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Effect of Cortisone and Hydrocortisone on the Biochemical Changes in the Fat Body and Haemolymph of the Silkworm, *Bombix mori* L.

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The effect of topical application with 10, 20 and 30 µg/ml cortisone and hydrocortisone to the fifth stadium larvae of the silkworm, *B. mori* on fat body glycogen, protein, total lipids, phospholipids, neutral lipids and haemolymph trehalose and protein has been studied. The fat body glycogen haemolymph trehalose significantly decreased in all the treated groups except in the 10 and 20 µg/ml treated groups. The fat body protein increased significantly in all the cortisone and hydrocortisone treated groups except in the group treated with 10 µg hydrocortisone. Whereas that of haemolymph protein significantly increased in all the groups treated with cortisone and hydrocortisone. The total lipids, phospholipids and neutral lipids of the fat body decreased significantly in all the groups treated with cortisone and hydrocortisone when compared with that of carrier control.

Key words : Cortione, Hydrocortione, Biochemical parameters, *Bombyx mori*.

Introduction

Insects were once considered as "mechanical black boxes" which function without hormones. Thanks to the pioneering works of Wigglesworth and many others, it is suggested that hormones control the metamorphosis and reproduction. For a long time, the endocrine system of insects was considered to be much less complicated than that of vertebrates (Swevers *et al.*, 1991). Subsequently it has been reported that the endocrine system of insect is much more complicated than previously assumed and in addition, it shares many common features with that of vertebrates (De Clerck *et al.*, 1983, 1984, 1987, 1988; Bradbrook *et al.*, 1990).

In recent years, the presence and activity of various vertebrate steroid hormones have been demonstrated in life system of many insects. Since the initial study of Mordue (1967), the effect of mammalian corticosteroids on insect growth, development and puffing pattern has been extensively investigated (Sang, 1968; Smith *et al.*, 1968; Rosinski *et al.*, 1978, Gawienowski *et al.*, 1987). The physiological effect of several glucocorticoids on mung bean seedlings (*Phaseolus aureus* Roxb.) was demonstrated to enhance the plant growth (Genus, 1980). Therefore, the treatment with glucocorticoids is not unusual as insects and their relatives are phylogenetically closer to mammals, than are plants. Infact, cortisol therapy has been shown to increase the mite (*D. bravis*) population in human skin (Sato *et al.*, 1965). Corticosteroids are known for its role in glucose metabolism in vertebrates (Turner and Bagnara, 1976). In insects, the effects of corticosteroids on the biochemical parameters are limited.

It has been reported that the glucocorticoids affect protein catabolism, free aminoacids as well as production of larger amounts of uric acid (Mordue, 1967). In vitro studies have demonstrated that glucocorticoids have the capacity to increase the output of free fatty acids from adipose tissue (Jeanrenaud and Renold, 1967). Recently, the effect of topical application with cortisone and hydrocortisone to the silkworm larvae showed significantly increased larval weight along with other enhanced larval, cocoon and adult parameters (Goudar and Kaliwal, 2000). However, there were no reports on the effect corticosteroids on the biochemical constituents of the silkworm, *B. mori*. Therefore, the present investigation is undertaken to find out the effect of cortisone and hydrocortisone on the fat body glycogen, haemolymph trehalose, fat body protein, haemolymph protein, fat body total lipids, phospholipids and neutral lipids of the silkworm, *B. mori*.

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Materials and Methods

The eggs of the silkworm, *B. mori* were obtained from the Rayapur, Dharwad, and Karnataka and reared in the laboratory by the improved method of rearing technique (Krishnaswami, 1978). The fifth instar larvae were divided into eight experimental groups including control groups. Each group consisting of five replications of 20 worms each. The hormones, cortisone and hydrocortisone were procured from M/S Sigma Laboratories Pvt. Ltd. Bombay and were dissolved separately in small quantity of acetone. The cortisone and hydrocortisone were diluted to form 10, 20 and 30 µg/ml solutions by adding acetone. The topical application was made on dorsal side of the larvae. Each larva in its group was topically applied with one of the three concentrations of cortisone and hydrocortisone on alternate day in the V stadium upto the spinning stage. In each application 5 ml of solution was used to treat 100 larvae. The larvae of carrier control was topially applied with acetone and served as carrier control for cortisone and hydrocortisone treated groups, while the normal controls did not receive any treatment. The treated carrier control and normal control larvae were utilised for the estimation of glycogen, protein, total lipids, phospholipids and neutral lipids from the fat body and protein and trehalose from the haemolymph.

The silkworm larvae were dissected in *Bombyx saline* at pH 6.5 on the 6th day of the fifth stadium. The fat body was immediately collected and used for the glycogen (Shiefter *et al.*, 1950), protein (Lowry *et al.*, 1951), total lipids, phospholipids and neutral lipids (Folch *et al.*, 1957) estimations. The haemolymph was collected by amputing one of the thoracic legs in a prechilled centrifuse tube and was used for the estimation of trehalose (Roe, 1955) and protein (Lowry, 1951). Anthrone positive carbohydrate in the haemolymph is considered as trehalose.

The experiments were designed by the complete randomised block design (CRBD) method and the data collected were subjected to the statistical analysis of variance (ANOVA) test to determine the significant difference between the treatment and control groups (Raghav Rao, 1983).

Table 1. Effect of cortisone and hydrocortisone on the biochemical constituents of the silkworm, *B. mori*

Treatment	Dose µg/ml	Fat body glycogen µg/mg	Haemolymph trehalose µg/ml	Fat body protein µg/mg	Haemolymph protein µg/ml	Fat body total lipids µg/100 mg	Fat body phospholipids µg/100 mg	Fat body neutral lipids µg/100 mg
Cortisone	10	15.999* (64)	245 (100)	18.888* (38)	8340* (178)	298.0* (95)	122.0* (92)	176.0* (97)
Cortisone	20	7.444* (29)	273 (111)	32.444* (66)	6000* (128)	289.3* (92)	117.6* (89)	171.6* (94)
Cortisone	30	4.333* (17)	588* (240)	26.222* (54)	9240* (197)	282.6* (90)	113.6* (86)	169.0* (93)
Hydrocortisone	10	13.555* (54)	490* (220)	52.888 (109)	6000* (128)	291.3* (93)	118.6* (90)	172.6* (95)
Hydrocortisone	20	11.111* (44)	490* (220)	15.555* (32)	6540* (139)	278.0* (88)	113.0* (85)	165.0* (91)
Hydrocortisone	30	6.333* (25)	630* (257)	15.999* (33)	5700* (121)	268.0* (85)	109.0* (82)	159.0* (87)
Carrier control	Acetone	24.888 (100)	245 (100)	48.444 (100)	4680 (100)	312.6 (100)	131.3 (100)	181.0 (100)
Normal control	-	9.333* (37)	357 (145)	42.222 (87)	2040* (43)	308.6* (98)	130.0* (98)	178.6* (98)
		S	S	S	S	S	S	S
S.Em ±		2.072	85.783	6.474	30.000	1.314	0.480	0.726
C.D. at 5%		4.456	184.833	13.920	64.500	2.826	1.032	1.562

* - Significant increase/decrease at 5%
** - Angular transformed values
S - Significant
S.Em ± - Standard error mean
C.D. - Criticle difference
Per cent increase/decrease over that of the carrier control in parenthesis.

Results and Discussion

Effect of cortisone and hydrocortisone on the fat body glycogen and haemolymph trehalose

In the present study, the treatment with all the three doses 10, 20, 30 µg/ml cortisone and hydrocortisone significantly decreased the fat body glycogen and it was dose dependent but the haemolymph trehalose was increased in all the treated groups except in the group treated with 10 and 20 µg/ml cortisone (Table 1). These results possibly suggest that all the three doses of cortisone and hydrocortisone might have stimulated the synthesis of glycogen by the fat body and its immediate release into the haemolymph, as trehalose. Therefore, increased amount of trehalose is found in the haemolymph.

Effect of cortisone and hydrocortisone on the fat body protein and haemolymph protein

Wigglesworth (1977) has stated that the fat body in insects is the main site of protein synthesis as well as the intermediating metabolisms of amino acids. In the present study, treatment with cortisone and hydrocortisone has resulted in decreased fat body protein in all the treated groups except in the group treated with 10 µg hydrocortisone. However, the haemolymph protein is increased in all the groups treated with cortisone and hydrocortisone when compared with that of carrier control (Table 1). The protein catabolism has been reported after the treatment with glucocorticoids in *Schistocerea gregaria* and *Tenebrio molitor* (Mordue, 1967). Ilan and Ilan (1973) showed that growth and development of insects are controlled by neuroendocrinal secretions. It could be anticipated that these secretions of neuroendocrine organs control the protein metabolism of an insect. On these bases the present results possibly suggest that at the given concentrations the cortisone and hydrocortisone might have stimulated the synthesis of protein by the fat body and its immediate release into the haemolymph for silkgland development since, the silkgland weight was significantly increased in the group treated with cortisone (Goudar and Kaliwal, 1999, 2000).

Effect of cortisone and hydrocortisone on the fat body total lipids, phospholipids and neutral lipids

The lipid in fat bodies is an energy reserves which can be mobilised rapidly during starvation, oogenesis, embryogenesis and moulting and is used to sustain continuous muscular activity (Gilbert and Chino, 1974). In the present study, treatment with cortisone and hydrocortisone has resulted in a significant decrease in the fat body total lipids, phospholipids and neutral lipids in all the groups when compared with that of carrier control (Table 1). The decreased total lipids, phospholipids and neutral lipids of the fat body might possibly be due to the inhibitory effect of the cortisone and inhibitory effect of the cortisone and hydrocortisone at a given concentration on the synthetic activity of the fat body. There was significant increase/decrease in larval fat body glycogen and haemolomphy protein of the normal controls when compared with the corresponding parameters of the acetone treated controls. This significant difference might be due to the effects of acetone since the acetone has considerable effect on the larval body weight and food utilization in silkworm, *B. mori.* (Padaki, 1991).

The possible mechanism of action of cortisone and hydrocortisone on the biochemical constituents of the silkworm, *B. mori* is due to their influence on nervous system or hormones, or the stimulation or inhibition of enzymes activity, or the induction or suppression of enzyme synthesis is not known. However, further investigation is essential to known the exact mechanism of action of vertebrate steroids on the biochemical constituents in the fat body and haemolymph of the silkworm, *B. mori.*

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