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EFFECT OF ZNIC ON PHYSIOLOGY OF HEART BEAT OF SILKWORM, *BOMBYX MORI. L*

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Abstract
Silkworm is an economic insect producing valuable silk. Generally the study of heart beat physiology provides information on supply of oxygen, food and removal of waste products. Hence the author aimed to study the rate of heart beat which is affected by environmental factors such as temperature, pH, heavy metals etc. zinc is known to be an essential element participating in various enzyme activity in an organism and there by affecting the heart also A number of other neuro hormones are also known to affect the heart beat in vitro, but they are not known to be involved in regulation of heart in the intact insect. In our study we exposed the silkworm at lethal, sub-lethal and sub-sub lethal doses of zinc. The results indicated that the rate of heart beat decreased significantly at the lethal dose. (12.26 mg/kg b.wt) and sub lethal dose. (2.45 mg/Kg b.w). However, the sub- sub lethal dose of Zinc enhanced slightly over control. It indicates that the metabolic activity of silkworm was increased at a very low level of Zinc ions. The decrease in the lethal and sub- sub lethal dose is due to zinc stimulation. On the whole it can be concluded that the zinc ions might have a role on cardio acceleratory peptides (CAPS).
Keywords- Silkworm, Heartbeat, Lethal, sub lethal dose.

I. INTRODUCTION

The circulatory system enable all parts of the body to receive the constant supply of oxygen, food etc and to have waste products removed promptly. The farm of heart beat varies greatly throughout the animal kingdom. In insects the heart is long dorsal tube divided into thirteen chambers, also with ostia. The frequency with which, the heart contracts varies considerably both within and between species. In general, the frequency of beating is higher in early than in later stage larvae. It also depends on the age within a stage of development. For example, in silkworm *(Bombyx)*, the rate drops from 80 beats per minutes in second stage larvae to about 50 in the fifth stage, and it drops sharply just before each moult except the last. In the pupa, the heartbeat falls to 10- 20 per minutes and remains at this low level until shortly before adult eclosion. A similar, but slightly less marked change occurs during the development of the migratory locust, Locusta, but the rate of beating tends to rise before a moult. In Lepidoptera, vary sharp increase in the heart rate occurs at eclosion, falling to a sustained but moderate rate when eclosion is complete.Activity usually stops above 45- 50^0 C and below 1- 5^0C. Within this range the rate is higher at higher temperatures. In Locusta, the rate of heartbeat is In addition to these intrinsic changes, the rate of heartbeat is affected by environmental also higher in the light than in the dark. It is common for the heart to stop beating, sometimes for a few seconds, but sometimes for 30 seconds or more. The heart of the young pupa of Anopheles sometimes stops beating altogether, and in old pupae no activity of the heart is observed.

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It is also common for the heartbeat to undergo periodic reversals, with waves of contraction starting at the front. When this occurs, blood is forced out of the 'incurrent' Ostia and at least in the more crickets, Gryllotalpa, powerful currents pass out of the sub terminal in current Ostia. In female Anopheles, 31% of heartbeats start at the front end of the heart. Reversal of heartbeat is rare in holometabolous larvae, but begin in the pupal stage or event at the larva- pupa ecdysis. Usually the rate of the heart beat is lower when the heart is pumping backwards. In the adult blowfly, Calliphora, the rates are about 175 beats per minute backwards compared with about 375 forwards.In pharate adult Lepidoptera, periods of fast forward beating lasting few minutes alternate with periods when the heart reverse and beats more slowly, but during the period of wing expansion following eclosion no reversals occur (Tublitz and Truman, 1985). Subsequently, periodic reversals are an essential feature of haemolymph circulation, a least in the Lepidoptera.The activity of the pulsatile organs may be different from that of the heart. The antennal ampullae of Periplaneta pulse at about 28 beats per minute, considerably slower than the heart rate. The wing pulsatile organs of Lepidoptera are only active during heart reversal when the mesothoracic organ and the heart pulsate at similar rates, although they are not necessarily in phase; the metathoracic organ pulsates more rapidly. At eclosion, the pulsatile organs pulsate more rapidly and without interruption. Dhondi et. al., (2012) reported that the toxic effect of roger resulted in the disturbance of regular working of heart beat of male crab, *Brytelphusa Guerini*.

The activity of the heart is basically myogenic although the myogenic pattern may be modulated neutrally or hormonally. As the segmental nerves leading to the heart contain the ramifying terminals of neurosecretory axons, it might be expected that their secretions exert modulatory effects (although it is possible that the heart also functions as a neurohemal organ).

Table: 1 Heart beat for minute in different instars of silkworm *Bombyx mori*

S. No	Age	No. of pulse/ minute
1	II	58
2	III	55
3	IV	51
4	V	45- 56

In addition, hormones released into the blood at points remote from the heart are known to affect it. For example, the increase in the rate of beating at the time of exclusion in Lepidoptera is at least partly due to peptides released in the haemolymph at this time.These cardioaceleratory peptides are produced in neurosecretory cells in the ganglia of the ventral nerve cord and released into the haeemolymph at the perivisceral neuro hemal organs. The same peptides also increase the heartbeat during flight. Their effects are synthesized by very low levels of octopamine which is also present in the haemolymph during wing inflation and flight (Prier, Beckman & Tulitz, 1994). A number of other neuro hormones are known to affect the heart rate in vitro, but they are not known to be involved in regulation of heartbeat in the intact insect.The direction of a beat, from back to front or vice versa, may be related to the distribution of blood pressures. If pressure at the front of heart is so high that back pressure is set up, the heartbeat is reversed. The direction of beat after transaction of heart adds support to this suggestion. The prevalence of a reversed beat in pupal stages possibly results from blockage of excurrent Ostia by histolysed tissues. In Anopheles, the direction of heartbeat is sometimes correlated with abdominal ventilation. If ventilation starts posteriorly, the heart beats forwards; if ventilation starts anteriorly, the heart beats from front to back. These changes might well be due to pressure.

Alternatively, or additionally, the direction of heartbeat might be related to the availability of oxygen. In the absence of a good oxygen supply, the rate of heartbeat is strongly reduced. The larva of

Bombyx has a better tracheal supply to the posterior end of the heart than to the anterior and reversals of the heartbeat do not normally occur. If, however, the posterior spiracles are occluded, so that the oxygen supply to the pupa, the tracheal system of the whole heart is poor and the rate of beating is low with reversals, while in the adult, the tracheal, supply is well- developed both anteriorly and posteriorly and the heartbeat is rapid, again with reversals.

II. MATERIALS AND METHODS

Estimation of the Heart Beat - Isolated Silkworm Heart Technique:

The silkworm Bombyx mori CSR2 X CSR4 obtained from the RSRS, Raptadu, Anantapur. Silkworms were feed four times (6.00, 16.00 and 22 hrs)on fresh mulberry (Morus Var. Victory 1) leaves except during moulting period. After IV moult, the larvae of same size and age were collected from the rearing tray and divided into batches and each batch consisting of 50 larvae.They were maintained at $25.5+1^{0}$C, fresh leaves of Morus alba (VI) collected from the garden and sprayed with zinc solutions of various concentrations prepared for the experimentation separately and dried under fan a room temperature. A batch of silkworm, were fed without zinc treated leaves as control. It is on established fact that at 120 hours (5^{th} day) after resumption from the IV moult the silkworm of V instar is very active. Hence, one the 6^{th} day i.e., at 144 after IV moult for V instar were fed with zinc treated leaves at different intervals of 6.00, 10.00, 16.00 and 22.00 hours. After words the V instar physiological and cocoon commercial characters were studied.

Krijgsman, et al. (1950) described the isolated technique of heart, the heart beat can be noted by dissecting the worm. The worm to be dissected was first made inactive by immersing in physiological solution for few minutes. Then the worms were fixed on a wax plate with its dorsal side down. This plate was placed in a shallow Petri- dish containing physiological solution. An incision was made at the posterior end of the insect. This was continued up to the thorax of the insect. The heart was exposed carefully by removed the digestive tract and fat tissue Then the heart was allowed to recover from shock and with resumption of regular heart rate in about 15- 20 min. the frequency of heart beat was taken. The values are expressed in beats/min. The rate of heart beat was calculated on 5^{th} day of V instar of silkworm, Bombyx mori.The physiological solution used by Naidu (1955) has been employed in our present investigation. The composition of the solution was NaCl 9.62 g K.Cl 0.7g, CaCl 120.5g, NaHCO3 0.18g, NaH2 0.01g/liter dextrose/g 2 drops of 0.1 NH4Cl per one liter of distilled water and room temperature were maintained. The data obtained for each parameter were analyzed for their significance according to the method or Duncan’s multiple Range test (Duncan, 1955). The significance was calculated at 5% level ($p<0.05$).

III. RESULTS AND DISCUSSION

The heart beat per minute in different instars of silkworm. *Bombyx mori* was given in table 1. The results in silkworm were showed in table2, treated with lethal dose of zinc (12.26mg/kg body wt), a decrease in the rate of heart beat was observed. The difference for the frequencies of heart beat between the control and zinc treated silkworm was focused to be significant ($P>0.01$). The frequency of heart beat in sub lethal dose (2.45 mg/kg body wt) was also decreased significantly. Whereas silkworm treated with sub- sub lethal dose (1.22 mg/kg body wt) exhibited significant increase in the rate of heart beat.The results indicated that the rate of heart beat decreased significantly at the lethal dose (12. 26 mg/kg body wt.) and sub lethal dose (2.45 mg/b.wt) also exhibited increased rate of heart beat. However, sub- sub lethal dose is due to zinc stress. Whereas the increase of heart beat in sub- sub lethal dose is due to zinc stimulation of metabolic activities.

The decreased rate of heart beat in the lethal and sub lethal dose of zinc can be attributed to the inhibition of the neurogenic heart of silkworm and sub- sub lethal dose might have stimulation effect on the neurogenic heart of silkworm. There must be neuro secretions that regulate the heart beat of the silkworm. Further, the inhibition or enhancement of heart beat is zinc is dose dependent.It is very well known that than one two neuro petides in lepidopteron insects, they are called cardio acceleratory peptides (CAPS), they are released from abdominal ganglia and these peptides stimulate the heart to increase in beat frequency. So, the zinc ions might have a role on cardio acceleratory peptides (CAPS) in silkworm. There are a number of other compounds that have shown to stimulate the heart including other neuro peptides and biogenic amines the important biogenic amine is octopamine because of the nature of the open circulatory system in silkworm in order for some of these other hormones to act they need to be circulated so if as a secondary role stimulate the heart, they will be circulated more quickly. Krijgsman, 1952 reported that choline esterase influences greatly on the heart beat of periplunata, cockroach, the grass hopper, melanoplus, the Honey bees and cricket, stenopermatus (Hamilton, 1939; Davenport, 1941).

IV. CONCLUSION

On the whole it is concluded that lethal and sub-lethal doses exhibited toxic manifestations in silkworm and these changes are attributed to the stimulation of neurogenic heart. The inhibition or enhancement of heart beat is dose dependent. The zinc ion levels might have shown effect on cardio-vasculatory peptides in silkworms. Further sub-sub lethal doses of zinc could contribute to optimum level of heart beats which in turn activates the metabolic status of silkworm for proper growth and development of silkworm.

Table. 2: The rate of heart rate in Vth instar of CSR2 X CSR4 race of silkworm *Bombyx mori* on exposure to Zinc

S. No	Dose	No. of pulse/minute
1	Control	46 c
2	Lethal	35 a (-23)
3	Sub- lethal	38 b (-17.3)
4	Sub- Sub lethal	52 d (+13.0)

* Each value is a mean of eight estimations.
** Percentage decrease (-/+) relative to control is given in parenthesis
*** Means within a column followed by the same letter are not significantly different (P>0.05) from each other according to Ducan's Multiple Range Test.

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