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Steroid Metabolizing Enzymes in the Testis of Diapausing and Adult Tasar Silkworm, *Antheraea mylitta*—A Histochemical Study

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Traces of Δ^5-3β-hydroxysteroid dehydrogenase 17β-hydroxysteroid dehydrogenase and 11β-hydroxysteroid dehydrogenase activities observed in the peritoneal sheath, epithelium and in spermatogoneal cells during the first and second month of diapause. Subsequently the enzyme activity was gradually increased in these spermatogenic components and extended to the spermatocytes, spermatids and spermatozoan bundles during later periods of diapause and adult after emergence. The spermatozoan bundle showed preferences for testosterone. An intense glucose-6-phosphate debydrogenase activity was observed in all the testicular components during diapause period and after emergence. The lipid droplets found in the peritoneal sheath and epethelium were larger than those found in the other components. These results indicate that the testis of *A. mylitta* has the ability to metabolize hydroxysteroids to corresponding ketosteroids.

Key Words: ***Antheraea mylitta*, Diapause, Testis, Spermatogenesis, Steroid metabolizing enzymes**

Introduction

Vertebrate type of hormonal steroids are shown to be present in some of the invertebrates (Sandor et al. 1975). The gonads of insects are capable of metabolizing steroids and possess the necessary enzymes, Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β HSDH), and 17β-hyroxysteroid dehydrogenase (17β-HSDH), (Lehoux et al. 1968; Lehoux & Sandor 1969, 1970, Dube & Lemonde, 1970). Presence of Δ^5-3β-HSDH by histochemical method is shown in a few species of insects (Lehoux & Sandor 1970, Trandaburu & Tasca 1976). Hurkadli et al. (1988a, b) have shown the presence of Δ^5-3β-HSDH, 17β-HSDH and 11β HSDH activity in the developing and adult testis of *Philosamia ricini* and adult testis of *Bombyx mori* by histochemical demonstration. In the present investigation an attempt has been made to localize steriod converting enzymes, Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH in the testis of diapausing pupa and adult *Antheraea mylitta*. In addition, the histochemical localization of glucose-6-phosphate dehydrogenase (G-6-PDH), lipids and cholesterol in the testis of this silkworm has also been studied.

Materials and Methods

For histological study, the silkworms of respective stages were killed, the testis removed and fixed in Bouin's fluid for 20-25 hr, embedded in paraffin wax and 4 μm sections were cut and stained with haematoxyline and eosin.

For histochemical studies, diapausing pupa and adult were decapitated, the testes of respective periods were removed and frozen over dry ice vapour at–50°C. For histochemical demonstration of Δ^5-3β-HSDH, 17β-HSDH, 11β-HSDH and G-6-PDH activity, the frozen sections were incubated in different media containing different substrates (table 1), prepared according to the procedure described by Baillie et al. (1966) and Hurkadli et al. (1988a,b). For the demonstration of lipids a few frozen sections were treated with Sudan Black B (Pearse 1968). Cholesterol was detected following the procedure of Schultz (Pearse 1968).

Parallel sections incubated in the media lacking the substrates served as controls. A few sections incubated in the medium containing DHA, co-enzyme, NBT and cyanoketone/isoxiozol, served as specific control for Δ^5-3β-HSDH activity.

Results

Each testis of *A. mylitta* consists of four follicles and is enclosed in a layer of connective tissue sheath or peritoneal sheath followed by a layer of epithelial cells around the lumen of each follicle. During the first and second months of diapause, each follicle consists of spermatogoneal cells, large number of primary spermatocytes and a few secondary spermatocytes. During the third month the secondary spermatocytes number is increased (figure 1). During the fourth month large number of spermatids and a few spermatozoan bundles make their appearance. During the fifth month of diapause and in the adult the testes contain a few spermatids and large number of spermatozoan bundles.

The results on histochemical reaction for the hydroxysteroid dehydrogenases are summarised in table 1. A weak Δ^5-3β-HSDH activity was found in the cells of peritoneal sheath, epithelium and traces of activity in spermatogoneal cells in the testis of first and second month diapausing pupa (figure 2). A weak activity was observed in the cells of peritoneal sheath and epithelial layers, spermatogoneal cells, primary and secondary spermatocytes and activity in traces was observed in the spermatids during third month of diapausing pupa. During fourth and fifth month of diapausing pupa and adult, a fairly intense activity was observed in all the testicular components (figure 3). After mating the enzyme activity was reduced in all the testicular components (figure 4). Of the two substrates used pregnenolone yielded more reaction than DHA in the epithelial layer.

The intensity and distribution of 17β-HSDH activity with testosterone and 17β-estradiol as the substrates and NAD as co-factor in the testis of diapausing pupae and in adults before and after mating is similar to that of Δ^5-3β-HSDH at respective stages (table 1). However, the spermatozoan bundles showed preference for testosterone.

A positive 11β-HSDH activity with 11β-hydroxyandrostenedione as substrate was similar to that of Δ^5-3β-HSDH with DHA as substrate in the testis at respective stages of diapausing pupa and adult moth.

An intense G-6-PDH activity was observed in all the testicular components of the testis of diapausing pupa and adult *A. mylitta*.

Large lipid droplets were present in the cells of peritoneal sheath and epithelium of the testis the during first three months of diapausing pupa. During fourth and fifth month of diapause and in adult there was a slight reduction in the size and number of lipid droplets in the cells of these two layers. Other testicular components showed small-sized lipid droplets at respective stages. The lipid droplets in different testicular components during diapausing pupa and adult periods gave positive histochemical test for cholesterol. Generally no diformazan deposition was observed in the sections incubated in the medium lacking the substrate but some times a faint reaction was obtained. The sections incubated in the medium containing DHA and isoxozol cyanoketone did not show diformazan deposition.

Discussion

Insects are unable to synthesize cholesterol from acetate but it has been shown that the phytophagous insects can obtain cholesterol from their diet (Svoboda et al. 1975). Our histochemcial findings have shown the presence of cholesterol positive lipids in the various components of the testis during diapausing and adult stages. Presence of large lipid droplets, in the cells of peritoneal sheath and epithelium during first three months may indicate the mobilization of lipids in the cells of peritoneal sheath and epithlium. The decrease in the last two months of diapausing pupa and adult may indicate that the lipids are transferred to other testicular components such as spermatogoneal cells, spermatocytes, spermatids and spermatozoan bundles to meet their metabolic requirements, since these two layers are considered to be the source of nutrients for the testis (Snodgross 1935, Salama 1976).

Barrington (1968) has stated that steroid biosynthesis is not an exclusive prerogative of vertebrates, since steroid biosynthesis is wide spread throughout the animal kingdom. A number of steroids have been isolated and identified from the prothoracic and pygidial glands of different species of aquatic beetles (Schildknecht & Korning 1968). The gonads of insects are capable of metabolizing steroid substances (Lehoux et al. 1968, Lehoux & Sandor 1969, 1970, Dube & Lemonde, 1970). *In vitro* and histochemical studies by Lehoux and Sandor (1970) have shown the occurrence of Δ by Lehoux and Sandor (1970)^5 -3β-HSDH in the testis of cockroaches, *Graphodorohina portentosa* and *Byrsotrea fumigata* and the cricket, *Gryllus assimilis*. However, there is no detailed information as to which of the testicular components show Δ^5-3β-HSDH activity, except that it has been histochemically reported in the spermatids and spermatozoa of two hemipterans, *Graphosoma italicum* and *Eurydema ventralis* (Trandaburu & Tasca 1976). The presence of Δ^5-3β-HSDH has been shown histochemically in the peritoneal sheath, epithelial layer, spermatogonial cells, primary and secondary spermatocytes, spermatids and sperm bundles of the adult testis of *B. mori* and developing and adult testis of *P. ricini* at respective stages (Hurkadli et al. 1988a, b). In the present investigation the presence of Δ^5-3β-HSDH activity in the testis of

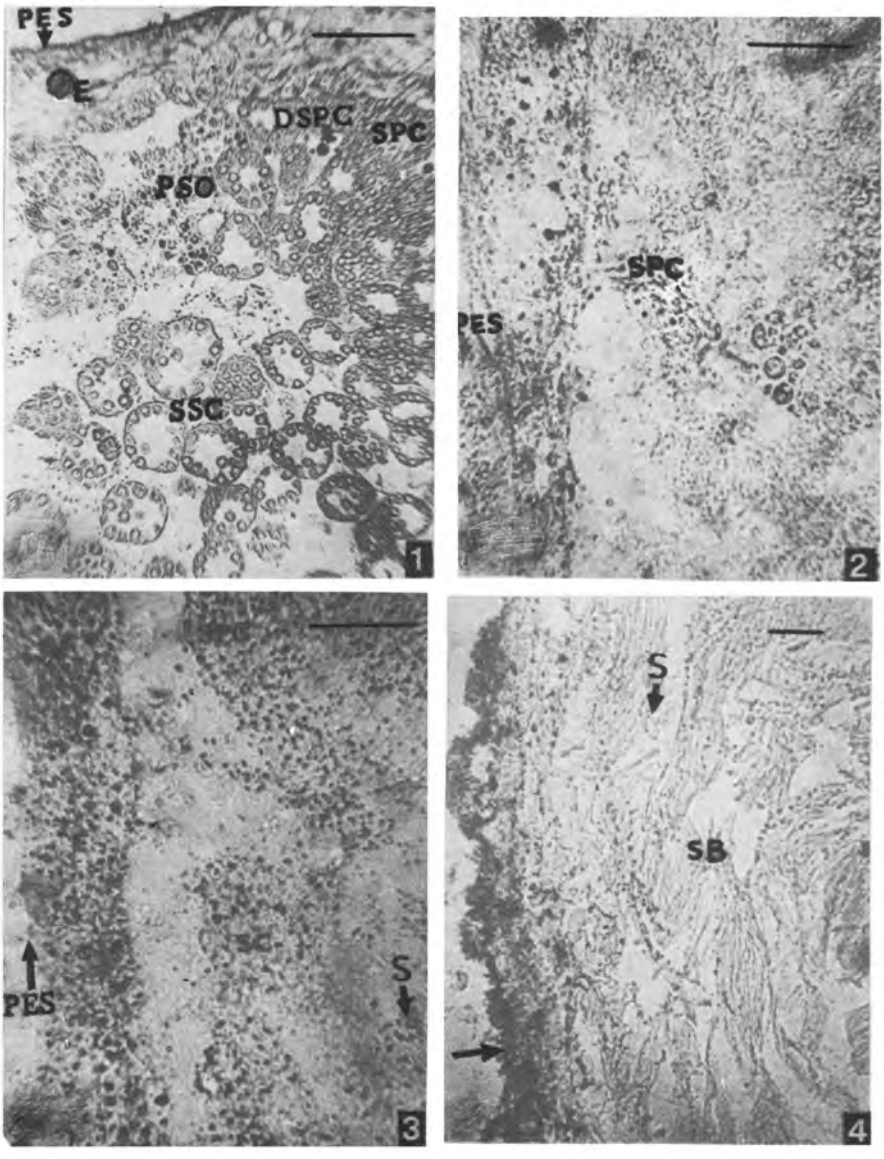

Figure 1 L S of the testis of two months old diapausing pupa of *A. mylitta* showing peritoneal sheath (PES), epithelium (E), spermatogoneal cysts (SPC), degenerating spermatogoneal cysts (DSPC), primary spermatocytes (PSC) and secondary spermatocytes (SSC)

Figure 2 Δ^5-3β-HSDH activity in the peritoneal sheath (PES) and epithelial cells (E) and in spermatogoneal cysts (SPC) in fresh frozen section of the testis of one month old diapausing pupa of *A. mylitta*

Figure 3 Fairly intense Δ^5-3β-HSDH activity in the peritoneal sheath (PES) and epithelial cells (E), spermatogoneal cysts (SPC), spermatocytes (SC) and spermatids (S) in fresh frozen section of the testis of four month old pupa of *A. mylitta* with DHA as the substrate

Figure 4 A weak Δ^5-3β-HSDH activity in the spermatids (S) and spermatozoan bundles (SB) in the fresh frozen section of the tesis of adult *A. mylitta* after mating, with DHA as the substrate (The scale line = 40 μm)

Table 1 *Activity of hydroxysteroid dehydrogenses in the testis of diapausing pupa and adult* ***A. mylitta***

*Developmental stages, Enzymes and the substrates**	*Intensity of reaction***			
	Peritoneal sheath and epithelial cells	*Spermatogonial cells*	*Primary and secondary spermatocytes*	*Spermatids and spermatozoan bundles*
DIAPAUSING PUPAL PERIOD				
1st and 2nd month				
Δ^5-3β- HSDH DHA	+	+	Not yet formed	Not yet formed
Pregnenolone	++	+	–do–	–do–
17β- HSDH				
Testosterone	+	+	–do–	–do–
17β-estradiol	+	+		
11β-HSDH				
11β-hydroxyandrostenedione	+	+	–do–	–d–
3rd month				
Δ^5-3β- HSDH, DHA	++	++	++	
Pregnenolone	+++	++	++	±
17β-HSDH,				
Testosterone	++	++	++	+
17β-estradiol	++	++	++	±
11β-HSDH				
11β-hydroxyandrostenedione	++	++	++	±
4th and 5th month				
Δ^5-3β- HSDH, DHA	+++	+++	+++	+++
Pregnenolone	++++	+++	+++	+++
17β-HSDH				
Testoserone	+++	+++	+++	++++
17β-estradiol	+++	+++	+++	+++
11β-HSDH				
11β-hydroxyandrostenedione	+++	+++	+++	+++
ADULT				
Δ^5-3β-HSDH DHA Pregnenolone	+++	++	++	+++
17β-HSDH				
Testosterone	+++	+++	+++	+++
17β-estradiol		++	***	***
11β–HSDH				
11β-hydroxyandrostenedione	+++	++	++	+++
After mating				
Δ^5 -β-HSDH DHA	++	++	++	+++
Pregnenolone	++	++	++	++
17 β-HSDH				
Testosterone	++	++	++	++
17β-estradiol	++	++	++	++

* All the chemicals are of Sigma grade, obatined from Sigma Co., USA

** Intensity of reaction is graded from minimum (+) to intense (++++) activity, (−) denotes absence of reaction and (±) denotes trace activity

diapausing pupa and adult moth of *A. mylitta* is very much similar to that of the testis of *B. mori* and *P. ricini*. From this study it may be suggested that the testis of diapausing pupa and adult *A. mylitta* possess enzyme or enzymes necessary to convert exogenous pregnenolone to progesterone and DHA to androstenedione.

17β-HSDH occurs in a number of tissues of invertebrates (Hathaway 1965, Idler et al. 1969). The testis, intestine, leg muscle, cuticle and fatty tissue of the cricket, *G. domesticus* are capable of transforming testosterone to androstenedione (Lehoux & Sandor 1969). Testis of *S. gregaria* bring about the interconversions of androstenedione to testosterone and 17β-estradiol to estrone (Dube & Lemonde 1970). In the present investigation 17β-HSDH activity has been histochemically demonstrated in the peritoneal sheath and epithelial cells, spermatogonial cells, primary and secondary spermatocytes, spermatids and spermatozoa. Our results further suggest that the testosterone and 17β-estradiol were equally well utilized by all the components of the testis except the spermatozoa which showed preference for testosterone. Similar observations were made in the developing and adult testis of *P. ricini* and adult *B. mori* by histochemical study (Hurkadli *et al.* 1988a, b). Thus the present histochemical results suggest the ability of the testis of diapausing pupa and adult *A. mylitta* to bring about the interconversions of androstenedione-testosterone and 17β-estradiol-estrone.

The occurrence of corticosterone and cortisol in the haemolymph of the cricket, *G. domesticus* has been reported. The presence of 11β-HSDH in the testis of diapausing pupa and adult *A. mylitta* is in conformity with that reported in the developing and adult testis of *P. ricini* and *B. mori* by histochemical method (Hurkadli et al. 1988a, b) and this result suggest the ability of the testis to metabolize 11β-hydroxysteroids. G-6-PDH activity indicates the presence of NADH generating system in the testis of *A. mylitta* (Hurkadli et al. 1988a,b).

In conclusion the present findings suggest that the testis of diapausing pupa and adult *A. mylitta* has the necessary steroid converting enzymes that can convert the exogenous hydroxysteroids to corresponding ketosteroids.

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References

Baillie A H, Ferguson M M and Hart D Mck 1966 Developments in Steroid Histochemistry; (New York: Academic Press)

Barrington E J W 1968 Phylogenetic perspectives in vertebrate endcrinology; in *Perspectives in Vertebrate Endocrinology* eds E J W Barrington and C G Jrgensen pp. 1-46 (New York: Academic Press)

Dube J and Lemonde A 1970 Transformations des steroids par la femelle adulte d'un insecte orthoptere, *Schistocerca gregaria* Forskal; *Gen. Comp. Endocrinol.* **15** 158-164

Hathaway R R 1965 Conversion of estradiol-17β by sperm preparations of sea urchin and oysters; *Gen. Comp. Endocrinol.* **5** 504-508

Hurkadli H K, Hooli M A and Nadkarni V B 1988a Steroidogenic cells in the testis of larva, pupa and adult eri silkworm, *Philosamia ricini*—A histochemical study; *Proc. Indian Acad. Sci*, **97** 189-196

——, —— and —— 1988b Hydroxysteroid dehydrogenases in the testis of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae): A histochemical study; *Proc. Indian natn. Sci.* **B54** 51-55

Idler D R, Sangaland G B and Kanazawa A 1969 Steroid desmolase in gonads of a marine invertebrate, *Placopecten magellanicus* (Gmelin); *Gen. Comp. Endocrniol.* **12** 222-230

Lehoux J G and Sandor T 1969 Conversion of testosterone Δ^4-androstene-17-dione by house cricket, *Gryllus domesticus*, male gonad preparations *in vitro*; *Endocrinology* **84** 652-657

—— and —— 1970 The occurrence of steroid metabolizing enzyme in invertebrates—A review; *Steroids* **16** 141-171.

——, ——. Lanthier A and Lusis O 1968 Metabolism of exogenous progesterone by insect tissue preparations *in vitro*; *Gen. Comp Endocrinol.* **11** 481-488

Pearse A G E 1968 *Histochemistry—Theoritical and Applied* Vol. I (London: Curtchill and Livingstone Ltd.)

Salama H S 1976 Spermatogenesis and testicular development in the gypsy moth, *Porthetria dispar* L. *Z. Angew. Entomol.* **81** 1-2-110.

Sandor T, Sonea S and Mehdi A Z 1975 The possible role of steroids in evolution; in *Trends in Comparitive Endocrinology* eds E J W Barrington; *Amer. Zool.* **15** (Suppl. 1) 227-253

Schildknecht H and Korning W 1968 Protective materials in the secretion from the prothoracic protective gland of *Mexican cybister* species; *Angew Chem. Int. Ed.* **7** 62-63

Snodgrass R E 1935 *Principles of Insect Morphology* pp 550-580 (New York: McGraw Hill)

Svoboda J A, Kaplanis J N, Robbins M E and Thompson W B 1975 Recent developments in insect steroid metabolism; *Ann. Rev. Entomol.* **20** 205-220

Trandaburu V and Tasca C 1976 Activity of 3β-hydroxysteroid dehydrogenase and some glycolytic enzymes occurring in the testis of *Graphasoma italicum* (Mull.) and *Eurydema ventralis*; *Rev. Roum. Med. Endocrinol.* **14** 203-205