International Journal on Recent and Innovation Trends in Computing and Communication ISSN: 2321-8169
Volume: 3 Issue: 7 4834 - 4837

# Effect of Cobalt Chloride on the Amino Acid Levels in Silk Gland of Silkworm, *Bombyx mori* L

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***Abstract:-*** The effect of cobalt on the total proteins, protease activity, free amino acids, alanine amino transferase (ALAT) activity and aspartate amino transferase (AAT) activity were studied. The increase of total protein content in silk gland may be either due to increased efflux or decreased proteolysis activity which might lead to accumulation of protein content. The decrease of protease activity level in silk gland may be due to lower rate of histolysis. The free amino acids showed a decrease in the silk gland of cobalt chloride treated larvae which indicates the faster mobilization of free amino acids into oxidative metabolism in the presence of cobalt chloride. The ALAT and AAT activity levels were elevated after cobalt chloride treatment indicating the active involvement in the protein synthesis.

***Key words:*** *Bombyx mori L., total proteins, protease activity, free amino acids, ALAT and AAT.*

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## I. INTRODUCTION

All other animals, growth and development in insects are also very closely associated with protein metabolism (Mansigh and Baquaya, 1971). Proteins are the most important and characteristic constituents of living matter. Proteins must be constantly supplied to the organisms for growth and they are maintained at constant levels because of the dynamic state of constant turn over (Dunlap *et al*., 1978 and Hershku and Ciechanover, 1982). Minor losses of proteins may result in several neurological disorders. Thus proteins play a unique role in determining the pattern of chemical transformations in biological systems. Studies were made to analyse haemolymph proteins in insects such as *Philosamia ricini* (Poonia, 1978), *Bombyx mori* L. (Venkata Reddy, 1984 and Seong *et al*., 1985) and *Calliphora* (Anderson, 1984). Siva Prasad (1987) reported the total and soluble proteins during metamorphosis of the silkworm. The protein levels in the fifth instar larvae of silkworm in different environmental conditions were reported (Maheswaramma, 1994).

Proteases are the most commonly found digestive enzymes in insects (Ann Sorensen *et al*., 1983). Several factors responsible for the secretion of the proteolytic enzymes have been investigated (Briegel and Lea 1975). A strong protease activity has been demonstrated in the digestive fluid of the silkworm *Bombyx mori* L. (Eguchi and Iwamoto, 1976 and Sasaki and Sazuki, 1982). Protease activity was reported in the fifth instar larvae of *Bombyx mori* L. (Maheswaramma,, 1994).

Amino acids play an important role in the osmotic homeostasis of blood (Beadle and Shaw, 1950). Insects, in addition to sugars and lipids, use amino acids as the readily available source of respiratory fuels (Bursell, 1963). The occurrence of free amino acids in high concentration in insect haemolymph was first observed (Nazari, 1902). Free amino acids in insect haemolymph was in general much higher than in vertebrate blood (Duchateau and Florkin, 1958 and Auclair, 1959). Florkin (1949) observed that a very high titre of free amino acids in the haemolymph is characteristic of winged insects. The amino acids in the silk gland of *Bombyx mori* L. were determined (Chitra and Sridhara, 1972). Effect of dietary amino acids in the haemolymph of the larvae was studied (Inokuchi, 1970).

The aminotransferases (ALAT and AAT) mediate the transfer of amino groups of the amino acids to α-oxoglutarate, oxaloacetate and pyruvate to form glutamate, aspartate and alanine respectively (Lehninger, 1978). Amino transferasses have been detected in the tissues and eggs of silkworm (Seshachelam *et al*., 1985). The activity levels of aspartate and alanine amino transferases showed an increase in silk gland, while they showed decreased levels in the central nervous system, muscle and haemolymph (Sivaprasad and Murali Mohan, 1990).

## II. MATERIAL AND METHODS

The indigenous polyvoltine silkworm, *Bombyx mori* L. of the race, Pure Mysore was used in the present investigation. Disease free layings of the silkworm were

obtained from Pl Station, Punganur, Andhra Pradesh, India. The silkworms were reared in the laboratory at 24-28 degree centigrade and with 12L: 12D photo period and 70-85% humidity. For feeding of treated larvae, the fresh mulberry leaves were dipped at least for one hour in cobalt chloride (COCl2); Ranbaxy, India) solution having a concentration of 500µg/ml/10 g of leaf and fed to silkworm at 10 A.M. on first day of each instar and fifth instar daily. The control group of larvae was fed with mulberry leaves soaked in physiological saline. The matured fifth instar larvae, prior to the day of spinning were collected and dissected in ice-cold insect ringer and the silk gland of the larvae was isolated and used for the present experiment.

The total proteins (Lowry *et al*., 1951), protease activity (Davis and Smith, 1955), free amino acid content was estimated by the method of (Moore and Stein, 1954) as described by (Colowick and Kaplan, 1957). The activity of aspartate (AAT) and alanine amino transferases (ALAT) was determined by the method of (Reitman and Fraenkel 1957) as described by (Bergmeyer, 1965) were assayed in the silk gland of silkworm larva.

## III. RESULTS

The data presented in the table reveal the changes in the total proteins, protease activity, free amino acids, ALAT and AAT in the silk gland after treatment with cobalt chloride.

**Total proteins**

Total proteins showed significant increase (P<0.001) after cobalt chloride treatment. The increase in protein content in silk gland was 35.33% over control.

**Protease activity**

Significant decrease was noticed in protease activity (P<0.001). The decrease in protease activity in silk gland was 16.07 % over control.

**Free amino acids**

Significant decrease was noticed in the amino acid content (P<0.001) after cobalt chloride treatment. The decrease in free amino acid content in silk gland was 26.39% over control.

**Alanine amino transferase (ALAT) activity**

Significant increase was noticed in ALAT activity (P<0.001). The increase in ALAT activity in silk gland was 35.65% over control.

**Aspartate amino transferase (AAT) activity**

The AAT activity level showed significant increase (P<0.001) in the cobalt chloride treated larvae. The increase in AAT activity in silk gland was 24.17% over control.

## IV. DISCUSSION

In the present investigation, treatment of cobalt chloride resulted in the increase of total protein content in silk gland may be due to either increased efflux or decreased proteolysis which might lead to accumulated protein content. The protein profiles of the cell are indicative of the physiological status of the animal (Harper, 1985) and there exists a dynamic equilibrium between the synthetic and degradative pathways associated with these molecules. Cobalt chloride possibly stimulate protein synthesis in fat body leading to an increase in its protein content and consequently more protein is released into the haemolymph from this organ. Thus the present result of enhancement of proteins in silk gland may be supported by the mobilization of more proteins from the haemolymph and fat bodies of cobalt chloride treated silkworms.

The activity levels of protease showed decrease in silk gland in cobalt chloride treated worms compared to control worms. Proteases are the enzymes responsible for the hydrolysis of proteins into amino acids. In other words proteases are known to participate in protein digestion in intestine and histolysis in other tissues. The decrease of protease activity level may be due to lower rate of histolysis.

After cobalt chloride treatment free amino acid levels showed a decrease in the silk gland which indicates the faster mobilization of free amino acids into oxidative metabolism in the presence of cobalt chloride. The increase in protein synthesis and decrease in free amino acid level indicates faster mobilization of free amino acids to meet the energy requirement i.e., TCA cycle through transamination. Amino acids in the silk gland serve as the pool for silk protein synthesis (Siva Rami Reddy *et al*., 1984; Prudhommo *et al*., 1985; Sehnal and Akai, 1990 and Siva Prasad and Murali Mohan 1990). The amino acids resulting from protein digestion are the most important raw material for the growth of silkworms especially for the growth of silk gland. The decreased free amino acid content may be due to decreased proteolysis (Venkata Rami Reddy *et al*., 1992).

The ALAT and AAT activity levels were elevated significantly after cobalt chloride treatment indicating the active involvement in the protein synthesis. Higher amino transferase activities are the indicative of higher conversion of amino acid pool and subsequently greater protein synthesis. The enhanced activity of ALAT and AAT reflected the general index of mobilization of free amino acids into gluconeogenesis and oxidation of amino acids respectively (Venkatarami Reddy *et al*., 1992 and Sinha *et al*., 1996). Thus it can be concluded that cobalt chloride enhanced the amino acid metabolism which effects the growth production of silkworm.

**Table:** Changes in the levels of total proteins (mg/gm wet wt), protease activity (µ mol tyrosine equivalents/mg protein/hr), free amino acids (µ mol tyrosine equivalents/gm wet wt), ALAT (µ mol pyruvate formed/mg protein/hr) and AAT (µ mol pyruvate

formed/mg protein/hr) in the silk gland of *Bombyx mori* L. when treated with cobalt chloride. Each value represents the mean of 6 individual observations. Mean ± S.D; '+' or '-' indicate the per cent increase or decrease over control respectively. 'P' denotes the statistical significance.

| S. No. | Components | Control | Experimental |
|---|---|---|---|
| 1 | Total proteins | 212.35±18.2 | 287.38 ±23.92<br>35.33<br>P<0.001 |
| 2 | Protease activity | 1.12±0.19 | 0.94±0.052<br>-16.07<br>P<0.001 |
| 3 | Free amino acids | 21.82±2.01 | 16.06 ±1.82<br>-26.39<br>P<0.001 |
| 4 | Alanine amino transferase (ALAT) activity | 1.15±1.02 | 1.56± 0.94<br>35.65<br>P<0.001 |
| 5 | Aspartate amino transferase (AAT) activity | 0.91±0.082 | 1.3±0.09<br>24.17<br>P<0.001 |

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