

Study of Proteins during Larval Development of *Mythimna Separata* (Walker)

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Abstract---Insect pest is a major task for the crop economy. *Mythimna separata* is an important and most destructive pest in India which also widely distributed throughout the Asia, Australia and Pacific islands. The process of embryogenesis results in the formation of larva. The total growing period of larva is 22 to 27 days passing through six successful larval instars. In insects as in other animals one major biochemical event resulting from cellular activation is proteins synthesis. Proteins provide the chief structural elements for the muscles glands and other tissues during larval development. The gradual increase in the amount of protein was observed upto seventeen days during early larval development and after words proteins were found increased. These results are discussed on the basis of protein metabolism and development of larva.

Keywords---Larval development, *Mythimna separate*, Protein metabolism,

I. INTRODUCTION

THE transformation of stored yolk components into active protoplasm results in the inner growth of egg. The growth during insect development is restricted to the larval development. The mass necessary for the final adult will be deposited during the feeding period of larva. In most organisms growth occurs through cell multiplication. However, in the insect larva, the general principle of growth by cell multiplication is modified. In endopterygote groups the growth of many organs may be attained through an enormous enlargement of the single cells. Often, but not obligatorily, this growth by increase in the dimensions of the cells concerns the specific larval organs which undergo breakdown at metamorphosis.[1]

The rigid integument cannot easily accommodate itself to the increasing size of a growing insect and must therefore be shed and renewed periodically. At each moult there is cast off not only the general cuticle that invests the body and its appendages externally, but also the various endoskeletal structures and the intima or lining of most of the tracheal system, fore and hind gut, ectodermal glands and different reproductive ducts. All these, together with hairs, scales and cuticular sensilla, are renewed by the underlying epidermal cells [9]. The moulting is controlled by ecdysteroid and follow a similar course in all insects [10].

A number of substances, particularly amino acids and vitamins, are essential for any development to take place; others while not essential, are necessary for optimal development. The balance between different constituents is

also important [2]. Dipteran larvae are known to accumulate lipids, glycogen and proteins during development [8]-[13]. The reason for storing these constituents in larva is fairly obvious, this material later on can be used during metamorphosis.

The haemolymph proteins and lipoproteins in Lepidoptera were studied by [14] - [4] studied the amino acid and protein changes in the haemolymph of developing fourth instar, *Chironomus tentanus*. As per Reference [12] the hormonal regulation of phase polymorphism and storage protein fluctuation in the common cutworm, *Spodoptera litura*. As per Reference [5] it is studied that the larval growth, food consumption and utilization of dietary protein in *Galleria mellonella*. and the Reference [11] shows changes in protein content in the haemolymph of healthy and pebrine infected larvae and pupae of *Antheraea myllita*.

II. MATERIAL AND METHODS

1. MATERIAL:

i. Selection of Material:

The *Mythimna separata* is an economically important pest causing damage to crops like sorghum, rice, wheat, sugarcane etc. This pest is also known to satisfy all requirements like moderate availability, easy breeding and maintainance in the laboratory. The proteins and proteases enzymes during stage of embryogenesis can be easily studied.

A. Larval Stages for Study :

The embryogenesis period of *M. separata* is of 5 days. The newly hatched larva was tiny, cylindrical and active. The larval skin was soft and larva was pale brown coloured with black head. The length of the larva ranged from 0.9 mm to 1.3 mm with an average of 1.27 mm. The larval instars were studied by transferring freshly hatched larvae into the specimen jars. The cut pieces of the fresh and tender maize leaves were provided as food. The food was changed after every 24 hours. Number of days covered by each larval instar were recorded.

The larval developmental period is of 22 to 27 days. The larvae moult five times thus had six larval instars. The larval developmental stages from 1-day to 25-day larvae (L1 – L25) were taken for the study of Proteins.

2. METHODS:

i. Estimation of Proteins

The preparation of larval homogenate and estimation of proteins by the method of [7].

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III. OBSERVATIONS

TABLE NO I
CHANGES IN PROTEINS DURING LARVAL GROWTH

Period of larval Growth (days)	Amount of Proteins (mg/gm of body wt.)
1	18
2	21
3	26
4	28
5	29
6	36
7	38
8	40
9	41
10	42
11	47
12	49
13	53
14	56
15	58
16	63
17	64
18	61
19	60
20	58
21	59
22	60
23	61
24	60
25	58

IV. RESULTS AND DISCUSSION

The total development of larvae is marked by six instars as developmental stages. From first to sixth instar each stage lasts

for 4, 5, 5, 3, 3 and 5 days respectively. The moulting in larvae is observed after 4, 9, 14, 17, 20 and 25 days of development.

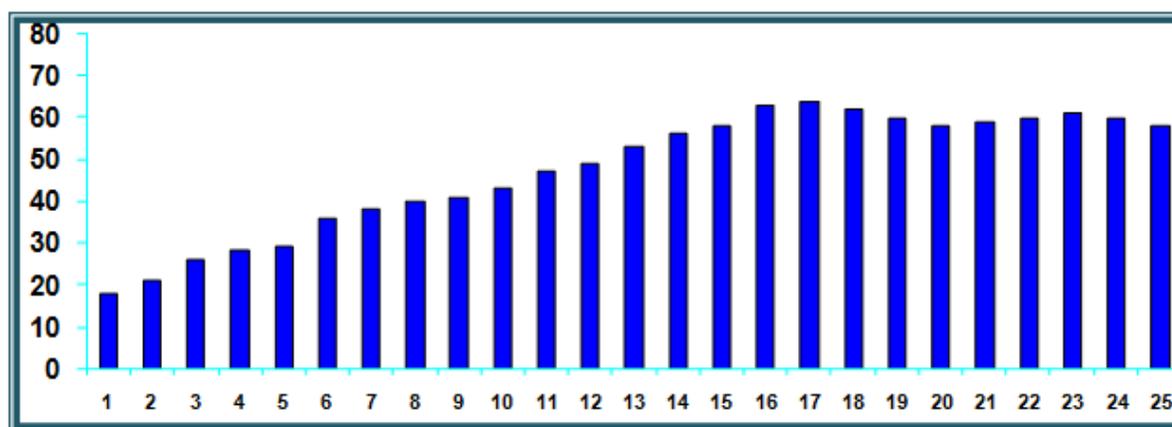
Changes in proteins during larval growth are represented in Table No. 1 and illustrated graphically in Fig. No. 1 Gradual increase in the amount of proteins from 1 to 17- day larvae was observed. After 17- day it remained relatively constant upto 25-day larvae.

According to Reference [13] the proteins provide the chief structural elements of the muscles, glands and other tissues. Various reports are available on showing that the total content of haemolymph proteins increases during larval development and decline rapidly with the advance of larval life with a concomitant appearance of protein granules in the fat body cells.

According to Reference [3] in some insects the increase is most rapid during the time approaching pupation. Reference [15] reported that the blood protein of *Bombyx* rises from 1.2 per cent in early third instar to 5.3 per cent in the late fifth instar. Apparently the same is true for *Samia cynthia* whose protein concentration, is according to Reference [6] increases rapidly from the third instar to a maximum in the spinning fifth larval instar.

In present work proteins are found to be increased in the early development of larva upto seventeen days and after words they remain constant upto 25th days. Our results are in good agreement with the findings of [15] - [6]- [3] and [13]. Increased amount of proteins upto 17 days of development suggests the synthesis of proteins required for the development of larva after words amount of proteins remained constant up to 25 days which indicates that there is less utilization of proteins for structural elements required for the further development of larva.

Amount of pr Mg. Proteins (Mg/gm of body wt.)



Period of Larval growth (days)
Changes in protein during larval growth of *Mythimna separata*

Fig. 1

REFERENCES

- [1] Agrell, I.P.S. & Lundquist, A.M. (1973). Physiological and biochemical changes during insect development. In : "*Physiology of Insecta*." (Rockstein, M. ed.). Academic Press, New York and London. **1**, pp.159-248.
<http://dx.doi.org/10.1016/B978-0-12-591601-1.50011-9>
- [2] Chapman, R.F. (1969). In : "*The Insects*". (ed.). The English Universities Press Ltd., London. pp. 70-106, 403-422, 675-691.
- [3] Chen, P. S. (1966). Amino acid and protein metabolism in insect development. In : "*Advances in Insect Physiology*". (Ed.by Beament, J.W.L., Treherne, J.E. & Wigglesworth, V.B.) Academic Press London and New York. **3**, pp. 53-132.
- [4] Firling, C.E. (1977). Amino acid and protein changes in the haemolymph of developing fourth instar *Chironomus tentans* *J. Insect Physiol.*, **23**, 17-2.
[http://dx.doi.org/10.1016/0022-1910\(77\)90103-2](http://dx.doi.org/10.1016/0022-1910(77)90103-2)
- [5] Jindra, M. & Sehnal, F. (1989). Larval growth, food consumption and utilization of dietary protein and energy in *Galleria mellonella*. *J. Insect Physiol.*, **35**, 35, 719.
- [6] Laufer, H. (1960). Blood proteins in insect development, *Ann. N.Y. Acad. Sci.*, **89**: 490-515.
<http://dx.doi.org/10.1111/j.1749-6632.1960.tb27574.x>
- [7] Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Rondal, R.J. (1951). Protein measurement with the Folin phenol Reagent. *J. Biol. Chem.*, 193-263.
- [8] Pearincott, J.V. (1960). Changes in lipid content during growth and metamorphosis of the housefly, *Musca domestica* Linnaeus. *J. Cellular Comp. Physiol.*, **55** : 169-174.
<http://dx.doi.org/10.1002/jcp.1030550208>
- [9] Richards, O.W. & Davies, R.G. (1977). In : "*Imms' General Textbook of Entomology*" (ed.). Chapman and Hall, London. **1**. pp. 234-262, 323-394.
- [10] Sehnal, F. (1985). Growth and life cycle. In : "*Comprehensive Insect Physiology, Biochemistry and Pharmacology*". (Kerkut, G.A. and Gilbert, L.I. ed.). Pergamon Press, New York. **2**, pp. 1-86.
- [11] Sinha U., Sinha A. & Shina, S. (1991). Changes in concentration of proteins carbohydrates in the developing healthy and pebrine infected embryos of tropical tasar silkworm, *Antheraea mylittad*. *Indian J. Sericulture*, **30**,(2), 155-156.
- [12] Tojo, S., Morio, M., Agui, N. & Hiruma, K. (1985). Hormonal regulation of phase polymorphism and storage protein fluctuation in the common cutworm, *Spodoptera litura*. *J. Insect physiol.*, **31**, 283.
[http://dx.doi.org/10.1016/0022-1910\(85\)90004-6](http://dx.doi.org/10.1016/0022-1910(85)90004-6)
- [13] Wigglesworth, V.B. (1972). "*The Principles of Insect Physiology*". (ed.). Chapman and Hall, London. pp. 593-662.
- [14] Whitmore, E. & Gibert, L.T. (1974). Haemolymph proteins and lipoproteins in Lepidoptera. A comparative electrophoretic study. *Comp. Biochem. Physiol.*, **47B**, 63-78.
- [15] Wyatt, G.R., Loughheed, I.C. & Wyatt, S.S. (1956). The chemistry of insect haemolymph. Organic components of the haemolymph of the silkworm, *Bombax mori* and two other species *J. gen. Physiol.*, **39** : 853-868.
<http://dx.doi.org/10.1085/jgp.39.6.853>