STUDIES ON THE NEUROENDOCRINE CONTROL OF EGG HATCHING AND METAMORPHOSIS IN *Philosamia ricini* (LEPIDOPTERA: SATURNIDAE)

ABSTRACT

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Based on the two families of neuroendocrine hormones, the steroid molting hormones, ecdysones and the sesquiterpene juvenile hormones, we present certain observations in the control of egg hatching and metamorphosis in the Eri silkworm, *Philosamia ricini*. The role of adenosine-3', 5'-monophosphate (cAMP) as a second messenger was also investigated.

In the present study, the obliterate effect of JH-III application on the eggs of *Philosamia ricini* was observed. Younger eggs tend to be more vulnerable to JH treatment but the percentage of hatching was also drastically reduced in older eggs as well. JH treatment to eggs also had a long term effect; most larvae that manage to hatch were not able to develop normally and may die at any stage during their postembryonic life.

Contrary to eggs treated with Juvenile hormone III (JH-III), 20-hydroxyecdysone (20-HE) treated eggs enhances hatching considerably. Although, certain stages of young eggs are susceptible to 20-HE treatment, the older treated eggs tend to response favorably in term of hatchability. Those larvae that hatched as a result of 20-HE treatment developed normally in normal time and the hormone does not seem to affect its postembryonic life as observed in JH treatment. Thus 20-HE may be regarded as a possible promoter of hatching in insects in addition to its role as a molting hormone.

In our observation, cAMP more or less mimicked the action exhibited by 20-HE, since the unhatched eggs produced as well as the pattern of effectiveness throughout the stages by both the treatments are almost similar.

The egg of *Philosamia ricini* is oval or laterally flattened ellipsoid. The freshly laid eggs are candid white in colour while the chorion is colorless and semi-transparent. Four distinct regions, the posterior and anterior poles, lateral flat sides and ventral (dorsal) edges, are easily distinguishable. The surface structure of the chorion is covered with a network pattern of fairly uniform polygons, mostly hexagonal but occasionally pentagonal or heptagonal units. The polygonal shape was common to the whole surface region. There is variation in the unit area of the polygon according to their location on the eggs.

The boundaries between polygons are made of ridges which had distinct aeropyles. The aeropyle number corresponds to the polygonal structure so that there are six aeropyles in a hexagonal structure, five in pentagonal, seven in heptagonal and so on. The variability in the size of aeropyles during egg development may be correlated with the metabolic rate of the developing embryo and the modification due to environmental conditions.

The study in term of hatching of the larvae from the eggs revealed that they gnawed their way out through the chorion membrane in accord with the process adopted by larvae of all lepidopteran insects. For the process of hatching there is no rupture of egg membrane. Line of weaknesses on egg surfaces were not observed nor the presence of egg buster or specialize spine or cuticle. The overall structure of the egg during the course of development until hatching was not observe to change except for the minute pores or aeropyles present on the ridges of the entire polygonal network.

In the present study, ligation during feeding stage (day 3) does not bring about pupal transformation other than the prodromal signs of pupation *viz.* gut purge, wandering, spinning, color change and lost of mobility in anal prologs, during their period of survival. The neck ligated larvae, showed normal developing pupae, while in the abdominal ligated larvae, the anterior part of the body bear a typical pupal character and the lower posterior region still retained the larval character of the last day 5^{th} instar. These larvae continue their developmental processes by undergoing spinning even when the ligated thread is still intact. The pupal cuticle bearing regions are interpreted as having received enough molting hormone, ecdysone while the posterior region in the later that still bear the larval cuticle are devoid of the molting hormone due to ligation. However, complete pupal cuticle formation occurred only when larvae were ligated long after gut purge (day 3 after gut purge). Thus, first critical secretion of prothoracicotropic hormone (PTTH) & ecdysone can be assumed to occur at day 3, 5:00 hr (during feeding) onwards and this is necessary for causing prodromal signs of pupation and for transition of feeding to post-feeding stage. While the second secretions that caused the molt from larva to pupal form was probably released three days after gut purge from 4:00 hr onwards.

Further, the phenomenon underlying gut purge and pupal ecdysis seemed to be under the control of circadian clock since, larvae that undergo gut purge on day-5 and day-6 pupated exclusively on day-9 and day-10 respectively, forming a time interval of exactly 4 days in between the events.

In the present study, mosiac characters in ligated larvae were also observed. The posterior part of the abdomen had perfectly formed and tanned pupal cuticle. Failure of the anterior part to pupate (anterior inhibition) can be attributed to respiratory deficiencies by injury of the tracheal system during ligation or the involvement of the posterior darkening factor (PDF) or still another factor, the anterior retracting factor (ARF).

In the present study, emergence of adult or eclosion from pupal case under natural condition occurred during the photophase period of the last day of adult development: beginning at 15:00 hours and continuing till 18:00 hours or later. Consistently, eclosion starts quite late in the cool afternoon period when light was comparatively reduced. The timing of eclosion was observed to occur after 9-11 hours exposure to light. It was also observed that, adult eclosion in *Philosamia ricini* was restricted within a time frame or gating during precise period of the day; if the pupa completes development while the gate is open, ecdysis can occur during the same day. By contrast, if development is completed after the gate (after 15:00hr) has shut; the insect must wait until the opening of the gate on the next day so that ecdysis can occur within the time frame of 15 hr-18hr.

In the present study, considering the time of eclosion commencing at 15:00 hours on the last day of adult development, the release of eclosion hormone (EH) in *Philosamia ricini* in all probability may occur at about 1 hr prior to adult emergence *i.e.* at 14:00 hours. This was substantiated by the fact that EH extracts prepared at about this time (1 hour before normal eclosion) triggers extensibility in isolated wings of pharate adult that had not been exposed to EH activity.

The curves of oxygen consumption during both the larval cycles (4^{th} and 5^{th} instars) showed an inverted U-shaped, where the descending part of the curve during moulting is regarded as a curve representing protein synthesis. JH treated larvae recorded lesser amount of O_2 consumed compared to 20-HE and cAMP treated larvae. Higher O_2 consumption in larvae treated with 20-HE and cAMP may be correlated to the specific action of these compounds directly or indirectly to the epidermal cells which may induce molting and thereby higher metabolic activity. Whereas, the JH-III treated larvae may not influence molting and they may only help to maintain larval character so metabolic activity is possibly reduced and hence lesser amount of O_2 consumption. However, JH-III treated larvae almost always showed highest O_2 consumption amongst the three treatments at the ultimate days of both the instars. But by this time development of the 20-HE or cAMP treated larvae had become more advanced with low metabolic activity and are at rest and on the verge of molting, therefore O_2 consumption is low. On the other hand, the

JH-III treated larvae at the same stage are quite active and probably high levels of metabolic activity or differentiation are in progress and hence higher demand of O_2.

In the present study, the pattern of O_2 consumption in cAMP treated larvae are more or less similar to 20-HE treatment and it may be possible that cAMP directly act *via* PTTH to induce indigenous 20-HE secretion and activation which may in turn stimulate cellular activity and thereby resulted in higher O_2 consumption.

Morphology and structural changes of integuments of 4th & 5th instars are quite similar throughout development. In the present study, the late larval integument with its structural maturity can be correlated to be induced by PTTH and ecdysteriod secretions, since, such hormones are known to be at their maximal levels prior to molting. At about this time probably the molting gel is secreted and the epidermal cells undergo a period of mitosis and cell division. The epidermal cell population becomes denser, the cells are more columnar and prominent, and their apical surface is thrown into a series of fine folds. It was also observed that intercellular spaces are more prominent than in younger stages and the epicuticle and endocuticle are distinguishable probably they are ready to be shed during the preceding molt. In contrast, low level of integumentary structures up to day-3 in both the instars in term of cuticular deposition revealed that they have not been subjected to ecdysteriod action and hence cellular activity was also low. At about this time, the cuticle looks smooth and pliable while the epidermal cells and intercellular spaces are also not prominent. The epicuticle and exodocuticle are not clearly distinct from each other making them difficult to identify.