nerve. If the repetitive stimuli be applied to the median nerve in the region of the third segment (fig. 1, ε′) augmentation is confined to the anterior segments, on the other hand Carlson ('05 b) has shown that stimulation at this point may be followed by inhibition of the ganglion and that both rate and force of the rhythmic contractions may be reduced. We have found that in spite of this inhibiting effect on the ganglion which is evidenced by

slower rate and weaker contractions of the posterior segments, those of the anterior segments may be considerably augmented, a result which can therefore be attributable only to the peripheral effects of the stimulation of the motor nerve fibers. Similar conclusions are to be drawn from the results of direct ganglionic stimulation described in the next section.

Stimulation of the heart ganglion. When the ganglion is stimulated repeatedly, preferably by repeated condenser discharges, a judicious selection of the rate and strength of stimuli results in partial or complete inhibition of the ganglion.[2] If the inhibition of the ganglion is partial it developed that mild simultaneous stimulation of any one or more of the motor nerves, those innervating the anterior segments for example, caused a definite augmentation of the contraction of those segments while the rest of the heart registered the inhibition of the ganglion. Here also the peripheral nature of the augmentor mechanism is evident; it is brought about solely through the stimulation of the motor nerves. It thus happens that stimulation of the ganglion alone, especially in its anterior portion where the results may be more readily recorded, often results in augmentation of the anterior segment contractions while other parts of the myocardium record the ganglionic inhibition due to the fact that intraganglionic nerve fibers which convey the motor impulses to the muscle are directly stimulated and cause their peripheral effects, although the ganglion is actually inhibited. Stimuli strong enough to produce the inhibition may, in the above procedure, produce tetanus of the innervated muscle and the augmented contractions appear superimposed on the tetanic contraction level thus produced. Examples of peripheral augmentation with partial ganglionic inhibition are illustrated in figure 3.

The deganglionated heart. With the preceding facts in mind it was a simple matter to devise a crucial experiment which eliminated the ganglion from consideration as a factor in the mechanism of augmented contractions considered herein. The ganglion was removed posterior to the middle of the third segment and cardiac muscle with its motor nerve was used

[2] A detailed account of this inhibitory phenomenon will be published later.

in the subsequent tests or the heart was merely transected at the middle of the third segment and the anterior portion which contains no ganglion was used; neither of these preparations show rhythmic beats, they are simple nerve muscle preparations, innervated by three motor nerves.

In one group of experiments rhythmic contractions of the myocardium were produced by very strong single induction shocks applied to one of the motor (lateral) nerves at a rate of twelve to twenty per minute. In spite of the fact that the stimulus was strong enough to excite all of the nerve fibers the

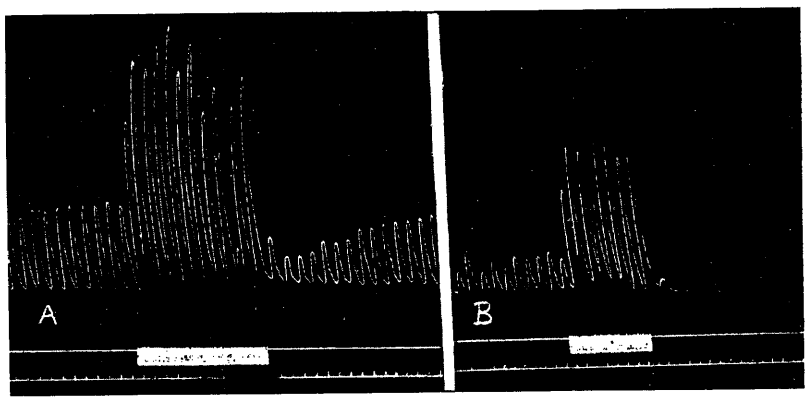

Fig. 3 Effects of direct stimulation of the Limulus heart ganglion. The augmentation is due to stimulation of intraganglionic motor nerve fibers. The ganglion itself is partially inhibited as is shown by the after effects of stimulation which in A are a reduced force, in B, decrease in both force and rate.

muscular response to these single shocks was very weak, but when the nerve was faradized with stimuli so weak that they fail to cause a contraction of the muscle the single shocks causes a considerably augmented contraction as may be noted in figure 4, A and C. It is evident that the augmented contraction is the result of a tetanus released by the action of the strong single shocks. One is reminded in this response of the Samojloff-Wedensky phenomenon—'Einzelreiztetanus' —as it appears in the simple muscle-nerve preparation (Samojloff, '30), but differs from it in a number of respects, principally in the fact that the augmentation is facilitated

equally well when the single shock is applied to one nerve and the faradization to still another of the motor nerves supplying the myocardium.

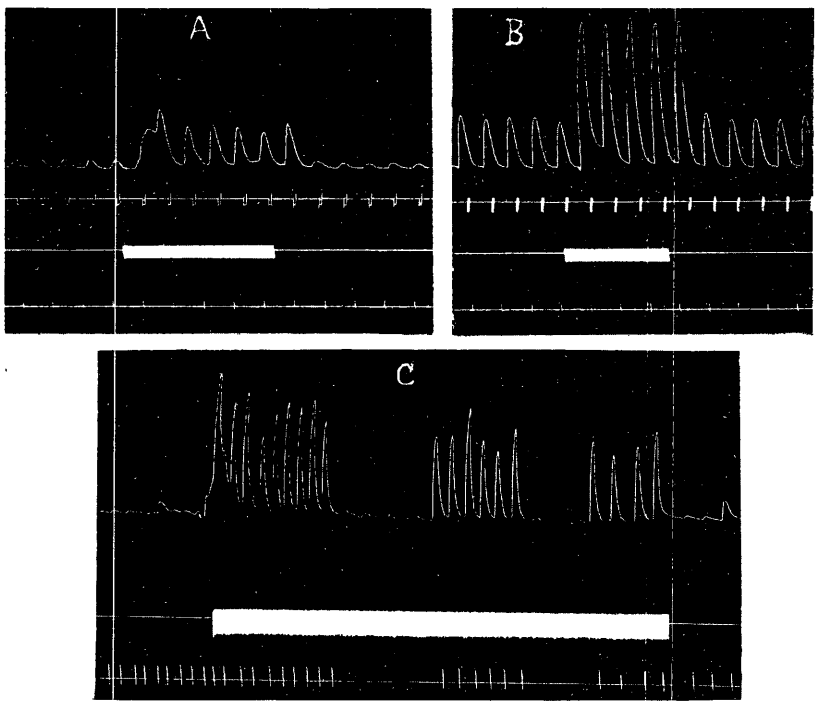

Fig. 4 The Limulus heart was completely deganglionated. In A, weak contraction was induced by rhythmic single induction shocks applied to one lateral nerve, mild faradization of the other lateral nerve caused augmentation of these contractions. In B, short periods of weak faradization rhythmically applied to the median nerve caused contractions similar to normal heart beats, these were likewise augmented by mild faradization of a lateral (motor) nerve: In C, peripheral augmentation is marked; the single induction shocks applied to one lateral nerve caused only just perceptible contractions; the faradic (augmenting) stimulus applied to the median nerve was subliminal.

The same augmentor results are even more strikingly demonstrated if rhythmic contractions are produced by repeated short periods of tetanic stimulation for example twenty shocks in 0.4 second repeated every 3 to 5 seconds. The heights of the short tetanic contraction may be graded as

desired by varying the strength of the stimuli. If now weak continuous faradic stimuli be applied to the stimulated nerve or to either of the other motor nerves there is a marked augmentation of the tetanic 'beat'' (fig. 4, B).

Not only is the ganglion removed from the picture in these experiments but it is clear that there is no summation of effects in the motor nerve fibers such as Wassiliew and Mogendowitch describe ('30), since the effect can follow the separate stimulation of two different nerves. Similarly Samojloff's conclusion that the Einzelreiztetanus was due to the removal of a block at the neuromyal junction does not apply here, and for the same reason that the effect follows stimuli applied to different motor nerves. This definitely limits the mechanism of augmentation to changes within the syncytial muscle itself.

Fractionate augmentation. Previous reference has been made to the fact that the augmentation is confined to those myal segments innervated by the stimulated motor nerve. It has been shown by Garrey ('32) that there is a further fractionate innervation of the myocardium, that while the median nerve is distributed equally to both lateral halves of the anterior segments, each of the lateral nerves affects predominantly its own lateral half of the muscle of these segments. Correspondingly it developed that stimulation of the median nerve caused augmentation of both lateral halves of the anterior segments. This was determined by pinning the heart firmly along its longitudinal median line and separately recording the contraction of the lateral halves. By this means it was also demonstrated that the faradic stimulation of each lateral nerve caused a predominant augmentation of the contractions of the ipselateral half of the corresponding myocardium (fig. 5). Owing to overlapping innervation there is some, although a lesser, augmentation on the contralateral side. This experiment furnishes a striking proof of the peripheral mechanism of the augmentation and like others considered above rules out of consideration all ganglionic participation in the phenomena of augmentation under consideration.

Augmentation of weakened heart beats. It frequently happened that hearts which had been isolated from the body for some time and subjected to much experimental manipulation showed a progressive weakening of the automatic beats. This condition is to be ascribed to ganglionic deterioration which often progressed to the point where no muscular contractions were recorded by the writing levers used. At any time during this progressive change repetitive stimuli applied to any of the three motor nerves to the anterior segments resulted in a considerable augmentation of their contractions.

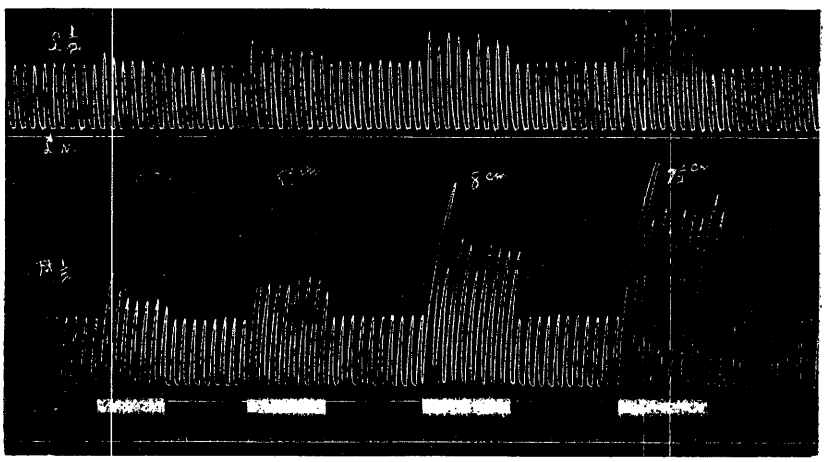

Fig. 5 Upper myogram from left half of the second segment of the Limulus heart, the lower from its right half. Weak faradization of the right lateral nerve caused predominant augmentation of the corresponding half of the myocardium.

Even in the later stages when there are no visible contractions they were elicited by the augmentor stimuli, indicating that normal subliminal ganglionic impulses become effective by the peripheral augmentor mechanism. The augmentation was confined to those segments receiving the motor nerve stimuli, the other segments remaining quiescent. Other procedures were employed to induce weak muscular contractions, for example any two of the three motor nerves of a vigorously beating heart could be cut thereby lessening the contractions

by reducing the number of pathways available to the ganglionic impulses and thus eliminating the fractions of the myocardium which respond to them. Under these conditions vigorous contractions may be induced during the application of the faradic stimulation to the cut nerves. A result such as this can be explained only as a restoration of additional contractile elements to participation in the contraction by a spread of the effective influence of the ganglionic impulses beyond the limits of their previous effectiveness. Obviously the all or none law does not apply to the contraction of the syncitial muscle and a contraction gradient is the norm in these muscles.

In another series of experiments the ganglion was removed piecemeal beginning at the posterior end. Such a procedure not only gradually reduces the amount of ganglionic tissue but it also progressively severs the motor nerves to the myocardium and as noted above this progressively weakens the beats to the point of extinction when the preparation becomes deganglionated. Augmentation of the contraction residuum can be demonstrated at any stage of this experiment with results identical with those just considered. Figure 6 illustrates the points in question.

Strength of stimulation and the augmenting effects. In the experiments described above arbitrary but effective strengths of augmenting stimuli were applied to the motor nerves. With strong stimuli a tetanus of the myocardium was produced and the augmented contractions were superimposed on the tetanic level (compare figs. 2 and 5). It was a noteworthy experience however that the tetanic level was rarely maintained but owing to rapid fatigue the base line of the tracing showed a return to the resting level of the normal beating heart. It was surprising to note however that the augmentation persisted in spite of the fatigued state of the muscle fraction under the direct influence of the nerve stimulated; the augmentation therefore must be due to an influence exerted upon other fractions of muscle than those fatigued and this leads to the conclusion that we are not

dealing with simple summation as conceived by Adrian and Lucas ('28) for the two stimuli do not act on the same point in the tissue.

If one defines as a threshold or liminal stimulus for a motor nerve one which produced a just detectable contraction, the experiments above described demonstrate that subliminal stimuli, too weak to produce a contraction, are still able to

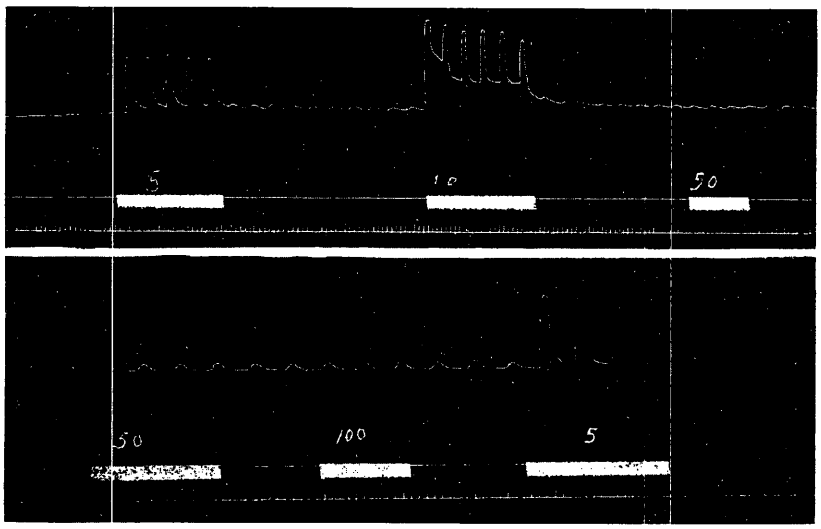

Fig. 6 Augmentation of weak contraction by repeated condenser discharge stimulation of a motor nerve. In A, the weak contractions are due to spontaneous deterioration of the preparation. In B, the posterior portion of the ganglion was removed until only weak contractions were recorded. The figures above the stimulation signal indicate the rate per second of condenser discharges. Only low rates were effective in these preparations. A = upper, B = lower tracing.

produce marked augmentation of the heart beats. Thus we have further demonstration that the phenomenon is not a simple summation of contractions but due rather to the establishment of a state or condition within the muscle which raises its excitability, facilitates the spread of the contractile process and thus renders more muscle active. To illustrate this effect a specific experiment may be presented. The lateral nerve was stimulated by condenser discharges from a Campbell stimulator set to deliver ten stimuli per second.

Intensities between 100 per cent and 30 per cent of the capacity of the stimulator tetanized the muscle proportionately. The rhythmic contractions were superimposed on the tetanic level but were augmented 100 per cent above the normal beats. At 20 per cent intensity there was a just perceptible tetanic effect but the beats were augmented 75 per cent. At 15 per cent intensity there was no perceptible tetanic effect, the base line being unchanged; these subliminal stimuli however increased the height of the beats some 50 per cent or more. For each heart there is however a range of intensity which produces maximal augmentation. This varies from heart to heart and with the type of stimulation.

Frequency of stimluli. Any detailed description of the effects of different frequencies of stimuli when applied to the motor nerves would carry us too far afield for present considerations. In brief augmentation may be obtained with a wide range of frequencies. The optional rate which produces the highest degree of augmentation doubtless varies with the characteristics of the stimuli used and also varies from heart to heart. In general it may be stated that frequencies of ten per second for stimulation with the Campbell stimulator (condenser shocks) usually produced maximum augmentation. Definite augmentation was induced by lower frequencies even down to one per second, a low rate which sometimes was very effective. Augmentation of the natural rhythmic beats was never observed to result from a single shock no matter in what phase of the heart cycle it was delivered nor when it was repeated at the frequency of the normal heart rate, i.e, every 5 seconds, unless of course the intensity was excessive and far above that used in these experiments. In this feature the results differ from the muscle-nerve responses described by Samojloff ('30). With high frequencies results are variable; at rates of thirty to fifty per second, definite augmentation is sometimes obtained, in other instances none at all, and it may be mentioned that these frequencies, and especially higher rates of 100 to 200 per second, when applied to the motor nerves, usually fail to produce any tetanic con-

traction of the cardiac muscle although not infrequently they do nevertheless result in augmentation of the beats, in other instances they do not however do so, e.g., in figure 6 frequencies of 50 and 100 per second did not cause augmentation although 5 and 10 did so.

Reciprocal augmentation. In the preceding description our attention has been confined to the augmentation of the height of heart beats by repetitive stimulation of a motor nerve, i.e., to an augmentation of the brief tetanic contraction which results from rhythmic ganglionic discharges. Attention should be further directed to the fact that the ganglionic discharge also renders the artificial augmenting stimuli more effective. This statement is based on the fact that the rhythmic contractions are not only higher but often the duration of each tetanic beat is distinctly prolonged beyond its normal duration, thus after the ganglionic discharge has ceased the muscle is still in the contraction phase; relaxation is prolonged and a distinct increase in the spread of the base of tracing is evident. This effect is more noticeable with fairly strong augmenting stimuli, especially if they have produced a mild grade tetanus, but the effect is evident after fatigue has set in and the base line has returned to the normal position. The same result often attends the use of the weak, subliminal augmenting stimuli. There is thus a definite increase in the effectiveness of the stimuli as a reciprocal effect of the normal neurogenic tetanizing impulse—an effect which outlasts the ganglionic outburst by a considerable fraction of a second and may even double the duration of each beat.

DISCUSSION

Previous work has proven that the normal rhythmic contraction of the Limulus heart is never a maximal one (Garrey, '32) hence summation of stimulation, and augmentation of contraction are always possible. The increase in the contractile response which has been described in this communication can take place only as a result of the increase in the number of contracting elements either because there

is a spread of the effective influence of the nerve impulses to involve more muscular tissue or because many other nerve fibers which are normally conducting ineffective impulses become effective when the augmenting stimulation is applied. We have concluded that the latter explanation is more likely. The evidence that this view is probably correct is strengthened by the fact already mentioned that frequently preparations which have been used for long experiments show no rhythmic responses by the mechanical recording methods used in this investigation, nevertheless, with the repetitive stimulation of any of the motor nerves, the masked rhythm becomes evident proving that impulses had been coursing down the nerve but that they became effective only after some changes in the muscle which raised the threshold of its excitability.

The augmentor mechanism is not the result of changes in excitability or of summation in the nerve fibers, since in the Limulus heart, as has been pointed out above, the augmentation can be induced by the stimulation of a nerve which is not carrying the rhythmic impulses from the ganglion. This summation of the effects of stimuli reaching the muscle over two different nerves likewise eliminates the nerve termination as the seat of the augmentor influence which was the explanation adopted by Samojloff in his analysis of the 'Einzelreiz-tetanus' of Wedensky (Samojloff, '30). The augmentation therefore appears to be the result of changes restricted to the muscle and we may speak of an enhancement of the peripheral excitatory state which has its analog in the enhancement of the 'central excitation state' which results from afferent nerve stimulation. Keller ('28) found that when a given afferent nerve (n. peroneus) was faradized with subliminal stimuli which produced no reflex response even a single strong stimulus applied to that nerve elicited the response, also Denny-Brown and Sherrington ('28) similarly found that the reflex elicitated by stimulation of the afferent musculocutaneous nerve was augmented by stimulating the saphenus nerve. These effects upon the central nervous system due to overlapping afferent effects in the 'moto-neurone pool'

seem to be directly related to the peripheral muscular effects described in this communication. In the Limulus heart there is a fractionate but overlapping distribution of motor nerves in the myocardium which may be referred to as the peripheral neuromyal pool within which additional stimuli even if they fail to produce contraction when acting by themselves enhance the effect of other stimuli.

A humoral concept has been invoked as the basis of the central augmentation (Sherrington) and likewise by augmentation of skeletal muscle due to a single strong stimulus to its motor nerve as described by Judin ('27). It is doubtful, however, whether such a secretory effect of the nerves is involved in the augmentation which we have observed and for two reasons; first because the onset of the augmentation is not progressive—it has none of the characteristics of a treppe —it is abrupt; second, the cessation of the augmenting stimulation terminates the effect with equal promptness, there is no suggestion of the prolonged effects with which we are familiar when the heart of vertebrates is augmented by stimulation of its augmentor nerves.

The phenomenon is far more suggestive of electrical changes, the building up of potential differences which increase the excitability of the muscle but fall short of actual stimulation, a state which when summed with similar states set up by effective stimuli result in the contraction of muscle elements which otherwise would not respond. Whatever the mechanism, we are forced to the conclusion that even subliminal stimuli while not in themselves causing contractions can nevertheless cause definite physiological alteration of the myocardium of the Limulus heart and that these augmenting effects may impinge on the muscle over any available motor nerve path.

If stimuli to nerve are subliminal in the sense that no contraction is elicited we must still admit that the nerves are transmitting effects to the muscle otherwise it is difficult to explain the fact that stimulation of another nerve provokes an augmented effect. It is difficult to harmonize the phenomena with the all or none effect on either muscle or nerve.

The conclusions reached above have been adopted as the most probable although we have not been unmindful of the possibility that a peripheral nerve net, if it should exist, may enter the picture. Carlson has reported evidence against the existence of any such structure in the Limulus heart ('08); at most, it could only leave us a choice of locus for the exhibition of the augmentor phenomena; the mechanism would remain the same and our general conclusions unaltered. The augmentation would consist in the opening of more nerve paths to play upon the muscular syncytium and evoke additional loci of contraction due to a pleurineural innervation of muscular syncytium.

SUMMARY

Repetitive, e.g. faradic, stimulation of any of the three motor nerves of the Limulus heart causes significant augmentation of the contractile response. Not only is this the case when the nerve is intact but also after severing the nerve's connection with the ganglion; thus demonstrating that the ganglion is not involved in the response which is due solely to efferent impulse impinging on the myocardium thus causing augmentation which is purely peripheral in origin. Experiments of varied types were devised to substantiate the finding. Subliminal as well as stronger stimuli effected the augmentation which was also demonstrated on deganglionated preparations. Some of the implications of the phenomenon are discussed.

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