THE MECHANISM OF THE NERVOUS REGULATION OF THE CRAYFISH HEART

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(With One Plate and One Text-figure)

INTRODUCTION

The existence of nerves regulating the frequency of the heart beat in crustaceans has been repeatedly reported (Jolyet & Viallanes, 1892; Dogiel, 1894; Connant & Clarke, 1896; see also Dubuisson, 1934). Carlson (1905, 1906), studying the nerves of *Panulirus* more extensively, came to the conclusion that two nerve pairs from the suboesophageal ganglion innervate the heart, the anterior pair being inhibitors, the posterior accelerators. The situation in crabs seems to be very similar, but the crabs have two pairs of nerves, instead of one, with accelerating properties (Connant & Clarke, 1896). Another nerve to which a regulatory function has been ascribed is the nervus cordis, or nerve of Lemoine (Lemoine, 1868).

It was found that the preparation of the crayfish (*Cambarus clarkii*) heart as used in the investigation of the effect of ions (Cole, Helfer & Wiersma, 1939) was very suitable for an investigation of the function of the regulatory nerves. In this preparation the nerves can easily be prepared and stimulated under conditions which allow a simultaneous registration of the heart beat. In such preparations, it was found possible to locate, in addition, tracts in the oesophageal commissures which have an inhibitory and acceleratory function.

METHODS

To study the nervous regulation of the heart, the crayfish is first eviscerated by opening the carapace in front of the cervical groove, and most of the chitin from the dorsal and lateral sides in front of the groove is removed. The stomach and 'liver' are pulled out, care being taken that no digestive juice escapes into the body cavity. The green glands and the muscles of the mandibles are taken out and the cavity is washed with physiological solution (van Harreveld, 1936). In all cases the crayfish were first made clawless, since it was found that otherwise the claws invariably became entangled with the recording thread.

Three ways of mounting the preparation have generally been used.

In the first method the crayfish is clamped in a vertical position, tail down. In this position continuous perfusion with physiological solution is carried out, as described by Cole *et al.* In order not to submerge the nerves during stimulation, a hole can be made on the ventral side of the animal at the level of the heart. The heart beats were recorded with a light heart lever, connexion being made by attaching a small pinch clamp to the heart. In some preparations the nerves may be damaged by this attachment, and stimulation of the nerves becomes without effect.

The second method, a variation of the first, consisted in clamping the crayfish at an angl of about 45 degrees, ventral side up. The clamp is pivoted on the cut chitin of the cervical groove and the motion of the heart is transmitted downwards. The kymograph records obtained are the reverse of those obtained with the other methods. This method has been found particularly advantageous in the investigation of the effects of drugs, in which rapid changes in the perfusion fluid are desired.

In the third method, which was used in the study of the effect of the stimulation of the central nervous system, the crayfish is clamped in a horizontal position. The heart is in a dorsal position and, as in the second method, the clamp is made to pivot on the cut chitin of the cervical groove. The perfusion fluid level is adjusted so that the oesophageal commissures are just covered, in which case the fluid does not quite reach the heart. Perfusion of this organ is, therefore, less perfect than in the other methods. In experiments of rather short duration, however, a comparison of this method with the first one did not show any significant difference. This method makes possible isolation of fibres in the oesophageal commissures (Wiersma, 1938).

The influence of the frequency of stimulation has been determined with two thyratron stimulators. Stimulations of 15 sec. duration have been regularly used and, in the tables and records, the number of beats are given for that interval unless specific times are given. The phenomena reported here have been consistently observed and the total number of preparations has been large.

RESULTS

The effect of stimulation of the peripheral inhibitors

By evisceration, the course of the pair of peripheral inhibitors from the suboesophageal ganglion to the heart is exposed for most of its length. The nerves enter the main body cavity through an easily recognizable square hole, situated above the suboesophageal ganglion (Text-fig. 1). From there they take a latero-dorsal course running along the ridge of chitin to which the flexor muscles in the ventral part of the thorax are attached. Near the point where the most lateral heads of this muscle group are attached, the course of the nerves becomes more caudad and crosses the surface of the extensor muscles in the thorax. These nerves probably correspond to the second superior nerves from the suboesophageal ganglion which Keim (1915) described for *Astacus*.

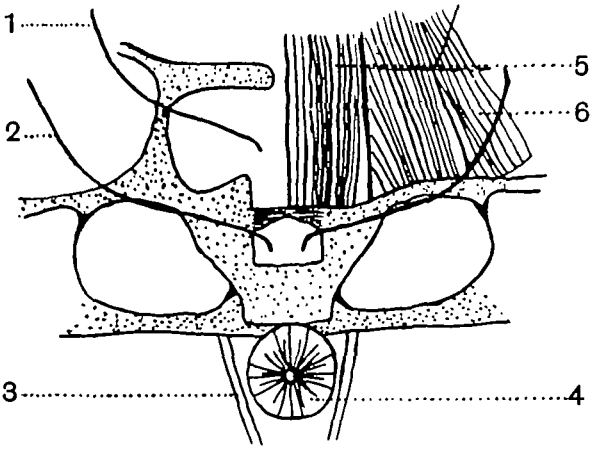
The nerve is easily freed from its attachment to the chitinous ridge, and can then be selectively stimulated by raising it on electrodes or by pressing a pair of electrodes against it.

In a fresh preparation, stimulation of one nerve with frequencies of about 40 per sec. normally results in a complete interruption of the heart beat. As with stimulation of the vagus of the vertebrates, such stoppage is not permanent, but is followed by a partial escape. In some cases a stoppage of several minutes was obtained. The onset of complete inhibition is either instantaneous, or one reduced beat may occur (Pl. 1, fig. 1:60), depending on the moment of the beat cycle when the stimulation is started. The heart normally starts beating immediately after the end of stimulation, usually at a frequency slower than normal, speeding up rather gradually (Pl. 1, fig. 1:60). This after-effect will be discussed in more detail later.

Variation in stimulus strength at a frequency which causes complete inhibition shows a close adherence to an all-or-none relation. Stimuli within the very narrow range between a strength resulting in complete stoppage and one without any influence may show an increase in the number of escape beats with decrease in stimulation strength.

It is quite possible that this difference is not due to a different number of nerve fibres stimulated but to slight variations in the threshold of stimulation. Since preparation of single fibres could not be performed, it is not known how many inhibitory fibres are in each nerve, but the number is most likely very small and might well be only one.

Complete stoppage of the heart occurs only at rather high frequencies. In fresh preparations the necessary frequency is about 40 per sec.; in older ones it may rise to 90 per sec. Slowing of the frequency of the heart beat becomes evident only with frequencies



Text-fig. 1. Diagram showing the course of the inhibitor and accelerator nerves in the antero-ventral portion of the thoracic cavity showing: 1, accelerator nerve; 2, inhibitor nerve; 3, oesophageal commissures; 4, oesophagus; 5, flexor muscles; 6, extensor muscle.

above 10 per sec. This is partly due to the fact that at low frequencies the effect develops so gradually that it becomes difficult to distinguish it from other spontaneously occurring changes. Frequencies of about 20 per sec. result in a gradually established slower rhythm which may be constant for several minutes. With frequencies between this value and one which completely inhibits, an initial stoppage is obtained which remains longer the higher the frequency. After this stoppage is over a regular rhythm is usually estab-

Table 1. *Influence of the frequency of stimulation of the peripheral inhibitor. Stimulation of 15 sec. duration.*

Stimulation frequency per sec.	Heart rate per min.			Stimulation frequency per sec.	Heart rate per min.		
	Before	During	After		Before	During	After
60	112	0	112	15	112	108	112
45	112	72	112	10	112	112	111
30	112	81	113	20	111	96	110
20	112	104	112	60	108	0	105
45	112	76	113				

lished which is lower the higher the frequency of stimulation. Table 1 shows determinations for a certain preparation in which the frequency between tests was unusually constant over a long period. Pl. 1, fig. 1 shows a typical set of records obtained on stimulation with different frequencies.

Stimulation of either the left or right inhibitor results in identical effects; when both are stimulated simultaneously, a stronger inhibition results than on stimulation of either alone.

In a series of experiments action potentials were led off during peripheral inhibition A hole was made in the carapace above the posterior part of the heart, and into it one lead-off electrode was inserted. The other was placed just anterior to the pericardial cavity. The action potentials were almost without exception single spiked, a '*T*' wave often following the spike. This '*T*' wave was, at least to a large extent, due to movement of the anterior electrode with each heart beat. During stoppage of the heart by inhibition, the electrocardiogram disappeared completely. When smaller beats occurred during inhibition, correspondingly smaller potential spikes were obtained.

The effect of stimulation of the peripheral accelerators

The accelerator nerves are more difficult to prepare than the inhibitors, since, on their entry into the main body cavity, they are covered by the flexor muscles in the thorax and emerge on the surface of the extensor muscles only after they have reached the more caudal part of the cavity (Text-fig. 1). These nerves would seem to correspond to the third superior nerves from the suboesophageal ganglion as described by Keim (1915) for *Astacus*. Near the heart they are in such close proximity to the inhibitors that selective stimulation is difficult. Preparations were made by cutting the insertion of the flexor muscles and carefully bending these back. Most of the experiments were performed by gently pressing the electrodes against the exposed nerve; care was taken to stimulate with rather weak currents to prevent simultaneous stimulation of the inhibitors. In a number of experiments the latter were removed for a considerable length in order to diminish further this possibility. Experiments in which the exposed part of the nerve was stimulated by lifting it on micromanipulated electrodes showed the same results.

During stimulation of the accelerator, both the frequency and amplitude of the beat are increased. In some cases during maximum stimulation the amplitude of the beats is lower than normal. In other hearts acceleration gave rise to prolonged beats, and the picture resembled more or less that of an incomplete tetanus. It is never possible to accelerate a heart above a certain limit; this maximum response tends to remain constant over short intervals, so that minor differences in frequency before stimulation have little effect on the frequency during stimulation. If a heart attains a slower rhythm after a longer period, acceleration cannot induce it to reach its former maximum, nor can a heart which is beating relatively slowly be speeded up to the same frequency which a heart, beating normally at a higher rate, can reach.

As with inhibition, the accelerating effect diminishes during prolonged stimulation. After a shorter stimulation the frequency may stay higher than normal for some time after the stimulation, returning gradually to its former value (Table 2). There is no difference in the effects of stimulation of the right and left accelerators. Simultaneous faradic stimulation of both has very little stronger effect that that of one; in almost every instance the frequency was only a few beats per minute faster than on stimulation of either one alone.

A study of the influence of the frequency of stimulation reveals that the accelerators differ markedly from the inhibitors, since low frequencies have a noticeable effect on the accelerators but not on the inhibitors. Stimulation rates as low as 1 per sec. gave an increase in frequency (Table 2A), and even a single stimulus could sometimes shorten the pause between two beats to a measurable degree. In such sensitive preparations frequencies of above 30 per sec. did not result in a further pronounced increase. In older

or less sensitive preparations, higher frequencies are necessary to obtain the same effects. Table 2B shows a typical set of responses of a heart to different frequencies of stimulation, frequent returns to 200 stimuli per sec. being made to test the maximum response. Pl. 1, fig. 2 shows a series of records in which the effects of different frequencies of stimulation are illustrated.

The action potentials led off during acceleration are, of course, more frequent than normal. In addition, there is a definite increase in the size of the spike, which accompanies the increase in the mechanical beat size. In the cases where prolonged beats occurred during acceleration, it was found that the accompanying action potentials showed more tops, and the more pronounced the mechanical prolongation was, the clearer the tops could be distinguished.

Table 2. *Influence of the frequency of stimulation of the peripheral accelerator. Stimulation of* 15 *sec. duration.*

Stimulation frequency per sec.	Heart rate per min.			Stimulation frequency per sec.	Heart rate per min.		
	Before	During	After		Before	During	After
A 7	52	66	58	B 30	48	64	52
5	50	64	56	200	56	66	58
2	50	62	54	20	52	62	58
1	54	64	62	200	52	72	56
B 200	48	68	48	20	52	60	52
100	40	64	48	7	52	56	52
60	40	64	48	7	44	48	48
45	32	68	44	200	44	72	66
200	40	68	44	7	48	56	52

Simultaneous stimulation of the inhibitor and the accelerator

In a number of experiments, the effects of simultaneous stimulation of the accelerator and inhibitor nerves were investigated. The combined effects were roughly the average of the individual effects. For instance, one preparation with a normal rate of 18 per 15 sec. was accelerated to 21 by maximal stimulation of the accelerator nerve and was depressed to 12 by stimulation of the inhibitor with a stimulation frequency of 30 per sec.; when both were stimulated at the same time, the beat rate was 16. Another preparation treated similarly had a normal beat rate of 15, an accelerated rate of 19, an inhibited rate of 9, and with both nerves stimulated together, a beat rate of 14 per 15 sec. When inhibition is complete, however, simultaneous stimulation of the accelerator is generally without immediate effect, although cases have been found in which a few escape beats were induced.

The after-effects of inhibition

After a heart has been stopped by maximal stimulation of the inhibitor, it generally returns gradually to its former frequency. This phenomenon is more apparent in hearts which are normally beating slowly. Immediately after stopping stimulation of the inhibitor, the heart invariably beats once. However, the pause between this beat and the following one is usually longer than normal, and for the first few beats this interval between beats may even lengthen somewhat before gradually returning to normal (Pl. 1, 1:60).

When acceleration and inhibition are applied and released simultaneously, the after-effect of inhibition largely disappears, although acceleration may not manifest itself at the time of stimulation of the nerves (Pl. 1, fig. 3). The delayed action of the accelerator nerve under these conditions suggests liberation of a substance, which can exert its influence at a later time.

The effects of eserin and acetylcholine

Perfusion of the heart with eserin (10^{-5}) yielded no obvious effect; in a few cases the beat frequency was slightly raised, in others it was lowered. Comparison of the acceleration produced by maximal stimulation of the accelerator nerve before and during perfusion with eserin shows that eserin enhances the effect of acceleration (Table 3). Eserin has no influence on the effectiveness of inhibition.

Table 3. *Comparison of the effects of stimulation of the accelerator nerve of eight different hearts before and after eserinization. Stimulation of* 120 *per sec. for* 15 *sec.*

	Before eserinization. Heart rate per min.			After eserinization. Heart rate per min.		
	Before	During	After	Before	During	After
A	24	104	92	28	116	84
B	84	96	88	72	108	84
C	92	104	96	92	108	84
D	36	84	48	32	104	68
E	68	80	68	64	88	72
F	72	84	72	32	92	60
G	72	92	76	68	116	80
H	72	84	76	72	104	80

In confirmation of Welsh (1939*a*) and Davenport (1941) perfusion with acetylcholine gave an immediate increase in frequency and amplitude of the beat. During perfusion complete inhibition can be obtained, but somewhat higher frequencies than those effective before the addition of acetylcholine are necessary. A certain preparation, for instance, could be completely inhibited by a stimulus of 40 per sec., but a stimulus of 50 per sec. was necessary during acetylcholine perfusion. Upon release of inhibition, the heart may enter a partial tetanus of brief duration.

The maximum degree of acceleration obtained by perfusion with acetylcholine and by stimulation of the accelerator is approximately the same. In Pl. 1, fig. 4, the slightly greater number of beats obtained by acetylcholine stimulation as compared with nervous stimulation is represented by a larger number of small alternate beats.

The nerve of Lemoine

This nerve is part of the so-called sympathetic system, which connects with the central nervous system mainly at the commissural ganglia situated about half-way between the 'brain' ganglion and the suboesophageal ganglion. There has been considerable discussion whether the nerve makes connexion with the heart or whether it stops at the anterior arterial valve (Lemoine, 1868; Police, 1908; Keim, 1915; Alexandrowicz, 1932; Heath, 1941). A regulatory influence has been ascribed to this nerve (Young, 1878; Plateau, 1880; Moquart, 1883) and also has been denied it (Jolyet & Viallanes, 1892; Connant & Clarke, 1896; Carlson, 1905). The negative evidence of the latter workers was obtained by stimulation of the 'brain' after transection of the oesophageal commissures.

In order to investigate the effect of the nerve of Lemoine on the heart, another method of preparation has been used. The tail was removed from the animal and the posterior side of the heart exposed, leaving the 'liver' completely intact. The carapace was carefully removed for a small distance on top of the stomach, exposing the nerve which is very thin, and which runs in the median line. The preparation was mounted head downwards and the clamp fastened to the posterior side of the heart. No effect whatsoever was noticed in such preparations upon stimulation of the exposed nerve with various frequencies. As a control the peripheral inhibitors and accelerators were subsequently stimulated in these preparations, with the expected effects. Several variations were made in order to exclude possible damage of the nerve by preparation, but in no case was any change in heart-beat size or rate found.

The inhibitors in the central nervous system

Stimulation of the oesophageal commissures frequently results in an immediate stopping of the heart. By dividing the commissure into bundles and stimulating these separately, it was found that this inhibitory function was the exclusive property of a very thin fibre bundle, consisting of three fibres at most. It is likely that only one of these, which is thicker than the others (diameter about 30 μ), is solely responsible. Single fibres in the commissure are, however, much sooner damaged than those in peripheral nerves, and this conclusion is, therefore, not absolutely certain.

The experiments on the effect of this inhibitory tract have been varied in many ways. In a number of experiments the peripheral accelerators were cut, and the central inhibitors more or less prepared. There was no difference between the left and the right side. On subsequent cutting of the peripheral inhibitor on one side, this situation was still unaltered, but the effect was diminished. Cutting of the other peripheral inhibitor abolished all inhibitory effect of central stimulation on the heart. It is thus certain that each of the inhibitory tracts in the centre makes connexion with both the left and right peripheral inhibitors. Stimulation of the central stump of a cut peripheral inhibitor never results in inhibition through the other inhibitor, which proves that synapses intervene.

In another set of experiments the influence of the frequency of central stimulation was investigated. It was found that the effects are much less constant than with stimulation of the peripheral inhibitor. One difference which was regularly observed was that central stimulation had in many cases a pronounced after-effect, when such an effect was absent or slight in peripheral stimulation. This indicates an after-discharge of the inhibitory ganglion cell. In fresh preparations the use of very low frequencies will often give slowing or complete stoppage; in older ones there is usually not much difference between the result of using the same frequencies on central or peripheral application. After cutting one of the peripheral inhibitors, it is necessary to use a considerably higher frequency to obtain the same effect as before the cutting; the required frequency may be about twice as high. Simultaneous stimulation of both central inhibitors results in a stronger effect than that obtained by stimulation of only one.

In these experiments proof is given that there is a tract from the 'brain' to the suboesophageal ganglion, which gives inhibition exclusively. It is certain that this is not the only tract in the central nervous system which connects with the peripheral inhibitors. In preparations in which the superior ganglion no longer functions, stimulation of the

tail still results in heart inhibition, showing the presence of a presumably similar tract in the lower part of the central nervous system. This inhibition also disappears on cutting the two peripheral inhibitory nerves.

The accelerators in the central nervous system

Preparation of the central accelerators is less satisfactory than that of the inhibitory tract, mainly because of the much shorter survival time. After the peripheral inhibitors have been cut, stimulation of the unprepared oesophageal commissures results in acceleration. After cutting the peripheral accelerators this effect disappears, although in some preparations a very prolonged stimulation has ultimately resulted in some speeding up of the heart. It is clear, however, that this is a different effect, which may be due to the release in the blood stream of a substance liberated somewhere else in the body.

The two accelerators are most likely the only nerves with this function in the crayfish. As in the case of the inhibitors, both oesophageal commissures contain fibres which are connected with each of the two accelerators, and no difference was found between the two sides. As mentioned, preparation of bundles in the commissures is difficult because of the short survival time, therefore no definite statements are possible about the specificity of the tracts, but it was conclusively shown that only a small part of the oesophageal commissure possesses this property.

In a number of experiments it was investigated whether or not cutting the peripheral inhibitors or accelerators results in a persistent change in the frequency. These experiments were largely negative: in the prepared animal there is thus no evidence of an inhibitor or accelerator tone.

Stimulation of a whole oesophageal commissure with different frequencies in preparations with the four peripheral heart regulators intact showed that low frequencies of stimulation cause a short lasting inhibition which is gradually displaced by an accelerated rhythm. This is always found with frequencies lower than 20 per sec. High stimulation frequencies (e.g. 60 per sec.) result in a stoppage of the heart for some seconds, after which a gradual increase in the beat rate takes place, but in these cases the frequency remains lower than the original.

DISCUSSION

From the results obtained by a number of workers with the hearts of both *Limulus* and the decapod crustaceans, and from the results described in this paper for the crayfish heart, a hypothesis with regard to the mechanism of the crayfish heart beat is proposed. This hypothesis is meant to serve only as a means of combining as many observations as possible. Most of its parts have already been proposed by others in the same or similar form.

The crustacean heart beat is of neurogenic origin. The intrinsic ganglion cell pool has a spontaneous rhythm. For each heart beat a series of impulses (volley) is sent down the nerve fibres connecting the ganglion cells with the heart muscle fibres. The frequency of the heart beat is determined by the frequency of volleys. The frequency of volleys is altered by the influence of the regulatory nerves by means of neurohumours. The acceleratory neurohumour is acetylcholine; the inhibitory neurohumour is unknown. During acceleration, the number of impulses in each volley as well as the frequency of volleys increases; during inhibition the reverse is true.

The neurogenic origin of the beat in *Limulus* has been generally accepted since the classic experiments of Carlson (1905–6), notwithstanding a few experiments which tend to show that a myogenic rhythm may be present under certain conditions (e.g. Hoshino, 1925). The similar experiments of Alexandrowicz (1932) have indicated the neurogenic basis for the beat of the crustacean heart. The unquestionable presence of ganglion cells adds weight to this view. In general, the results described by us and other workers conforms much better to the theory of neurogenic origin than that of myogenic.[1]

The spontaneous rhythm of the ganglion cell pool is not as well established. That such an automatic rhythm may be present in structures comparable to these has been shown by Weiss (1941). Gerard & Libet (1939) also are of the opinion that synchronizations like these are frequent in central nervous systems and bring evidence that the factor responsible for the synchronization is electrical rather than chemical.

That volleys of impulses are set up in the nerve fibres leading from the ganglia to the muscle fibres of the heart has been shown in *Limulus* by Heinbecker (1933), and by Armstrong, Maxfield, Prosser & Schoepfle (1939). In the case of the crustacean heart, the evidence is only indirect (see, however, Rijlant, 1932). The partial suppression of the beat height during slowing by inhibition and the increase in height during acceleration can be readily explained by a decrease and increase, respectively, in the frequency of nerve-fibre discharges in each volley, accompanied by a change in the total number of impulses per volley. The prolonged beats sometimes found in older heart preparations can be explained by an excessively prolonged duration of each volley. In maximum acceleration, the rest periods between different volleys may become so small that the heart fibres never completely relax, resulting in tetanic contractions.

It seems likely that acetylcholine is the medium by which the accelerator nerves produce their effect because of the identity of the effects of nerve stimulation and perfusion with acetylcholine. This view is held by a number of authors (Welsh, 1939*a*, 1939*b*, in *Carcinus* and *Panulirus*; Davenport, Loomis & Opler, 1940, in *Astacus*; Davenport, 1941, in *Cancer*; Obreshkove, 1942, in *Daphnia*). Further support of this view is given by the fact that eserin enhances the effect of stimulation of the accelerator nerve. It seems clear that the acceleratory effect is mediated through the ganglia rather than through the muscle fibre directly. Garrey (1942) has shown that in *Limulus* acetylcholine does not act on heart-muscle fibres, although it accelerates the heart if the ganglion is perfused; likewise, acetylcholine has no effect upon crustacean peripheral muscle fibres (du Buy, 1935; Bacq & Nachmansohn, 1937).

The humoral nature of the inhibiting substance is more doubtful. None of the substances tried so far have given a comparable result. Its humoral nature can be inferred only from the after-effect of inhibition, an effect which is often but by no means always present. It is not inconceivable that inhibition has a different, e.g. electrical, nature, but this will have to be studied further. No evidence has been found that the inhibition of the heart is like the typical peripheral inhibition of the crustaceans. The close correlation between the action potentials and the beat size does not fit in with peripheral inhibition. The location of the inhibitory effect is, without much doubt, in the ganglion.

Many authors have claimed that the electrocardiogram of the crayfish regularly shows

[1] In this connexion it may be pointed out that in the peripheral muscles of the crustaceans, conduction in the muscle fibres may well be absent (see Wiersma, 1941). If this is true, too, for heart-muscle fibres, the neurogenic origin would be imperative.

a number of tops and have concluded that the heart beat is tetanic in nature. Dubuisson (1934), however, has shown that under normal conditions the electrocardiogram exhibits a single top. Our observations wholeheartedly agree with those of Dubuisson. It has been shown that in peripheral nerve-muscle preparations in crustaceans a quick succession of impulses gives rise to a smooth, high action potential rather than to a series of tops (Wiersma & van Harreveld, 1938). The same might be the case here. Variation in the number and the frequency of the impulses in the intrinsic nerve fibres would then readily explain the respective increase or decrease in size of potentials during acceleration and inhibition. The many topped action potentials of the prolonged beats would reflect the irregularities in the discharge of the ganglion cells.

SUMMARY

A preparation is described in which the peripheral inhibitor and accelerator nerves of the crayfish (*Cambarus clarkii*) heart can be separately or simultaneously stimulated. The effect of different frequencies of stimulation were investigated; complete stoppage of the heart was obtained only with rather high frequencies (45 per sec.); maximal acceleration, with lower frequencies (30 per sec.). No difference in effects between left and right nerves was found. Perfusion with acetylcholine and stimulation of the accelerator nerve produce identical effects. Perfusion with eserin does not influence the normal heart beat but enhances the effect of acceleration.

The nerve of Lemoine has no regulatory influence on the heart.

Special inhibitory and acceleratory tracts have been prepared in the suboesophageal commissures. Each of these tracts makes heterolateral as well as homolateral connexions. The influence of the frequency of stimulation on the central tracts has been studied.

An hypothesis with regard to the mechanism of the crayfish heart beat and its control is presented.

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REFERENCES

ALEXANDROWICZ, J. S. (1932). *Quart. J. Micr. Sci.* **75**, 181.
ARMSTRONG, F., MAXFIELD, M., PROSSER, C. L. & SCHOEPFLE, G. (1939). *Biol. Bull. Woods Hole*, **77**, 327.
BACQ, Z. M. & NACHMANSOHN, D. (1937). *J. Physiol.* **89**, 368.
BUY, H. G. DU (1935). *Amer. J. Physiol.* **114**, 224.
CARLSON, A. J. (1905). *Biol. Bull. Woods Hole*, **8**, 123.
CARLSON, A. J. (1906). *Amer. J. Physiol.* **15**, 127.
COLE, W. H., HELFER, R. G. & WIERSMA, C. A. G. (1939). *Physiol. Zoöl.* **12**, 393.
CONNANT, F. S. & CLARKE, H. L. (1896). *J. Exp. Med.* **1**, 341.
DAVENPORT, D. (1941). *Physiol. Zoöl.* **14**, 178.
DAVENPORT, D., LOOMIS, J. W. & OPLER, C. F. (1940). *Biol. Bull. Woods Hole*, **79**, 498.
DOGIEL, J. (1894). *Arch. mikr. Anat.* **43**, 223.
DUBUISSON, M. (1934). *Arch. int. Physiol.* **40**, 181.
GARREY, W. E. (1932). *Amer. J. Physiol.* **136**, 182.
GERARD, R. W. & LIBET, B. (1939). *Livro de Homenagem*, p. 288. Rio de Janeiro.
HARREVELD, A. VAN (1936). *Proc. Soc. Exp. Biol., N.Y.*, **34**, 428.
HEATH, J. P. (1941). *J. Morph.* **69**, 481.
HEINBECKER, P. (1933). *Amer. J. Physiol.* **103**, 104.
HOSHINO, N. (1925). *Pflüg. Arch. ges. Physiol.* **208**, 245.

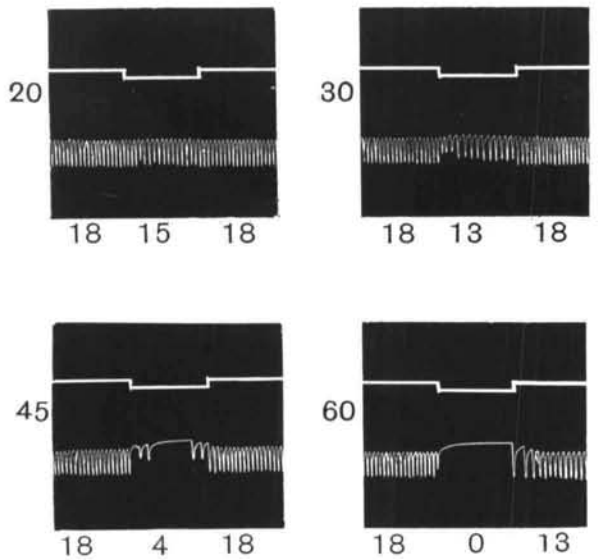

Fig. 1

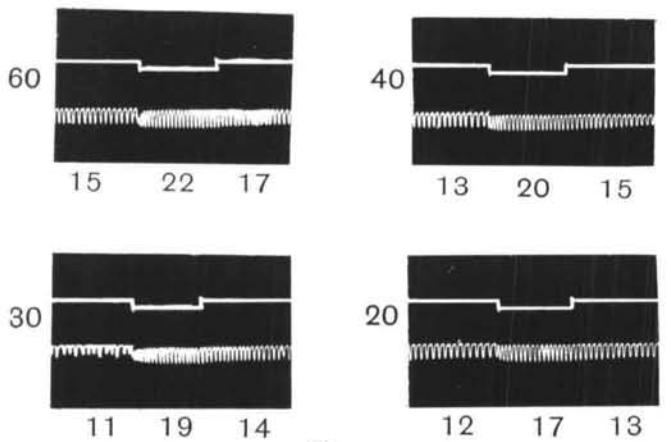

Fig. 2

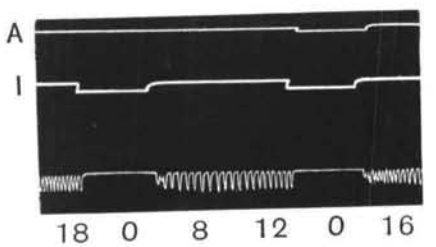

Fig. 3

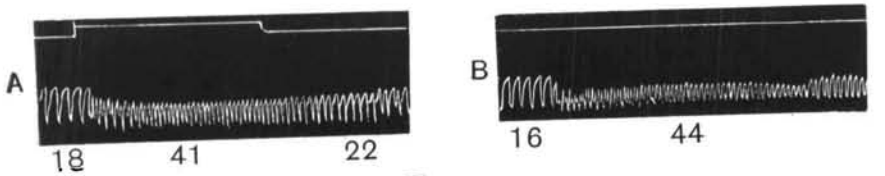

Fig. 4

WIERSMA AND NOVITSKI—THE MECHANISM OF THE NERVOUS REGULATION OF THE CRAYFISH HEART (pp. 255–265).