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The impact of cobalt chloride on the activity levels of dehydrogenases in the gonads of silkworm, *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 silkworm *Bombyx mori* L. Lactic acid content was decreased with a significant increase in the pyruvic acid content in the testes and ovaries of experimental silkworm 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 silkworm 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 silkworm.

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 cyanocobalamin 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 Krebs'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 channeled 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 PI 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 silkworm.

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 silkworm. 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 silkworm. ($P < 0.001$). 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 is 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 glutarate 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 Krebs'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 silkworm 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 (μ mol formazan formed /mg protein/hr) in the testes and ovaries of silkworm *Bombyx mori* L. treated with cobalt chloride. Each value represents the mean of 6 individual observations. Mean $\pm$ S,D: '+' and '-' indicate the per cent increase and decrease respectively over control. 'P' denotes the statistical significance.

S. No.	Component	Control		Exptl (Cobalt chloride treatment)	
		testes	ovaries	testes	ovaries
1	Lactic acid	0.982 $\pm$ 0.072	1.07 $\pm$ 0.055	0.801 $\pm$ 0.061 -18.43 P<0.001	0.95 $\pm$ 0.049 -11.21 P<0.001
2	Pyruvic acid	0.062 $\pm$ 0.008	0.072 $\pm$ 0.007	0.075 $\pm$ 0.005 +20.97 P<0.001	0.081 $\pm$ 0.005 +12.5 P<0.001
3	Aldolase activity	0.94 $\pm$ 0.041	0.74 $\pm$ 0.041	1.15 $\pm$ 0.062 +22.34 P<0.001	0.90 $\pm$ 0.062 +21.62 P<0.001
4.	LDH activity	0.31 $\pm$ 0.01	0.34 $\pm$ 0.009	0.38 $\pm$ 0.019 +22.58 P<0.001	0.41 $\pm$ 0.012 +20.59 P<0.001

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

S. No.	Component	Control		Exptl (Cobalt chloride treatment)	
		testes	ovaries	testes	ovaries
1	SDH activity	0.032 $\pm$ 0.004	0.022 $\pm$ 0.001	0.041 $\pm$ 0.007 +28.13 P<0.001	0.028 $\pm$ 0.001 +27.27 P<0.001
2	ICDH activity	0.80 $\pm$ 0.05	1.21 $\pm$ 0.05	0.91 $\pm$ 0.09 +13.75 P<0.001	1.43 $\pm$ 0.07 +18.18 P<0.001
3.	GDH activity	0.078 $\pm$ 0.004	0.102 $\pm$ 0.006	0.099 $\pm$ 0.007 +26.92 P<0.001	0.12 $\pm$ 0.008 +17.65 P<0.001

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