

STUDIES OF CARDIO-REGULATION IN THE COCKROACH, *PERIPLANETA AMERICANA*

BY T. MILLER* AND P. N. R. USHERWOOD

Department of Zoology, The University, Glasgow

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INTRODUCTION

The circulation of haemolymph in insects is maintained by a system of muscular pumps and fibromuscular septa. The most important muscular pump is the dorsal vessel which collects blood from the abdominal cavity and discharges it into the head. In cockroaches the dorsal vessel is a straight tube comprising the 'heart' in the abdominal and thoracic segments and narrowing to form the aorta in the head.

In the American cockroach, *Periplaneta americana*, there are three heart chambers in the thorax and nine chambers in the abdomen. A heart chamber is defined as the region of the dorsal vessel between two pairs of ostial valves. Four of the chambers in the middle of the abdomen give off paired segmental vessels which are innervated and therefore possibly muscular. By means of the segmental vessels some of the intracardial haemolymph is distributed laterally in the abdomen. The walls of the heart consist of a single layer of multinucleate striated muscle cells. The muscle fibres are about $3\ \mu\text{m} \times 40\ \mu\text{m} \times 200\ \mu\text{m}$, and are arranged in a semicircular fashion. The fibres are often connected by intercalated disks similar to those found in vertebrate heart muscle (Edwards & Challice, 1960) and in moth heart muscle (Sanger & McCann, 1968). References to the detailed anatomy of *Periplaneta* heart can be found in the comprehensive reviews by Jones (1964) and McCann (1970). Especially pertinent works cited in these reviews are those of Alexandrowicz (1926), McIndoo (1939, 1945) and Johnson (1966).

The cardiac nervous system of *P. americana* consists of paired lateral cardiac nerve cords running along the entire length of the dorsal vessel, one on either side, and the processes from paired segmental nerves which originate lateroposteriorly at each central ganglion. The paired segmental nerves join the respective right and left lateral cardiac nerve cords opposite the ostial valves in each heart chamber. Each lateral cardiac nerve cord contains nerve cells which are usually bipolar and are of two types. Ganglion cells, which are probably motor in function, send axons to the myocardium and also send processes anteriorly and posteriorly along the lateral cardiac nerve cord. The second type of nerve cell is probably neurosecretory. It also sends processes along the lateral cardiac nerve cords, but as yet there is no evidence that these cells innervate the myocardium (Miller, 1968).

McIndoo (1945) reported that the lateral cardiac nerve cords in the American cockroach contain about 32 nerve cells. It is not clear as yet how many of these neurones

* Present address: Department of Entomology, University of California, Riverside, California 92502.

are ganglion cells. The neurosecretory fibres in the lateral cardiac cords can be clearly distinguished from the processes of the ganglion cells. The latter are white in colour whereas the neurosecretory fibres which are grouped into a bundle have a blue appearance (Miller & Thomson, 1968). Axons containing neurosecretory material have been found also in the segmental nerves (Johnson, 1966; Miller & Thomson, 1968).

In primitive insect orders such as the Dictyoptera the cardiac nervous system reaches its most complex form. In higher insects such as Diptera and Lepidoptera intrinsic cardiac neurones are either few in number or are absent altogether and the cardiac nervous system is greatly reduced. The complexity of the cardiac nervous system of the cockroach is rather surprising in view of the myogenic nature of the heartbeat in this insect. The rhythmic activity of the heart muscle is not qualitatively altered by removing the cardiac nervous system (Miller & Metcalf, 1968*a*) so presumably both the central neurones, which send axons to the heart via the segmental nerves, and the intrinsic cardiac neurones act either directly or indirectly as cardio-regulators. By analogy with the vertebrate heart and other arthropod hearts it might be anticipated that cardio-regulation in the cockroach would involve both excitatory and inhibitory neurones.

Carlson (1905) found evidence for inhibitory innervation of the heart of the grasshopper, *Dictyophorus reticulatus*, although his evidence was based solely on the results of visual observations. Lasch (1913) also found evidence of neural inhibition of an insect heart, in this case a larval heart of a beetle, *Lucanus* sp. Steiner (1932) demonstrated that the segmental nerves and lateral cardiac nerve cords of *P. americana* both serve as cardio-acceleratory pathways in response to brain stimulation.

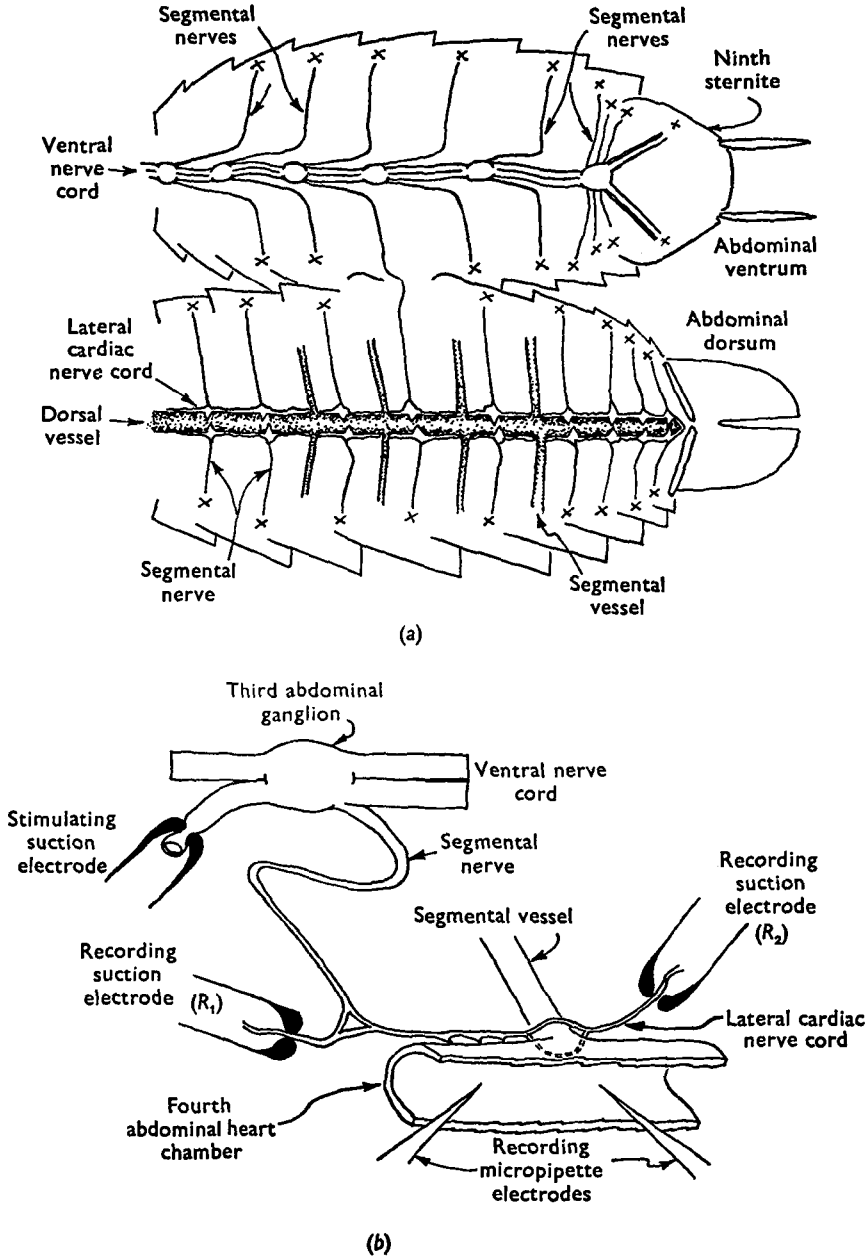
The object of this publication is to describe further studies of the innervation and mode of operation of the cockroach heart. Particular emphasis has been placed in these studies on tracing neuronal pathways between the central nervous system and the heart muscle.

MATERIALS AND METHODS

Heart preparations from large nymphs (♂ and ♀) of *P. americana* were found to be more satisfactory than those from adults, provided that the nymphs were neither newly moulted specimens nor at a stage just prior to a moult (Richards, 1963). Only the abdominal portion of the dorsal vessel was studied and usually experiments were restricted to the fourth abdominal chamber of the heart (Text-fig. 1). The heart was prepared for examination in much the same way as in previous studies (e.g. Miller & Metcalf, 1968*a*) although in some preparations the nervous connexions of the fourth abdominal heart chamber with the central nervous system, i.e. the segmental nerves, were left intact.

In preparations which included the central nervous system the abdomen was opened by cutting along the right pleural region. Next, the dorsum was pinned down on wax and the ventrum was folded back using the pleural region as a hinge. This manipulation exposed the left segmental nerves which innervate the left side of the abdominal heart. The segmental nerve from the third abdominal ganglion which joins the heart at the fourth abdominal heart chamber was left intact. The rest of the connexions between the central nervous system and the heart were severed, including connexions of all the segmental nerves from the ventral ganglion on the right side.

The dorsal diaphragm was usually removed to allow access to the lateral cardiac nerve cords in the vicinity of the fourth abdominal chamber of the heart. Finally, in many experiments, the third abdominal ganglion was isolated from the rest of the central



Text-fig. 1. (a) Diagrammatic representation of the abdominal heart preparation of an adult male cockroach including central nervous connexion. An incision is made around much of the lateral circumference of the abdomen leaving only the neural connexion between the third abdominal ganglion and the fourth abdominal heart chamber. The dorsum is lifted away from the ventrum as illustrated, and the gut is removed. Cut ends of nerves are indicated by crosses. A detail view of recording and stimulating electrode positions is shown in (b).

nervous system by cutting the central connectives between it and the second and fourth abdominal ganglia (Text-fig. 1*b*).

The electrical activity of the lateral cardiac nerve cord on one side of the heart was recorded as shown in Text-fig. 1 by a suction electrode positioned about halfway between the segmental blood vessel of the third abdominal heart chamber and the ostial valve of the fourth chamber. A second suction electrode was placed on the lateral cardiac nerve cord near the ostial valve of the fifth abdominal chamber of the heart. The lateral cardiac nerve cord was cut in these two places and the cut ends of the cord were drawn into the suction electrodes.

The electrical activity of the myocardial cells was recorded with glass intracellular microelectrodes (10–25 M Ω resistance) filled with either 3 M potassium chloride or potassium propionate. The muscle fibres were usually impaled from the outside of the heart but in some preparations the fourth abdominal chamber was slit open along the right side so that access to the muscle fibres could be gained from the lumen side of the heart (Text-fig. 1*b*). The inner wall of the dorsal vessel, unlike the outer wall, is not covered by connective tissue fibres and could be more readily penetrated by the microelectrodes. Contractions of the heart were monitored (isotonically) using a capacitance device (Miller & Metcalf, 1968*b*).

The heart preparations were mounted in a Perspex bath and perfused continuously with saline of the following composition: NaCl, 188 mM/l; KCl, 20 m-moles/l; CaCl₂, 8 m-moles/l; MgCl₂ or MgSO₄, 1.8 m-moles/l. Previous studies have shown that the cockroach heart functions quite satisfactorily in this saline even though it is not buffered (Miller, 1969*b*). This is clearly advantageous in view of the high concentration of calcium in the saline.

RESULTS

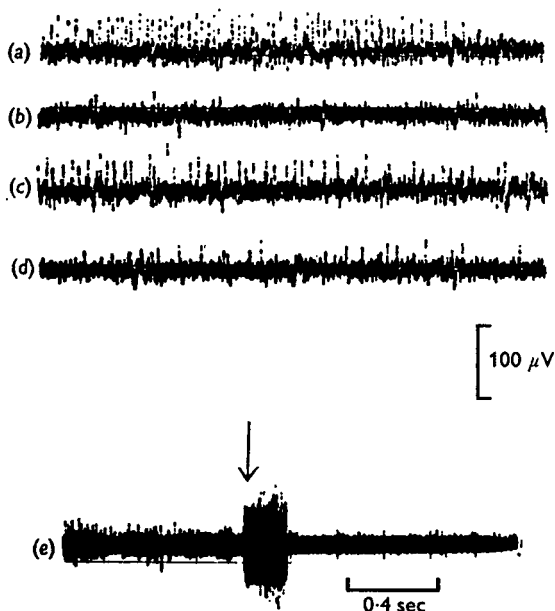
After perfusing a fresh heart preparation for several minutes with physiological saline, relatively uniform rhythmic contractions of the dorsal vessel were obtained. However, in preparations in which the fourth abdominal chamber was still coupled to the central nervous system by one or both of its segmental nerves, the rhythmic activity of this chamber and the chambers immediately adjacent to it was occasionally punctuated by an increase in the amplitude of the contractions and slight changes, usually increases, in the frequency of the contractions. These transient changes in the characteristics of the heart beat occurred simultaneously with activity in some of the skeletal muscles of the abdomen, namely those which were still connected to the central nervous system, notably the dorsal and ventral longitudinal muscles. Small potentials were recorded from both the segmental nerve and the lateral cardiac nerve cord during the periodic spontaneous output from the central nervous system to the heart. These potentials disappeared when the segmental nerve was cut (Text-fig. 2). The influence of the central nervous system on the contractions of the heart and of the abdominal skeletal muscles was greatest in fresh preparations and gradually diminished as the preparation aged.

Responses to stimulation of the segmental nerves

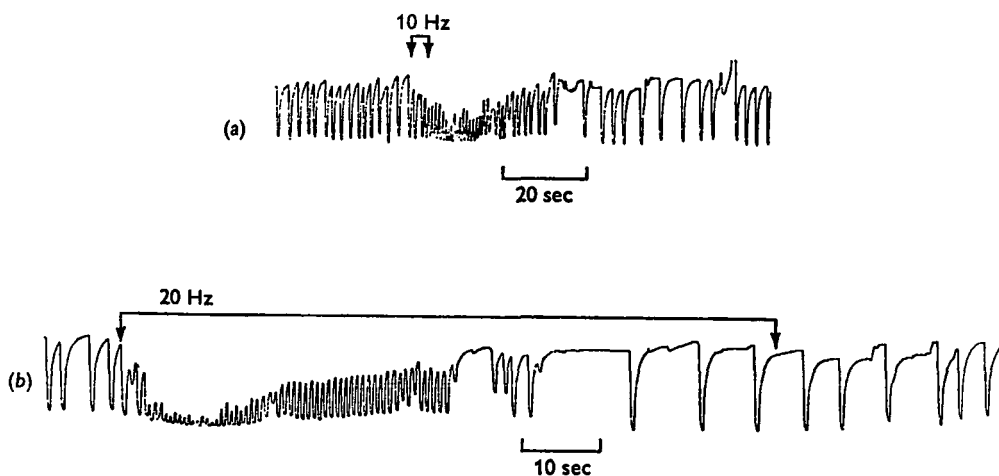
Stimulation of the segmental nerve occasionally resulted in an increase in the frequency of the small potentials recorded from neurosecretory fibres of the lateral

cardiac nerve cord. In some preparations where stimulation of the segmental nerve was accompanied by an increase in the heartbeat rate, the cardiac ganglion cells of the lateral cardiac nerve cord were activated as well.

The effects of segmental nerve stimulation on the rhythmic contractions of the fourth abdominal chamber of the heart were characterized by an initial increase in the



Text-fig. 2. Extracellular recordings (R_1 , cf. Text-fig. 1 *b*) from the left lateral cardiac nerve cord. In (*a-d*) the connexion between the left segmental nerve and the central nervous system was intact. In (*e*) this connexion was severed (at arrow) and a marked fall in the level of activity of the lateral cardiac nerve cord was seen.



Text-fig. 3. Isotonic mechanical recordings of the contractions of the third abdominal heart chamber. The central nervous system was not included in the preparation. The right segmental nerve which innervates this chamber was stimulated (*a*) at 10 Hz for 2 s, and (*b*) at 20 Hz for 82 s.

rate of beat followed by a decrease to a level below the basal rate (Text-fig. 3*a*). A higher peak increase in the frequency of the contractions was obtained by increasing the stimulus intensity (Miller, 1969*a*). The duration of the stimulation period did not qualitatively affect the response of the fourth abdominal chamber of the heart although the hyperactive phase was increased in duration when the period of stimulation was lengthened (Text-fig. 3*b*). In one preparation (see Text-fig. 3*a*) the segmental nerve was stimulated at a frequency of 10 Hz for about 2 s. The fourth abdominal chamber was initially contracting about 30 times/min but on stimulation of the segmental nerve the frequency of the contractions quickly increased to a maximum of 90/min. This was followed by a short period of subnormal activity when the contraction frequency decreased to about 12/min. When the duration of the stimulation period was increased to 82 s the frequency of contractions increased to about 105/min (Text-fig. 3*b*). This high frequency was maintained for about 40 s but then the frequency fell abruptly. At the end of the period of stimulation the frequency slowly increased again and reached a value of about 24 contractions/min after 28 s.

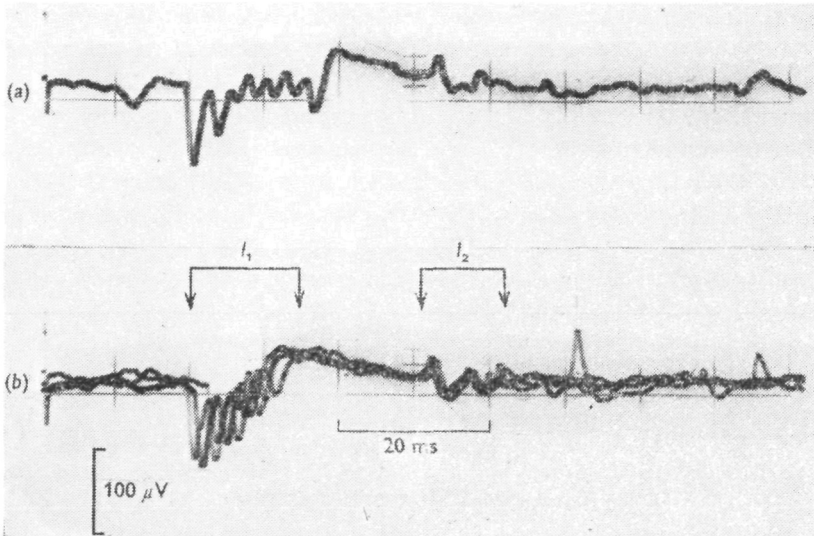
The changes in the heartbeat of the cockroach during segmental nerve stimulation are somewhat reminiscent of the changes in frog heartbeat during vagal stimulation, although in the latter case inhibition is followed by excitation. Possibly the segmental nerve of the cockroach like the vagus nerve of the frog contains excitatory and inhibitory fibres which make direct contact with the heart muscle. Alternatively the connexions between the central nervous system of the cockroach and the heart may be indirect, information from the central nervous system being relayed to the heart via neurones in the lateral cardiac nerve cord.

It was noticeable on occasions that, provided all the connexions between the central nervous system and the heart were intact, stimulation of the segmental nerve (left or right) of the third abdominal ganglion affected not only the fourth abdominal chamber of the heart but the entire abdominal portion of the dorsal vessel. The responses of the thoracic part of the heart were not examined at this time but there remains a distinct possibility that the entire heart is affected by stimulating just one segmental nerve. The possible significance of this will be discussed later. Even when the only connexion between the heart and the central nervous system was via one of the segmental nerves to the fourth abdominal chamber of the heart, stimulation of this nerve affected more than one chamber. It appears therefore that information from one segmental nerve propagates for some distance up and down the lateral cardiac nerve cord.

Activation of cardiac ganglion cells by central stimulation

Two classes of potential, large and small, were recorded from the segmental nerve when this nerve was stimulated electrically. The small potentials are apparently associated with activity of neurosecretory fibres in the segmental nerve whilst the large potentials occur in the ordinary (i.e. non-neurosecretory) fibres of the segmental nerve. Since both sets of fibres join the lateral cardiac nerve cord it is possible to record their activity with suction electrodes applied to this region of the cardiac nervous system. These electrodes also recorded the activity of the intrinsic cardiac ganglion cells and the neurosecretory neurones of the lateral cardiac nerve cord. In other words, it should be possible to identify four classes of potential although on occasions it was not possible to distinguish clearly between the different potentials.

Stimulation of any one of the four connectives of the third abdominal ganglion could activate both sets of fibres in the segmental nerve with the appearance of large and small potentials in the lateral cardiac nerve cord. However, the patterning of these evoked potentials varied from preparation to preparation and depended to some extent on which connective was stimulated. Text-fig. 4 illustrates the responses recorded from the left lateral cardiac nerve cord of the fourth abdominal chamber of one such preparation during stimulation of the left posterior connective. A group of large potentials was recorded at the anterior suction electrode (R_1) on the lateral cardiac



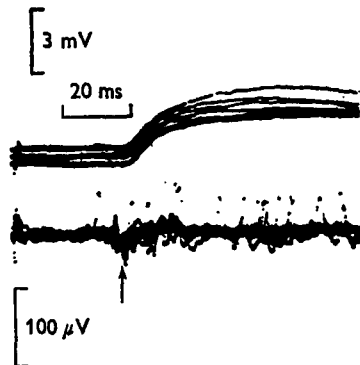
Text-fig. 4. Extracellular recordings (R_1 , cf. Text-fig. 1 *b*) from the lateral cardiac nerve cord during electrical stimulation of the left posterior connective of the third abdominal ganglion. In (*a*) a single trace is illustrated whereas in (*b*) several traces are superimposed. Each trace begins with a stimulus artifact at the left margin and two groups of impulses, I_1 and I_2 , can be distinguished in (*b*).

nerve cord (Text-fig. 1) after a latency of about 19 ms. In the absence of any synapses in the pathway from the connective to the recording site this would constitute an apparent conduction velocity of about 0.74 m/s. Although with repeated stimulation it was found that the latencies of these large potentials were not consistent (Text-fig. 4 *b*) in this preparation, in other preparations they were very consistent. Perhaps these large potentials resulted from indirect excitation of a unit or units within the abdominal ganglion. The groups of large potentials evoked by stimulating the central connective was followed by one or more smaller potentials. These small potentials occurred after a delay of about 50 ms which gives an apparent conduction velocity of about 0.28 m/s. Unlike the large potentials, these small responses always had a very constant latency. Perhaps the pathway from the connective to the lateral cardiac nerve cord for the small potentials is direct, although there are alternative possibilities. It could be argued, of course, that the small potentials resulted from activation of neurones within the lateral cardiac nerve cord following the arrival of the large potentials from the central nervous system. This seems unlikely, however, in view of the inconsistent latencies of the large potentials seen in Text-fig. 4. Also in this particular preparation the large

responses were evoked at slightly lower stimulus intensities than the small potentials. It was noticeable that in some preparations stimulation of a central connective failed to evoke either the large or the small potentials.

Evidence for direct innervation of the myocardium by axons from the central nervous system

It became clear from these studies that two classes of neurones send axons along the segmental nerve and that the fibres in the segmental nerve are descending rather than ascending. The next step was to determine whether these neurones innervate the myocardium directly. To do this it was necessary to ensure that the intrinsic activity of the lateral cardiac nerve cord and of the heart muscle fibres was reduced to a minimum. This was done by isolating the fourth abdominal chamber of the heart with its portion of lateral cardiac nerve cord from the rest of the cardiac system. The segmental nerve connecting the left lateral cardiac nerve cord on the fourth abdominal chamber with the central nervous system was left intact. The heart was then slit open longitudinally on the right side. A recording suction electrode was placed on the



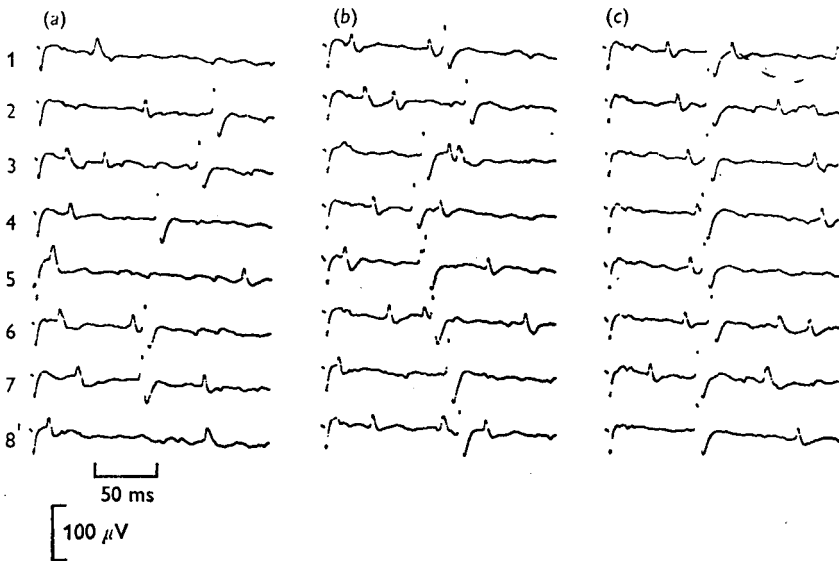
Text-fig. 5. Several superimposed traces of intracellular recordings (upper trace) from a fibre of the fourth abdominal chamber of the heart and extracellular recordings (lower trace) from the lateral cardiac nerve cord (R_1 , cf. Text-fig. 1*b*). The left anterior connective of the third abdominal was stimulated electrically at 1 Hz. Each stimulus was followed by a depolarizing postsynaptic potential in the heart muscle fibre preceded by a small potential in the lateral cardiac nerve cord (arrow).

anterior stub of the left lateral cardiac nerve (R_1) and a stimulating suction electrode was placed on the left anterior connective of the isolated third abdominal ganglion (Text-fig. 1). An intracellular microelectrode was used to record the activity of muscle fibres from the lumen side of the fourth abdominal chamber of the heart. This electrode was inserted near the centre of the chamber. In other words, the recording suction electrode on the lateral cardiac nerve cord was proximal to the junction between the segmental nerve and lateral cardiac nerve whereas the intracellular electrode in the heart muscle was distal to this junction. In actual fact an attempt was made to locate the two types of electrode at points equidistant from this junction.

When the connective was stimulated with a single shock a small potential was recorded at the proximal suction electrode (R_1) on the lateral cardiac nerve cord (Text-fig. 5). This potential appeared with a latency of about 27 ms. About 1.7 ms)

later, a large depolarizing synaptic potential was recorded by the muscle electrode. As mentioned previously, in some preparations stimulation of a central connective evokes both large and small potentials in the segmental nerve and lateral cardiac nerve cord. However, muscle synaptic potentials did not occur in association with the large potentials.

It could be argued that the latency of about 1.7 ms between arrival of the 'small' potentials at the myocardium and the appearance of muscle synaptic potentials is too long to be accounted for by delay at a single chemical synapse. Possibly a second neurone is interposed between the neurosecretory fibres from the central nervous system, from which the small potentials are recorded, and the muscle fibres. However,



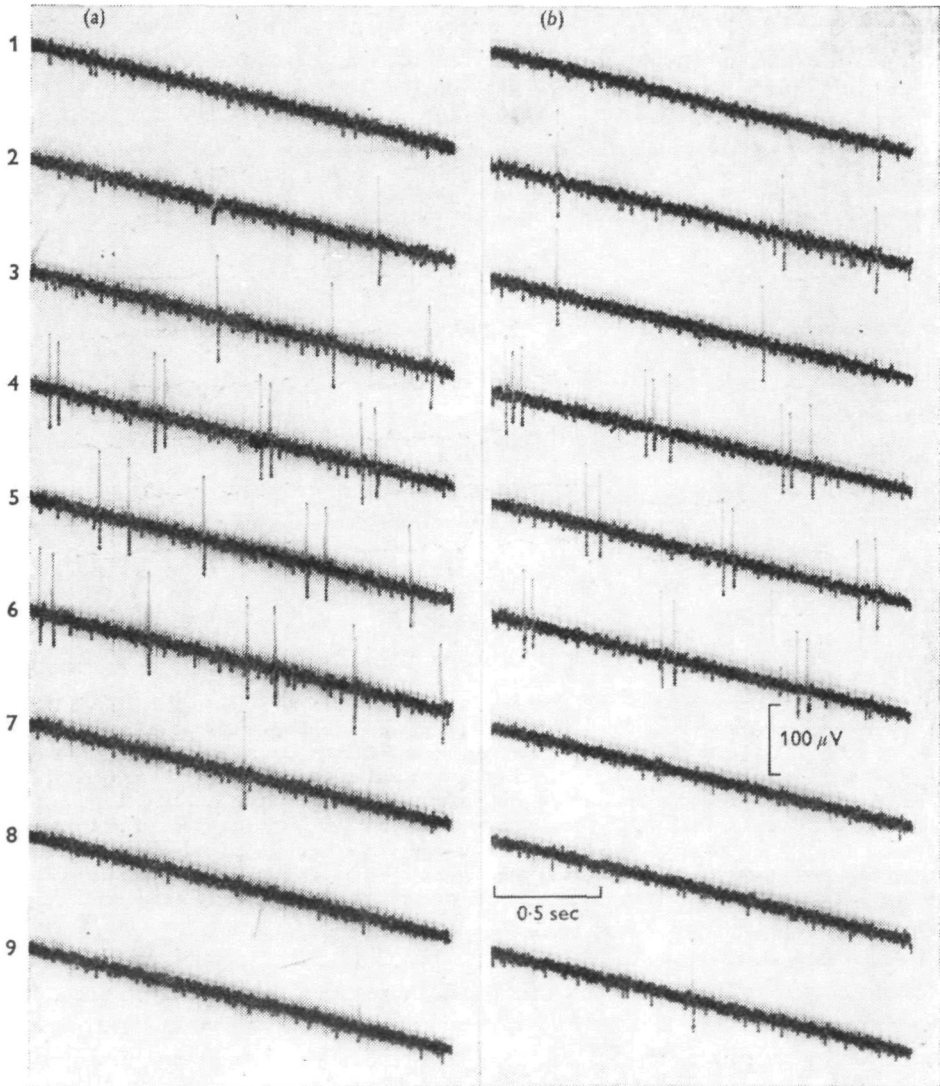
Text-fig. 6. Activation of a cardiac ganglion cell through central nervous stimulation via the left anterior connective of the third abdominal ganglion. Recordings made at electrode (R_1 , cf. Text-fig. 1*b*). The stimulus intensity was constant for (*a* 1-8), increased for (*b* 1-8) and increased yet again for (*c* 1-8). Each trace begins with and was triggered by a large potential recorded from an ordinary axon originating in the central nervous system and travelling down the segmental nerve and along the lateral cardiac nerve cord. Note the reduced and more consistent delay of the large biphasic potentials with increasing stimulus intensity. These potentials were from a cardiac ganglion cell. The other activity (small biphasic potentials) probably comes from one or more neurosecretory cells in the lateral cardiac nerve cord.

the nerve fibres which innervate the muscle probably narrow considerably towards their terminations and this factor alone could be responsible for a large part of the apparent synaptic delay. In any case there is no reason to suppose that events at synapses between neurosecretory neurones and cardiac muscle fibres will follow the same time course as those at synapses between motor neurones and skeletal muscle fibres in the cockroach (Roeder, 1953).

Responses of cardiac neurones to central stimulation

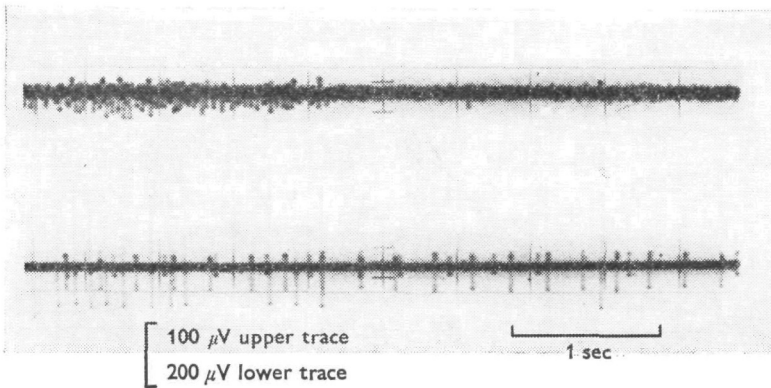
In the preparations described above, stimulation of any one of the four connectives associated with the third abdominal ganglion had a clear effect on the activity of the

ganglion cells in the lateral cardiac nerve cord. For example, in one experiment, stimulation of the left anterior connective evoked a large nerve potential which was recorded by the anterior suction electrode on the lateral cardiac nerve cord (Text-fig. 6). The apparent conduction velocity of this nerve response was of the order of 4 m/s, so presumably it was the result of activity in one or more of the ordinary axons which enter the lateral cardiac nerve cord from the segmental nerve. At a stimulation intensity just above threshold and at a stimulation frequency of 1 Hz, this large nerve potential



Text-fig. 7. Effect of central nervous stimulation on the firing frequency of cardiac ganglion cells. The left anterior connective of the third abdominal ganglion was stimulated (a) at 2 Hz and (b) at 1.4 Hz at a slightly higher intensity. The activity of the ganglion cells before and after stimulation is illustrated in traces 1-3 and 7-9 respectively while the responses during stimulation are illustrated in traces 4-6. In this preparation the activity of the cardiac ganglion cells was influenced by excitation of neurosecretory fibres which pass down the segmental nerve and along the lateral cardiac nerve cord.

was sometimes followed, after a variable interval, by discharge of a cardiac ganglion cell. When the stimulus intensity was raised to about twice the threshold intensity the cardiac ganglion cell fired in response to every command arriving from the central nervous system along this pathway. Nevertheless the delay between the arrival of the central information and the appearance of the response of the cardiac ganglion cell was still quite variable (96 ± 23 ms) and it was not until the stimulus intensity was raised to three times the threshold intensity that the timing of the ganglion cell impulse became relatively consistent (77 ± 7 ms).



Text-fig. 8. Relationship between spontaneous activity of neurosecretory fibres in the segmental nerve (upper trace) and activity of cardiac ganglion cells in the lateral cardiac nerve cord (lower trace). Recordings from R_1 and R_2 respectively (cf. Text-fig. 1*b*).

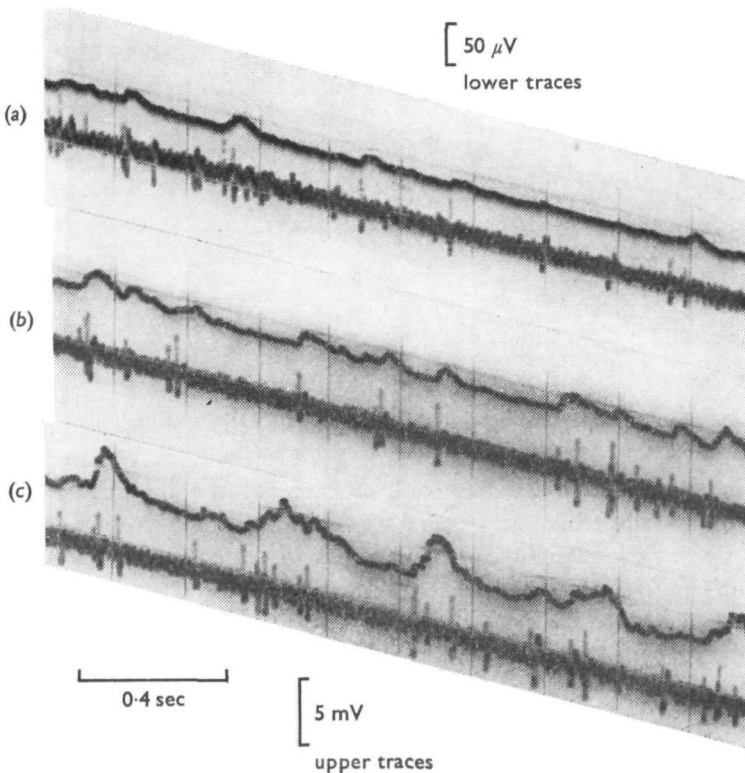
At these high stimulus intensities one or more of the intrinsic neurosecretory neurones in the lateral cardiac nerve cord were also activated. It seems possible from these results that as the intensity of the stimulus to the connective is raised more of the fibres which go to the lateral cardiac nerve cord are activated. As a result the input to the cardiac ganglion cell or cells is increased and a more consistent ganglion-cell response is established. If this is the case then it is perhaps somewhat surprising that the potential recorded from the fibres from the central nervous system did not increase in amplitude when the stimulus intensity was raised. Of course fibres of very different conduction velocity could have been recruited. Indeed, our data suggest that the cardiac ganglion cells are activated via neurosecretory fibres as well as via axons of ordinary neurones.

At stimulation frequencies less than 5 Hz firing of the cardiac ganglion cells followed stimulation of the central connective in a one-to-one fashion. Sometimes the ganglion cells initially responded repetitively to a single command from the central nervous system (Text-fig. 7). However, with repeated stimulation of the central connective the 'excitability' of the ganglion cell fell and a single one-to-one relationship between stimulus and response was established. At stimulation frequencies greater than 5 Hz the ganglion cells failed to respond every time the connective was stimulated. Apparently the breakdown in communication seen at the high stimulation frequencies occurred at the ganglion cell level rather than centrally. The portion of lateral cardiac nerve cord lying alongside the fourth abdominal chamber of the heart contains more than one cardiac ganglion cell. These cells are normally spontaneously active and can

be controlled by commands from the central nervous system (Text-fig. 8). Following a brief period of stimulation of a central connective the level of this spontaneous activity was transiently increased and then fell to below normal (cf. Text-fig. 3).

Intracellular recordings from myocardial cells

When the wall of the heart was penetrated from the outside by an intracellular microelectrode a potential difference, between recording electrode and bath reference electrode, of up to +30 mV was recorded, before the appearance of the 'negative-inside' potential difference usually obtained from excitable cells. The latter ranged



Text-fig. 9. Upper traces: changes in electrical activity of a muscle fibre of the fourth abdominal chamber of the heart during recovery from dissection 'shock'. (a) Recording made soon after completion of dissection; (b) recording made 10 min later; (c) recording made 12 min later. Lower traces: extracellular records of activity of the lateral cardiac nerve cord at R_1 obtained simultaneously with intracellular records from heart muscle fibre. Note increase in activity of lateral cardiac nerve cord and muscle fibre with time.

from -30 to -50 mV and was the only potential difference recorded during penetration of the heart muscle fibres from the lumen side of 'open-heart' preparations. The positive potentials recorded initially during entry from the outside of the heart were probably due to the presence of connective tissue and pericardial cells on the outer wall of this structure.

The majority of the hearts studied recovered from the dissection within 30 min. Other hearts recovered more slowly than this and only developed rhythmic contrac-

tions after an hour or more in cockroach saline. A few hearts remained inactive for several hours. In completely inactive hearts the resting potentials recorded from the muscle fibres were usually about -50 mV. Even in the absence of contractions, depolarizing synaptic potentials were clearly present (Text-fig. 9a). As the heart slowly recovered, the synaptic responses increased in magnitude and the time course of the depolarizations became quite complex, presumably due to activation of electrically excitable components in the membrane of the muscle fibres (Text-fig. 9b, c). The membrane potential of the muscle fibres then began to oscillate spontaneously as contractions returned. In the fully recovered preparation a number of different electrically excited responses occur which, together with the depolarizing synaptic potentials, produce a complex pattern of potential changes.

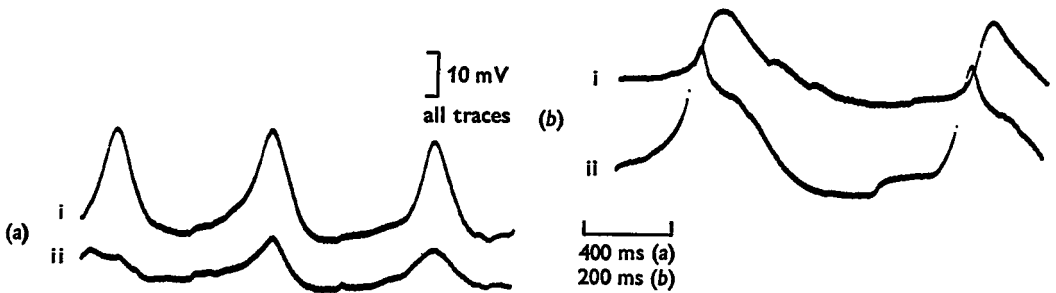
Synaptic potentials recorded from muscle fibres of the fourth abdominal chamber of the heart were associated with impulses travelling down to the heart from the central nervous system along neurosecretory fibres in the segmental nerve, and with activity of cardiac ganglion cells in the lateral cardiac nerve cord. So far only depolarizing postsynaptic potentials have been recorded from the heart muscle fibres of the cockroach. Possibly some of these are depolarizing inhibitory postsynaptic potentials.

In Pl. 1 a depolarizing postsynaptic potential accompanied by a graded electrically excited response ending in a phase of hyperpolarization followed each stimulus. A second synaptic potential was attenuated when it closely followed this complex response. This was not entirely due to synaptic fatigue since the time course of the second synaptic potential was markedly shortened, which suggests that the conductance of the muscle membrane was high at this time. In a normally contracting preparation the synaptic input from the fibres of the lateral cardiac nerve cord is ineffective in initiating depolarizing electrically excited responses in the muscle when it occurs during the early pre-potential phase of the intrinsic muscle response (Miller, 1968). Only synaptic potentials occurring during the late pre-potential, or immediately before the rapid depolarization phase are effective in initiating the depolarizing electrogenesis which triggers contraction (e.g. Text-fig. 12).

For analysis of local cardiac motor units the fourth abdominal heart chamber was prepared as described above except that all nervous connexions with the central nervous system were severed. The left lateral cardiac nerve cord was cut just posterior to the segmental vessel in the third chamber and opposite to the ostial valves in the fifth chamber. Spontaneous cardiac nervous activity was recorded anteriorly at suction electrode R_1 (Pl. 2, top traces) and synaptic potentials were recorded from the lumen side of the myocardium in the fourth chamber (Pl. 2, bottom traces). Usually motor impulses of three different sizes were recorded from the portion of lateral cardiac cord lying on the left side of the fourth chamber. In the example illustrated in Pl. 2, two impulses originated distally and travelled proximally, while a third impulse originated from the lateral cardiac cord at a point inside the suction electrode and travelled distally. Simultaneous intracellular records from the myocardium showed that each of these three impulses was accompanied by a postsynaptic potential. In this case the heart was quiescent; that is, spontaneous contractions were absent.

A continuous record of 99 consecutive synaptic potentials, including those in Pl. 2, were used to plot the frequency distribution of the potential amplitudes (Text-fig. 10). Since the synaptic events were the result of spontaneous cardiac neurone activity,

electrode was inserted into a fibre of the fourth abdominal chamber just beneath the right segmental blood vessel. A second microelectrode was positioned just anterior to the right segmental vessel near the midventral line in chamber five about 2.3 mm from the microelectrode in chamber four. The left lateral cardiac nerve cord adjacent to the middle abdominal chambers of the heart was removed. When the heart had recovered and was beating spontaneously, very similar electrical patterns were recorded from chambers four and five (Text-fig. 11 *a*). Similar results were obtained by recording from two fibres in the same heart chamber (Text-fig. 12). However, when all nervous connexions between the fourth and fifth chambers were eliminated by severing the right and left lateral cardiac nerve cords near the ostial valves of the fifth abdominal chamber (i.e. almost exactly midway between the two microelectrodes) the intrinsic

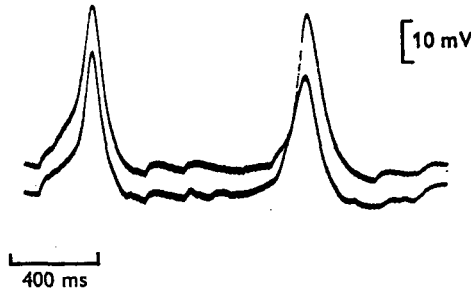


Text-fig. 11. Intracellular recordings obtained simultaneously from abdominal chamber four (i) and abdominal chamber five (ii). (*a*) Nervous connexion between the two chambers was intact. Note synchronous synaptic and electrically excited responses. (*b*) Nervous connexion between the two chambers was severed. As a result the electrically excited responses and, more significantly, the synaptic responses are no longer so well synchronized.

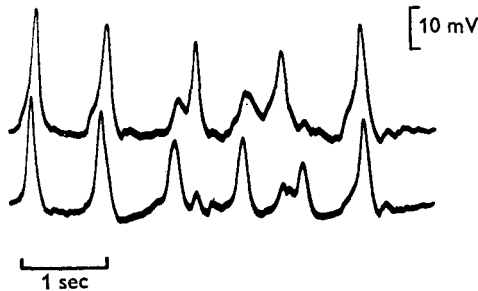
electrical activity of the two fibres, although similar in detail, was now out of phase, the onset of the depolarizing electrically excited response of the fibre in the fourth chamber being slightly ahead of that of the fibre in the fifth chamber (Text-fig. 11 *b*). At the same time marked differences in the synaptic events recorded at the two recording sites were now evident. One interpretation of such observations is that the activity of the cardiac nervous system ensures that each chamber of the heart receives, at all times, a similar synaptic input from local motoneurones. This implies that groups of neurones innervate local areas of the dorsal vessel, but that they are responsive to the activity of distal neurones in the lateral cardiac nerve cord. As a result the intrinsic electrical responses of all heart chambers are indirectly synchronized.

The depolarizing electrically excited responses of the muscle fibres of the cockroach heart are graded potentials and accordingly propagate in a decremental fashion. Since tight junctions between the fibres probably exist, it follows that by means of electrical coupling limited transmission of information between adjacent fibres occurs. Electric coupling between the muscle fibres of the heart is clearly illustrated in Text-fig. 13. Electrodes were placed proximally and distally in the fourth abdominal chamber about 1.5 mm apart. In this particular preparation the heart was recovering from dissection and the heartbeat was highly uncoordinated. The anterior and posterior parts of the fourth abdominal chamber were contracting independently at 61 beats/min and 76 beats/min respectively. Depolarizations recorded from the muscle fibre in the

proximal part of the fourth chamber spread to the fibre in the distal part and clearly influenced the activity of this fibre. At the same time the membrane events in the proximal fibre were influenced by events in the distal chamber. As a result a complex pattern of responses was recorded at the two sites. At this time the synaptic events recorded at the two sites were also markedly different but, when the heart had fully recovered, these responses, and the electrically excited responses, were more or less identical for both fibres.



Text-fig. 12. Intracellular recordings obtained simultaneously from the anterior (*a*) and posterior (*b*) regions of the fourth abdominal heart chamber. Note simultaneous synaptic and electrically excited responses.



Text-fig. 13. Intracellular recordings obtained simultaneously from the anterior (*a*) and posterior (*b*) regions of the fourth abdominal heart chamber during recovery from dissection 'shock'. In this preparation the two parts of the chamber were beating at different rates. More complex electrically excited responses of the two muscle fibres from which the records were obtained. At times these responses occurred simultaneously in both fibres but at other times they were markedly non-synchronous.

DISCUSSION

Until recently it has been generally assumed that the cockroach heart is neurogenic. Undoubtedly this conclusion derives to some extent from the presence of an elaborate cardiac nervous system in this insect (Alexandrowicz, 1926; McIndoo, 1945) and from the pharmacological properties of the cockroach heart (Krijgsman & Krijgsman, 1951; Miller & Metcalf, 1968*a*). According to Senff (1967) intrinsic pacemaker potentials do not occur in the cockroach myocardium and he concluded that the contractions of this organ are controlled by the cardiac nervous system which supplies the heart with rhythmic bursts of information. However, Smith (1969) found that tetrodotoxin

eliminates all cardiac nervous activity but does not abolish the rhythmic contractions of the cockroach heart and concluded that the cardiac nervous system is not the heart pacemaker. Miller (1969*b*) has also questioned the notion of a nervous pacemaker and has demonstrated that the denervated heart is still capable of coordinated rhythmic activity. According to McCann (1970) evidence for myogenicity based on the results of denervation studies is unsatisfactory because of the impossibility of removing all the fine nerve endings of the cardiac nervous system. However, it is unlikely that nerve endings as such could generate patterned activity, and even if this were the case it is very unlikely that such activity would result in coordinated contractions of the heart. The arguments for myogenicity in the cockroach heart therefore seem quite compelling although McCann (1970) believes the distinctions made between myogenic and neurogenic hearts are somewhat artificial. Nevertheless, if it is assumed that spontaneity is a property of cockroach heart muscle then we are confronted with the problem of the presence in this insect of a very complex cardiac nervous function.

In the cockroach a heart chamber in the middle abdomen is influenced, in part, by six cardiac ganglion cells which lie in the lateral cardiac nerve cords adjacent to each chamber, i.e. three cells on either side. There is undoubtedly considerable interaction between the ganglion cells in any one chamber and between ganglion cells of adjacent chambers, although local nervous pathways coupling the right and left lateral cardiac nerve cords do not exist. However, to a great extent it seems reasonable to visualize the cockroach heart as a series of interconnected repeating units (Alexandrowicz, 1926), each unit comprising a chamber with its lateral cardiac nerve cords and associated segmental nerves. The cardiac ganglion cells send processes in both directions along the lateral cardiac nerve cord and also send axons to the myocardium where synaptic contacts with the heart muscle fibres are made. There is anatomical evidence that the muscle fibres are multiterminally innervated although this is somewhat difficult to demonstrate electrophysiologically in view of the electrical coupling between the fibres.

The nervous input from the cardiac ganglion cells during normal heart activity occurs during diastole and consists of bursts of postsynaptic potentials (Miller, 1968). These depolarizing (excitatory?) postsynaptic potentials are clearly associated with bursts of action potentials in the lateral cardiac nerve cord which result from the activity of cardiac ganglion cells. This nervous input to the heart appears to exert a loose control over the timing of the spontaneous electrically excited responses of the heart muscle fibres with different intrinsic rhythms. Synchrony between chambers could also be achieved through nervous coordination of the activity of the ganglion cells. Rhythmic bursts of synaptic potentials have been recorded from heart muscle fibres of *Limulus* (Parnas, Abbott & Lang, 1969) but in this animal the heart muscle is electrically inexcitable and is not spontaneously active. Thus, the cardiac nervous system of *Limulus* controls not only the timing of the muscle contractions but also their duration and magnitude.

In the cockroach although individual ganglion cells can be spontaneously active, the formation of rhythmic bursts of action potentials recorded from the cardiac ganglion cells of the lateral cardiac nerve cord probably does not occur spontaneously. It is more likely that ganglion-cell activity is coordinated in response to mechanical deformation of tissues in the vicinity of the lateral cardiac nerve cord (Miller, 1968).

In a peristaltic heart the responsiveness of the ganglion cells to stretch could be useful in generating and regulating the peristaltic wave of activity of the heart. In hearts where the chambers of the myocardium contract simultaneously, the output from the cardiac ganglion cells could be used to trigger the next contraction and to eliminate any timing errors between chambers or parts of a chamber. One drawback to these suggestions is the fact that when the cockroach heart is denervated the contractions are still perfectly coordinated. Indeed the pattern of rhythmic contractions is much more regular in denervated preparations since in the absence of a neural input the spontaneous electrically excited responses of the heart muscle fibres are much more evenly spaced (Miller, 1969*b*).

On the basis of available evidence it seems not unreasonable to assume that one task of the axons from the central nervous system which innervate the myocardium and communicate with the cardiac nervous system is to regulate the heartbeat rate. According to Johnson (1966) neurosecretory fibres which contain large electron-dense granules are found in the segmental and lateral cardiac nerves, but these fibres do not synapse with either the myocardium or the intrinsic cardiac neurones. Probably they release their contents into the environment of the lateral cardiac nerve cord at sites located along the length of this cord. They may also be associated with the pericardial cells which line the outer surface of the myocardium (Johnson, 1966). We have found that the other type of neurosecretory fibre, i.e. those fibres containing small granules, synapse with the heart muscle and each fibre usually innervates more than one chamber of the heart. As well as influencing the electrical and mechanical activity of the heart by releasing a chemical transmitter at the nerve-muscle synapses, these neurones also probably release their neurosecretory contents into the haemocoel (Johnson, 1966). Perhaps it is by this means that they influence the activity of the cardiac ganglion cells. Alternatively, they may synapse with the cardiac ganglion cells as suggested by our electrophysiological data. The ordinary axons found in the segmental nerves and lateral cardiac nerve cords also apparently synapse with the cardiac ganglion cells but they do not innervate the muscle fibres of the heart. Since each chamber of the heart is innervated by a pair of segmental nerves there is seemingly no problem of assuring synchronous inputs to both sides of all the heart chambers, if this is necessary. This could be achieved centrally by linking the neurones which go to the heart with a common interneurone. There is some evidence, however, that the central nervous system contains common (excitatory?) neurones which directly innervate more than one heart chamber, presumably by sending branches down a number of segmental nerves. This could explain why stimulation of a single segmental nerve can affect the entire heart.

In many respects the innervation of the cockroach heart resembles that of cockroach visceral muscles (Brown & Nagai, 1969). In common with longitudinal muscle of the rectum of *Periplaneta*, the cockroach heart is innervated by axons containing neurosecretory granules. Similar axons innervate the spermathecae of *Periplaneta* (Gupta & Smith, 1969). These axons either synapse with the effector or form release sites in its vicinity. The nature of the transmitter released at the synaptic sites is unknown. However, it has been shown that the heart (Miller & Metcalf, 1968*a*) and the rectum (Freeman, 1966) of *Periplaneta* are sensitive to the same range of aromatic amines, including 5-hydroxytryptamine, dihydroxyphenylamine, adrenaline and nor-adren-

aline. In each case these molecules have an amine moiety two carbon atoms away from the aromatic grouping.

It appears from this and previous studies that the control of rhythmic contractions of the cockroach heart is organized on three levels. First, the muscle itself has the capacity for spontaneously generating rhythmic electrical and therefore mechanical activity. Probably each muscle fibre normally undergoes endogenous rhythmic activity and coordination between fibres is partly achieved through the electrical coupling between the fibres. The rhythmic input from the cardiac ganglion cells is superimposed on the myogenic rhythm whilst neurones from the central nervous system influence both levels of activity. The need for such a complex arrangement is difficult to understand. Possibly the cardiac nervous system in the cockroach controls a multitude of physiological processes, many of which have yet to be clarified.

SUMMARY

1. The heart of *Periplaneta americana* is segmentally innervated from the central nervous system by three types of neurone. Two of these types of neurones are neurosecretory; one type contains large granules, the other small granules. The segmental nerves are paired structures which join paired lateral cardiac nerve cords. Both types of neurosecretory neurone liberate their contents in the lateral cardiac nerve cords. The neurones with the small granules also synapse with the myocardium as well as with intrinsic cardiac neurones in the lateral cardiac nerve cords. The third type of neurone from the central nervous system is an ordinary efferent neurone and it synapses with the cardiac ganglion cells.

2. A heart chamber is associated with about six cardiac ganglion cells, three on either side. These send processes up and down the lateral cardiac nerve cord and make synaptic contact with the myocardium.

3. The myocardium is multiterminally and polyneuronally innervated, and electrical coupling between muscle fibres appears to be the rule. The fibres are spontaneously active and generate spike-like electrically excited responses. The timing of the electrically excited responses is influenced by the input from the cardiac ganglion cells which evoke a burst of synaptic potentials during diastole.

4. Control of the cockroach heart appears to be organized on three levels. The basic rhythm is myogenic. The timing of the contractions is influenced by inputs from the intrinsic cardiac ganglion cells possibly via a feedback mechanism involving the contractions of the heart muscle. Finally, the activities of the heart muscle and the cardiac ganglion cells are influenced by inputs from the central nervous system.

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EXPLANATION OF PLATES

PLATE 1

Complex intracellular recordings (upper traces) from a fibre of the fourth abdominal chamber of the heart during repetitive stimulation of the left anterior connective of the third abdominal ganglion. Extracellular recordings from the anterior region of the lateral cardiac nerve cord adjacent to the fourth abdominal chamber of the heart (i.e. at R_1) are illustrated in the bottom traces. (a) Single stimulus; (b) stimulation at 5 Hz; (c) stimulation at 10 Hz; (d) stimulation at 17 Hz; (e) stimulation at 20 Hz; (f) stimulation at 40 Hz.

PLATE 2

Simultaneous extracellular recordings from R_1 (upper trace) and intracellular recordings from a fibre of the fourth abdominal chamber of the heart (lower trace) showing relationship between firing of cardiac ganglion cells and appearance of synaptic potentials in the cardiac muscle fibres.

