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# STUDIES ON BIOCHEMICAL CHANGES DURING LARVA TO PUPA TRANSFORMATION IN ANTHEREAEMYLITTADRURY (DABA TV)

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**ABSTRACT**

*The present study has been carried out to analyze the biochemical variations inAnthereaemylittadrury (Daba TV) during larval to pupal transformation. In this study, various biochemical assays have been performed inhaemolymph and fat body of silkworms during 1st to 6th day of 5th instar larvae, spinning day 1, spinning day 2 and pre pupal stages. The results revealed that during larval to pupaltransformationa drastic variation in proteins, aminoacids, haemocytes and acid phosphatase activity has been identified. An increase in protein content of both haemolymph and fat body was noticed upto 6th day of fifth instar larvae but a gradual reduction in protein content was observed during first day of spinning, second day of spinning and also in prepupal stage. A reduction in amino acid levels of both haemolymph and fat body was recorded till 6th day of fifth instar larvae whereas an increase is noticed in spinning day 1, 2 and prepupa. Present results also shows an increase in haemocyte count till 6th day of fifth instar and a later decrease in spinning day 1, 6 and pupalstages.Significant increase in the activity of Acid phosphatase was observed in the fifth instar from 1st to 6th days and also during spinning day 1, spinning day 2 and prepual stages.*

***Keywords: Haemolymph, Fatbody, Proteins, Aminoacids, Haemocytes, Acid phosphatase, Transformation.***

## I. INTRODUCTION

Anthereaemylitta Drury a lepidopteran insect of the Saturniidae family produces tasarsilk(Vanya silk) of commercial importance[1]. The insect is having 44 ecoraces distributed along central India with varied phenotypic, physiological and behavioral characters. It is a polyphagousinsect and feeds on Terminaliatomentosa , Terminaliaarjuna etc.,

In insects, the growth and development is associated with protein metabolism [2]. In insects various biochemical changes occurs in the total concentration of haemolymphaminoacids and proteins during metamorphosis[3]. In silkworms, the protein synthesis activity of the body wall and the midgut decreased when the larvae began to moult and increased from the midstage of the moulting period [4]. Recently, some work has been carried out on the haemocytes and protein changes in tasar silkworm[5]. The growth of silkworm is correlated with the synthesis of carbohydrates, proteins, amino acids and various enzymes[6]. During fifth instar the larvae spin a cocoon for protection. After this larva change into the pupa, finally adult moth emerges from cocoon. Various proteins are synthesized in the fat bodies and haemolymhof silkworm which are required to participate in physiological process.During insect metamorphosis, profound biochemical changes occur in the haemolymph,

particularly in the levels of proteins[7]. It is evident that profound changes in protein metabolism take place at various periods during insect development. Transformation from larva to pupa at the time of metamorphosis is accompanied by characteristic variations in the patterns of aminoacids, peptides, and proteins [8]. Physiological and biochemical studies on insect development have provided a reasonable picture of the causal relationships of protein synthesis, growth, and differentiation [9].

## II. MATERIALS AND METHODS

The present work was performed by collecting Daba T.V cocoons as per the standard norms such as weight, colour, size of cocoons and length of the peduncle from the forest patches of Jakaram, Warangal District, during the third crop. The cocoons were preserved in the wire mesh cages of size 2 ft x2 ft x2 ft under temperature of 29±1 °C and humidity 70 % ± 1 %. The emerged moths were tested for pebrine disease [10]. Disease free layings were collected and incubated at 25- 30°C temperature and 70- 75% humidity and reared on Terminaliaarjuna plantation till cocooning.

First day to sixth day 5th instar larvae, spinning day 1(SP1), spinning day 2(SP2) and pre pupa(PP) were used for experiment. Haemolymph was collected in the test tubes by cutting the proleg.1mg of Thiourea was added to the test tubes to prevent melanisation. After centrifugation at 15000 rpm for 15min the supernatant was collected and stored at -20°C for further analysis. The fat body was isolated in cold condition by using Bodenstein's Ringer solution. 20% (W/V) homogenates of fat body was prepared in 50mM Tris -HCl Buffer (pH- 7.0) and centrifuged at 10,000 rpm for 20 min. Supernatant was collected for quantitative estimation of biochemical components. Total proteins and Aminoacids were estimated according to the standard procedures [11,12]. Enzyme likeAcid phosphatase was estimated according to [13].

For haemocytes estimation the haemolymph was drawn into Thoma white blood pipette upto 0.5 mark and diluted upto 11 mark with tauberyeager fluid. The pipette was shaken for 5 minutes and first three drops were discarded. 0.5 ml of the haemocyte suspension was mixed with 0.5 ml of PBS and 0.5 ml of trypan blue. 0.2ml of this suspension is placed on hemocytometer and number of viable cells were counted under the microscope. The viable cell does not permit the entry of dye this distinguishes dead cells from living. Percentage of viable cells was calculated as

% of viable cells: =Number of viable cells / Number of viable cells + Number of dead cells X100

## III. STATISTICAL ANALYSIS

Each assay was replicated 3 times. Values were expressed as mean ± SE.

## IV. RESULTS AND DISCUSSION

Table 1 shows protein concentration in haemolymph and fat body of fifth instar larvae and also prepupa. Total protein concentration varied in haemolymph and fatbody during day 1 to day 6 of 5th instar larvae and spinning day 1,day 2 and prepupal stage. Total protein concentration of both haemolymph and fat body increased up to day 6$^{th}$ dayof fifth instar larvae from first day but a gradual reduction in protein content is observed during first day, second day of spinning and also in prepupal stage. During metamorphosis the protein concentration decreases gradually in haemolymph [14].Different types of storage proteins are seen in silkworm which

involved in metamorphosis of silkworm.The total volume of protein increases exponentially with age in haemolymph of honey bees [15]. High transaminase activity results in high protein content [16]

Table 2 shows aminoacid concentration in haemolymph and fat body of fifth instar larvae and also prepupa. Variation in aminoacidconcenteration observed in haemolymph and fatbody during day 1st to day 6th day of 5th instar larvae and spinning day 1, day 2 and prepupal stage. Total aminoacid concentration of both haemolymph and fat body found decreased up to day 6th day of fifth instar larvae from first day but a gradual increase in aminoacid content was observed during first day ,second day of spinning and also in prepupal stage. Decrease in amino acids correlates with the increase in proteins. Decrease in the free amino acid content may indicate the possibility of active feeding of amino acid in Kreb's cycle and glycolytic pathway to meet the emergent energy needs as well as their utilization in the production of some new proteins synthesized to cope with the low temperature stress [17,18].The decrease in amino acids in the fat body and silk gland could be their conversion to pyruvate by enzymes [19].

Acid phosphatase used as a marker enzyme for lysosomes and for apoptosis, involved in the degradation of insect tissues and thus involved in metamorphosis or transformation [20].Present results shows that acid phosphatases vary in the fat body of the silkworm during larval to pupaltransformation.The activity of Acid phosphatase increased gradually till sixth day of fifth instar larvae followed byspinning day 1 and spinning day 2 and pre pupal stage (Fig 1).

Haemocytes are circulating cells and found in the haemolymph of insects responsible for the defense mechanism against foreign body [21].Haemocytes perform various physiological functions in the insect body, changes in total haemocyte counts of particular insect directly or indirectly affect the insect [22]. The haemolymph is store house of various kinds of proteins and enzymes [23]. These proteins functions as the main reservoirs for the supply of amino acids during larval moults and metamorphosis [24].Results also show that haemocyte count varies from larval to pupal transformation. The total viable haemocytes count increased from first day to sixth day of fifth instar larvae whereas it got decreased in first day spinning, second day spinning and in prepupal stage (Fig.2).

## V. CONCLUSION

Thus in conclusion protein levels, viable haemocytes are more in larvae but less during spinning and pupal stages. The amino acid levels are more in larvae and less in pupa whereas the activity of acid phosphatase is higher in pupa than in larva.

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**Table1 Amino acidle vels in haemo lymph and Fat body during transformation from fifth in star larva to pupa of *Anthereae my littadrury (Daba T.V)***

| Source | 1st Day | 2nd day | 3rd day | 4th day | 5th day | 6th day | SP 1 | SP2 | PP |
|---|---|---|---|---|---|---|---|---|---|
| Haemolymph (mg/100ml) | 998.6 ±3.5 | 989.4± 2.5 | 982.8± 3.6 | 975.6±4. 2 | 962.8± 3.8 | 942.4±3. 4 | 972.8±4. 6 | 985.6±2. 8 | 992.6±4. 5 |
| Fat body (µg/100mg) | 2.78± 0.18 | 2.63± 0.15 | 2.54±0. 14 | 2.49±0.1 6 | 2.45±0.1 2 | 2.38±0.1 8 | 2.58±0.1 5 | 2.64±0.1 8 | 2.76± 0.12 |

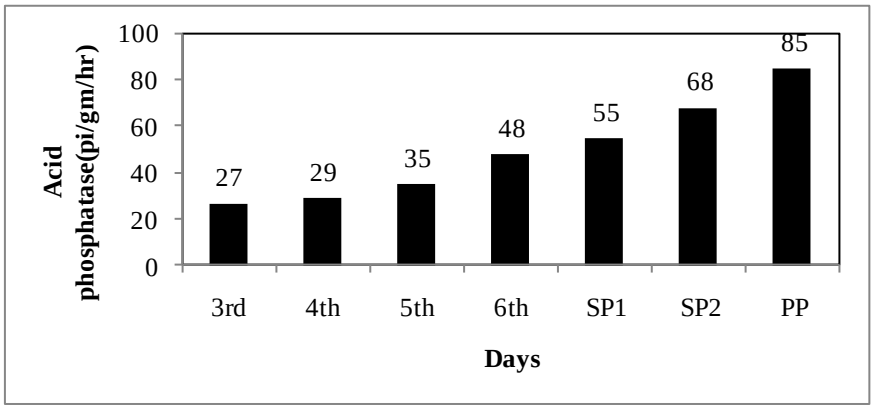
SP1-Spinning day 1, SP2-Spinning day 2, PP-Prepupa

**Table 2 Protein levels in thehaemolymphand fat body during transformation from fifth instar larva to pupa of *Anthereaemylittadrury (Daba T.V)***

| Source | 1st day | 2nd day | 3rd day | 4th day | 5th day | 6th day | SP 1 | SP2 | PP |
|---|---|---|---|---|---|---|---|---|---|
| Haemolymph (mg/100ml) | 18.5±0 .5 | 19.6±0. 6 | 25.6±1. 4 | 27.8±0. 5 | 32.5±0.6 | 35.8±0.8 | 16.6±0.4 | 12.4±0. 8 | 10.6±0. 5 |
| Fat body (mg/g) | 78.5±1 .25 | 82.2±1. 15 | 86.2±2. 08 | 98.8±1. 12 | 108.8±1. 08 | 112.6±2. 14 | 48.4±1.1 5 | 41.8±1. 16 | 38.6±2. 06 |

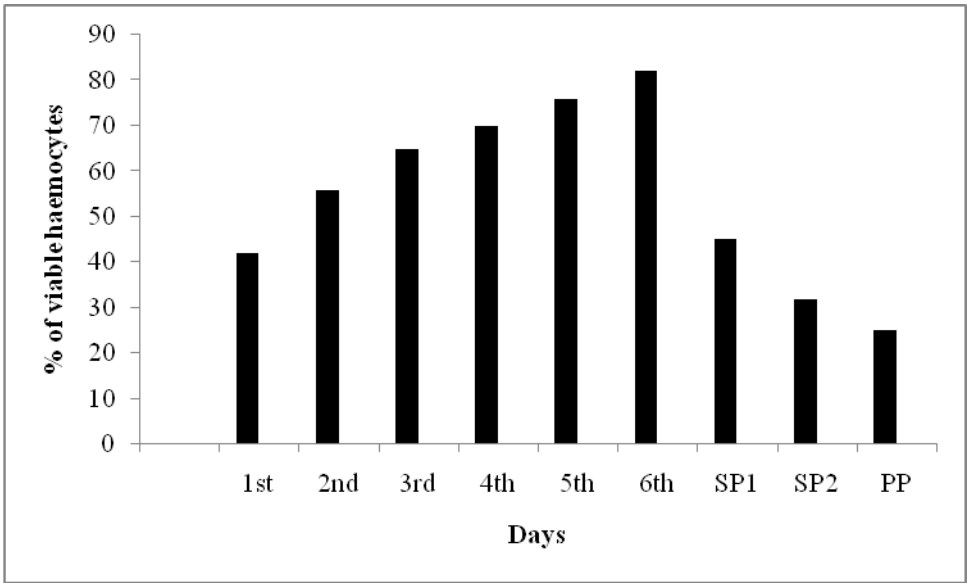
SP1-Spinning day 1, SP2-Spinning day 2, PP-Prepupa

**Fig .1 Acid phosphatase activity in fat body during larva to pupa transformation**




SP1-Spinning day 1, SP2-Spinning day 2, PP-Prepupa

**Fig.2 Total viable haemocyte count during transformation of fifth instar larva to pupa in *Anther eae my litta drury (Daba TV)***




SP1-Spinning day 1, SP2-Spinning day 2, PP-Prepupa