The impact of cobalt chloride on the activity levels of dehydrogenases in the gonads of silkmoth, *Bombyx mori* L.

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__ABSTRACT:__ The administration of cobalt on the lactic, pyruvic acid contents and activity levels of aldolase, lactate dehydrogenase (LDH), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (ICDH), and glutamate dehydrogenase (GDH) were studied in the gonads of silkmoth *Bombyx mori* L. Lactic acid content was decreased with a significant increase in the pyruvic acid content in the testes and ovaries of experimental silkmoth was suggestive of the formation of pyruvate either from lactate through elevated operation of NAD-LDH activity or from amino acids through transamination (Pushpa Rani, 1997). The enhanced aldolase activity in testes and ovaries of treated silkmoth indicate the active degradation of hexoses to trioses further activating the glycolysis. The activity levels of all the dehydrogenases were enhanced which showed the increased oxidative metabolism in the testes and ovaries of silkmoth.

**Key words:** *Bombyx mori* L, cobalt, lactic acid, pyruvic acid, aldolase, LDH, SDH, ICDH and GDH.

__I. INTRODUCTION__

The oxidation of carbohydrates beyond the level of pyruvate is reported by a series of dehydrogenases (Gilmour, 1961). In insects cyanocobalamine stimulates gluconeogenesis (Rockstein, 1978) in *Bombyx mori* L. Lactate is one of the end products in anaerobic glycolysis of the larval midgut (Ito and Horie, 1959). The presence of thyroid hormones and the hormonal precursors in some invertebrates without thyroid, such as gorgonid corals, polychaete worms, insects and mollusks were reported (Gorbman et al., 1954; Berg et al., 1958; Frieden, 1981).

The dehydrogenases involved in both glycolytic and pentose phosphate path way were studied in larval tissues of silkworm, *Bombyx mori* L. (Horie, 1987 and Venkatarami Reddy et al., 1992). GDH is an enzyme active in nitrogen and energy metabolism of tissues. In uricotelic animals such as insects, GDH may act as a key enzyme linking carbohydrates and amino acid metabolism (Male and Storey, 1982 and Prezioso et al., 1985). Succinate dehydrogenase is the oxidative enzyme involved in Kreb’s cycle in mitochondria and this dehydrogenase is considered as an index of aerobic metabolism (Fukuda et al., 1958).

The Aldolase activity favours not only the operation of glycolysis but also glycerol synthesis (Lehninger, 1978). Aldolase is an enzyme of glycolytic path way. It cleaves phosphorylated hexoses to triose phosphates. The enhanced aldolase activity in silk gland and gonads after thyroxine treatment indicate active conversion of hexoses to trioses, which are channelled to glycolysis (Hemavathi, 2001). The results indicate that there is active degradation of hexoses to trioses further activating the glycolysis (Nagoka et al., 1995 and Pushpa Rani, 1997).

__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. Testes and ovaries of the silk moths were isolated and used for the present experiment.

The pyruvic acid (Friedmann and Haugen, 1942), lactic acid (Barker and Summerson, 1941 as modified by Huckabee 1961) and aldolase activity (Bruns and Bergmeyer, 1965), the activity levels of LDH (Srikanthan and Krishna Murthy, 1955 as modified by Reddanna and Govindappa, 1979), SDH (Nachlas et al., 1965), ICDH (Korenberg and Pricer, 1951), and GDH (Lee & Lardy, 1965) were assayed in the testes and ovaries of silkmoth.

### III. RESULTS

The data presented in the tables 1 and 2 reveal the changes in the lactic acid, pyruvic acid contents and the activity levels of LDH, SDH, ICDH and GDH in the testes and ovaries of silk moth after treatment with cobalt chloride.

- **Lactic acid**
  - There was a significant decrease (P<0.001) in the lactic acid content in testes and ovaries of silkmoth. The percent decrease in the lactic acid was 18.43% and 11.21% in testes and ovaries respectively over control.

- **Pyruvic acid**
  - Pyruvic acid content showed significant increase in testes and ovaries of silkmoth. (P<0.001). The percent increase in pyruvic acid was 20.97% and 12.5% in testes and ovaries respectively over control.

- **Aldolase activity**
  - There was a significant increase (P<0.001) in the level of aldolase activity after treatment with cobalt chloride in testes and ovaries of silk moth. The percent increase was 22.34% and 21.62% in testes and ovaries respectively over control.

- **LDH activity**
  - The activity level of NAD-LDH showed significant (P<0.001) increase in testes and ovaries of silk moth. The percent increase was 22.58% and 20.59% in testes and ovaries respectively over control.

- **SDH activity**
  - Significant (P<0.001) increase was noticed in SDH activity after cobalt chloride treatment. The percent increase was 28.13% and 27.27% in testes and ovaries respectively over control.

- **ICDH activity**
  - Significant (P<0.001) increase was noticed in ICDH activity level. The percent increase in testes was 13.75% and in ovaries was 18.18%.

- **GDH activity**
  - The activity level of GDH was high (P<0.001) after treatment with cobalt chloride. The percent increase was 26.92% and 17.65% in testes and ovaries respectively over control.

### IV. DISCUSSION

The decreased lactic acid content with enhanced pyruvic acid content suggest the formation of pyruvate either from lactate through elevated operation of NAD-LDH activity from amino acids or through transamination (Hemavathi, 2001). The aldolase plays an important role in the continuation of glycolysis at the level of FDP. The elevation in aldolase activity in the treatment indicates active turnover of hexoses to trioses which are channeled to glycolysis (Geetha Bali and Chandra Sekhar, 1988). The increase in NAD- dependant LDH activity in the testes and ovaries revealed the possibility of formation of pyruvic acid from lactate.

The SDH and ICDH activity levels were found to be significantly increased in testes and ovaries of *Bombyx mori* L. after treatment. This can be attributed to the increased formation of succinate as evidenced from enhanced ICDH activity. SDH in a FAD dependant enzyme, which facilitates the conversion of succinate to fumarate (Fukuda et al., 1958). The importance of NAD-LDH activity is an index of anaerobic metabolism and that of FAD-SDH and NAD-ICDH as aerobic metabolism. The increase in SDH activity in testes and ovaries is in synchrony with glycolytic pathway which indicates that TCA cycle is also elevated in tune with pyruvate production during treatment, which may be due to increased energy demands (Raja Sekhar 1993 and Hemavathi et al, 2004). The increased activity levels of GDH in testes and ovaries suggest the increased oxidation of glutamate. It might be due to increased oxidative deamination of amino acids through keto gluterate facilitate the addition of amino acids into TCA cycle (Hemavathi et al., 2004).
Thus the results obtained from the above investigation showed that the aerobic and anaerobic metabolisms were stepped up due to cobalt chloride treatment. It can be concluded that both the aerobic and anaerobic metabolisms were elevated after the cobalt treatment. This suggests that cobalt chloride has triggered the intake of hexoses to glycolytic and Kreb’s cycle. Thus cobalt chloride has co-operative interaction on the biochemical mechanism of silk protein synthesis and oxidative metabolism. In the present experiment the testes and ovaries of silkmoth showed the response to cobalt treatment. The results of the present study indicate that the cobalt chloride has a stimulatory effect on tissue metabolism in *Bombyx mori* L. Hence cobalt chloride treatment can be viewed as an inducer of tissue oxidative metabolism which is vital for the expression of optimal commercial characters by the silkworm.

**Table-1:** Changes in the lactic and pyruvic acid contents (mg/gm wet wt) and the activity levels of aldolase (µ mol/mg protein/hr), and LDH (µmol formazan formed /mg protein/hr) in the testes and ovaries of silkmoth *Bombyx mori* L. treated with cobalt chloride. Each value represents the mean of 6 individual observations. Mean± S.D: ‘+’ and ‘-’ indicate the per cent increase and decrease respectively over control. ‘P’ denotes the statistical significance.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Component</th>
<th>Control</th>
<th>Exptl (Cobalt chloride treatment)</th>
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<td></td>
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<td>testes</td>
<td>ovaries</td>
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<tr>
<td>1</td>
<td>Lactic acid</td>
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<tr>
<td>2</td>
<td>Pyruvic acid</td>
<td>0.062±0.008</td>
<td>0.072±0.007</td>
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<td>3</td>
<td>Aldolase activity</td>
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<td>0.74±0.041</td>
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<td>4</td>
<td>LDH activity</td>
<td>0.31±0.01</td>
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</table>

**Table-2:** Changes in the activity levels of SDH, ICDH and GDH (µmol formazan formed /mg protein/hr) in the testes and ovaries of silkmoth *Bombyx mori* L. treated with cobalt chloride. Each value represents the mean of 6 individual observations. Mean± S.D: ‘+’ indicate the per cent increase over control. ‘P’ denotes the statistical significance.

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<tbody>
<tr>
<td></td>
<td></td>
<td>testes</td>
<td>ovaries</td>
</tr>
<tr>
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<tr>
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<td>ICDH activity</td>
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<td>3</td>
<td>GDH activity</td>
<td>0.078±0.004</td>
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V. REFERENCES