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# ON THE POINT OF ORIGIN OF HEART BEAT IN THE SCORPION, HETEROMETRUS FULVIPES C. KOCH

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### INTRODUCTION

IN scorpions the heart beat is known to be neurogenic as evidenced by the presence of a cardiac ganglion and its spontaneous rhythmic burst activity related to the contractions of the heart muscle in 1:1 ratio<sup>1,2</sup>. Since the heart of the scorpion is tubular and the cardiac ganglion is on the mid-dorsal line of the heart, it is of interest to find whether the generation of the spontaneous impulses from the cardiac ganglion is simultaneous throughout the ganglion or spread from one region to the other in a regular sequence.

Previous work on scorpions showed that the heart beat is maximum in the posterior bits of the heart compared to those in the anterior bits<sup>3,4</sup>. Similarly alterations in the activity of the cardiac ganglion in the anterior and posterior parts have also been reported<sup>3</sup>. The present study is aimed at finding of such gradient activities in the heart beat and the cardiac ganglion in the scorpion are related.

#### MATERIAL AND METHODS

Hearts were isolated from the scorpions belonging to Heterometrus fulvipes C. Koch and maintained in the ringer medium<sup>5</sup>. Spontaneous electrical activity of the cardiac ganglion was studied by recording the electrical activity at different regions. The differences in characteristics like burst frequency, burst amplitude, burst duration and interburst intervals in the spontaneous electrical activity of the cardiac ganglion recorded at various levels were determined by analysing the recorded film.

Acetylcholinesterase (AChE) activity was determined in the two halves of the heart muscle based on the method of Metcalf<sup>6</sup>. Succinic dehydrogenase (SDH) activity was estimated by the application of the method described by Nachlas et al.<sup>7</sup>. Protein content of the heart muscle was estimated by the method of Lowry et al.<sup>8</sup> to represent the enzyme activities per milligram protein.

#### RESULTS AND DISCUSSION

Earlier observations by the author<sup>9</sup> on the heart beat of the scorpion, *H. fulvipes*, indicated that there is a regular sequence in the cardiac cycle. The peristaltic wave commenced at the posterior part of the heart, and it gradually spread to the anterior end of the heart. The extent of contraction and relaxation of the heart decreased in the postero-anterior direction of the heart.

Recordings of the spontaneous electrical activity at different regions of the cardiac ganglion showed the presence of a postero-anterior gradient as in Fig. 1. The activity was maximum at the 7th ostial region of the cardiac ganglion and it gradually decreased anteriorly. As such, the activity was mostly in the form of regular bursts with few units firing in the interburst period at the posterior part of the ganglion. Such a typical burst of activity disappeared gradually towards the anterior end, especially at the 1st ostial region of the ganglion.

The characteristics of the burst also showed variation at different regions of the cardiac ganglion (Fig. 2). Burst frequency did not show any typical gradient and it was more or less fluctuating between 60-68 bursts/min (Fig. 2a). The burst amplitude, however, showed a typical trend in that it decreased in the postero-anterior direction. It had a maximum burst amplitude value of 580  $\mu$  Volts at the 7th ostial region whereas the burst

amplitude at the 1st ostial region was only  $150 \mu$  Volts (Fig. 2h). Besides, the number of units firing in a burst also decreased postero-anticriorly.

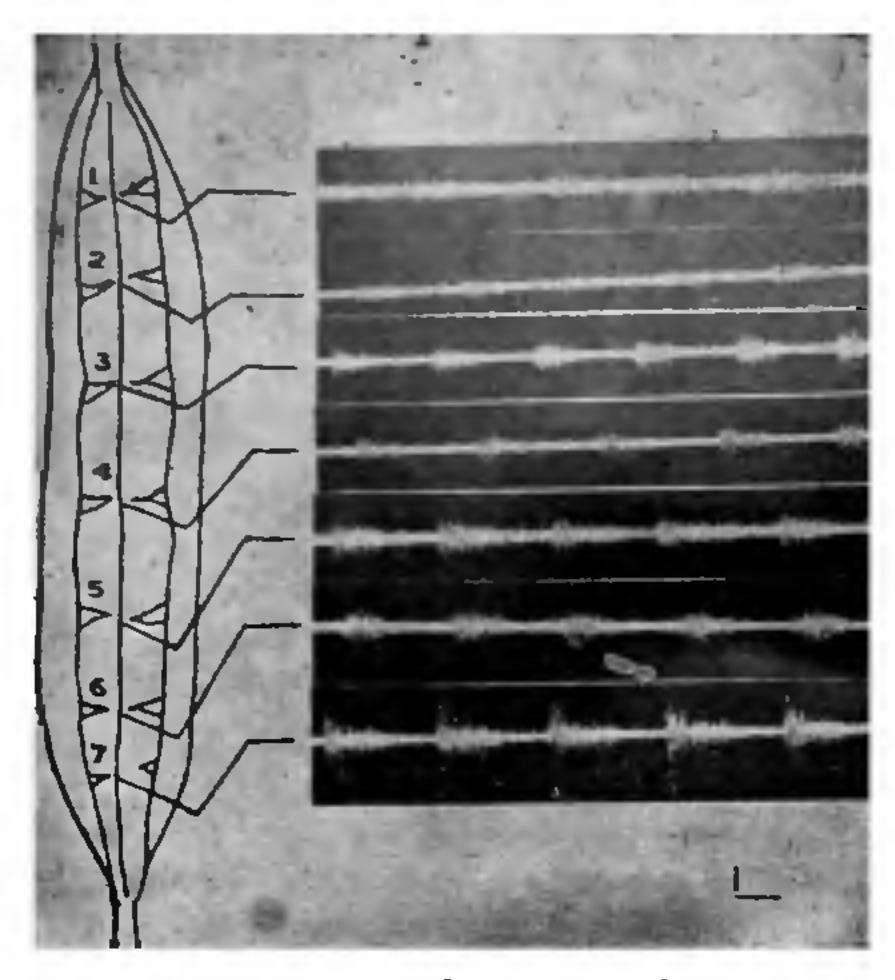


Fig. 1. Spontaneous electrical activity recorded from different regions (as indicated in the figure given on the left side) of the cardiac ganglion to show the postero-anterior gradient. Time: 0.5 sec; Calib:  $50 \mu v$ .

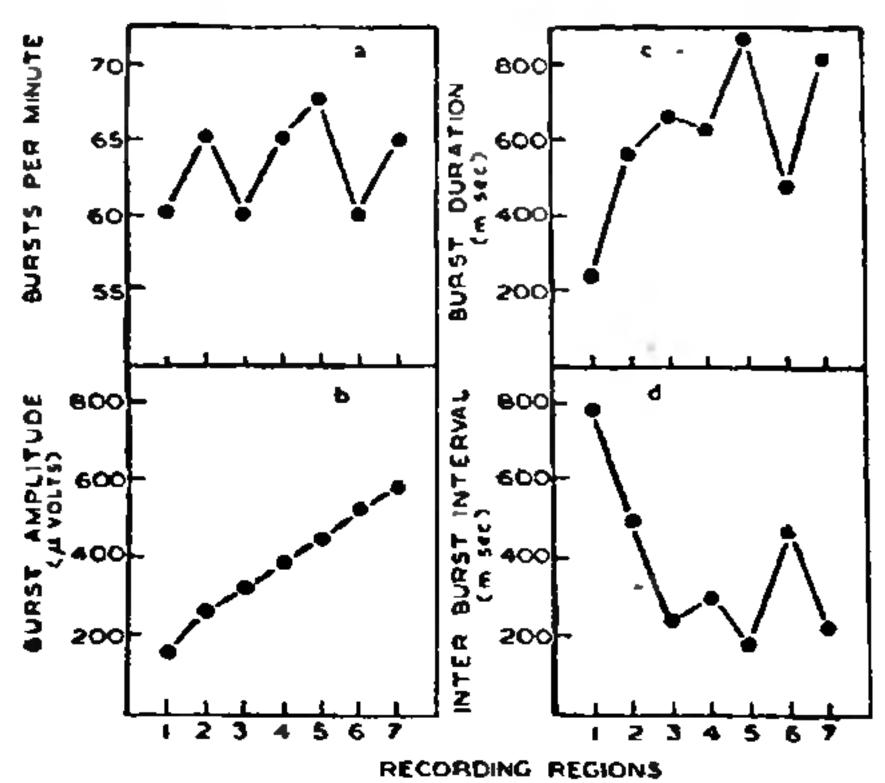


Fig. 2. Graph showing the differences in the various parameters of the spontaneous activity recorded at different regions of the cardiac ganglion.

The units firing in the interburst interval also decreased both in number and amplitude towards the anterior end of the cardiac ganglion. The burst duration was also fluctuating around a mean value of 670 m. sec. at all regions excepting at the 1st ostial region where the burst duration decreased to 270 m. sec. (Fig. 2 c). The interburst interval

was low at the posterior region of the cardiac ganglion. The average interburst interval was 285 m. sec. from the posterior region up to the 3rd ostial region. However, the interburst interval increased at the anterior region of the cardiac ganglion where it was 500 m. sec. and 800 m. sec. at the 2nd and 1st ostial regions respectively (Fig. 2 d).

AChE enzyme activity in the heart was maximum in the posterior half of the heart, and less so in the anterior half (Table I). The differences in the enzyme activities in the two halves of the heart were statistically checked and found to be significant.

TABLE I

Acetylcholinesterase and succinate dehydrogenase activities in the anterior and posterior halves of the heart values expressed are the average ± S.D. of

6 observations

	Acetylcholinesterase*		Succinate dehydro- genase†	
	Anterior half of the heart	Posterior half of the heart	half of	Posterior half of the heart
Enzyme activity 't' value	±8·65	214·273 ±10·35 ·6954		5.922 ±0.49
Inference	p<0-001 Activity more in posterior part		t=9.9633 P<0.001 Activity more in posterior part	

\* Acetylcholinesterase =  $\mu$  moles of Ach hydrolysed/mg protein/hr, † Succinate dehydrogenase =  $\mu$  moles Formazon/mg protein/hr.

It is of interest to note that electrical activity of the cardiac ganglion, enzyme activities of the heart muscle and the heart beat follow similar activity patterns. A typical postero-anterior gradient is seen. An inference is that the activity levels may be related to one another and perhaps interdependent.

It is suggested that the rhythmicity in various physiological processes of the scorpion might be due to rhythmicity in the central nervous system may be brought about by the agency of neurosecretory material of the cephalothoracic nerve mass11. The effect of neurosecretory material on the AChE activity of the central nervous system and the enzyme present in the central nervous system of the scorpion are said to be closely related to the electrical activity of the central nervous system<sup>12-13</sup>. As in the above the rhythmicity of the heart beat which has been shown to be influenced by the rhythmicity of the cardiac ganglion, may be influenced by a neurosecretory system.

It is known that AChE is found in the nervous tissues of invertebrates<sup>14</sup>. It is also known that

the levels of activity of the nervous system is related to the activity of the enzyme<sup>10</sup>. The enzyme activity is related to the amount of Ach synthesized and released. Since the electrical activity of the cardiac ganglion shows variations it is reasonable to expect changes in the AChE activity also. High level of activity necessitates the availability of energy in larger amounts which steps up the oxidative pathways. Besides, high levels of Ach, leading to increased AChE activity, it also stimulates the cellular respiration leading to release of higher amounts of energy<sup>15</sup>. Thus the variations in the heart beat at different regions seem to be due to the acetylcholine synthetic and energy yielding processes leading to variations in the electrical activity of the cardiac ganglion.

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is dedicated to the sweet memory of late Prof. K. Pampapathi Rao.

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## CHEMICAL COMPONENTS OF EUGENIA JAMBOLANA STEM BARK

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LUGENIA JAMBOLANA (Syn. Syzygium cumini) belongs to the family Myrtaceae and the common Indian name is 'Jamun'. The stem bark was examined by Sengupta and Das1 who isolated betulinic acid, friedelin, epi-friedelanol,  $\beta$ -sitosterol and eugenin, a fatty acid ester of epi-friedelanol. However the name eugenin for the ester was not appropriate since it had already been used long ago for 2-methyl-5-hydroxy-7-methoxy chromone which was isolated from Eugenia caryophyllata<sup>2,3</sup>. The bark is astringent and is used in sore throat, bronchitis, asthma and dysentery; therefore in order to study its phenolic constituents, the stem bark (400 gm) was cut into coarse powder and extracted with petroleum ether and alcohol. Identification of compounds was confirmed whenever possible by direct comcarison with authentic samples using mixed m.p. and I.R. spectra.

The petroleum ether extract yielded a brown oil (15 g) which was subjected to column chromatography over silica gel using petroleum ether-benzene as cluants and 4 compounds A, B, C, D were obtained. Compound A (500 mg), crystallised from chloroform-methanol as colourless needles, m.p. 309

311°,  $[a]_0 + 7^\circ$  (CHCl<sub>3</sub>) and was identified as betulinic acid. Compound B, after crystallisation from CHCl<sub>3</sub>-MeOH (350 mg) as needles had m.p. 255-56°  $[a]_D - 20^\circ$  (CHCl<sub>3</sub>). It gave positive Lieberimann-Burchard test. It was identified as friedelin. Compound C (50 mg) crystallised from CHCl<sub>3</sub>-MeOH as needles, m.p. 295-300°,  $[a]_D + 14^\circ$  (CHCl<sub>3</sub>). Its identity as friedelan-3 a-ol was confirmed by comparison with an authentic sample, and by the preparation of its acetate. Compound D, crystallised from methanol as colourless needles (70 mg), m.p. 135-36°,  $[a]_D - 30.6^\circ$  and it was identified as  $\beta$ -sitosterol.

Alcohol extract.—From the alcohol extract, was obtained ethyl acetate soluble and ethyl acetate insoluble fractions. The former was concentrated to a syrup which was a mixture of three compounds (TLC). They were separated by column chromatography over silica gel using ethylacetate and different proportions of ethyl alcohol as eluates to yield E, F and G. Compound E (200 mg), crystallised from aqueous ethanol and had m.p. 177–179°, It gave a brown colour with alcoholic ferric chloride and deep red colour with magnesium and