Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 97, No. 3, May 1988, pp. 189–196.

Steroidogenic cells in the testis of larva, pupa and adult eri silkworm, *Philosamia ricini*—A histochemical study

H K HURKADLI*, M A HOOLI and V B NADKARNI
Department of Zoology, Karnatak University, Dharwad 580 003, India
*Karnataka State Sericulture Development Institute, Thalaghattapura, Bangalore 560 062, India

MS received 30 November 1987; revised 16 March 1988

Abstract. The testis formed of 4 follicles develops as a definitive organ on the first day of IV instar. The localization of Δ^5-3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase and 11β-hydroxysteroid dehydrogenase, was limited to peritoneal sheath and epithelial layer of the testis of IV and V instar larva. From 3rd day of pupation upto the 8th day of pupation the enzyme activity extended to the cysts containing spermatogonical cells, primary and secondary spermatocytes and subsequently to the spermatids and spermatozoan bundles from 9th day of pupation and continued thereafter in the adults. Glucose-6-phosphate dehydrogenase activity was observed in all the testicular components during larval, pupal and adult stages. These results indicate that the developing and adult testis of eri silkworm, *Philosamia ricini* has the ability to metabolize hydroxysteroids.

Keywords. Eri silkworm; testis; spermatogenesis; steroidogenic cells.

1. Introduction

In recent years vertebrate type of hormonal steroids are shown to be present in arthropods, molluscs and echinoderms (Lehoux and Sandor 1970; Thompson *et al* 1973; Sandor *et al* 1975). In the last few years evidence has been accumulated to show that the gonads of insects are capable of metabolizing enzyme systems (Lehoux *et al* 1968; Lehoux and Sandor 1969; Dube and Lemonde 1970). *In vitro* conversion of Δ^5-3β-hydroxysteroids by the testis of cockroaches, *Gramphadorhina portentosa*, *Byrostria fumigata* and the cricket, *Gryllus assimilis* (Lehoux and Sandor 1970) has indicated, albeit indirect, the existence of Δ^5-3β-hydroxysteroid dehydrogenase (HSDH) activity and in fact it has been histochemically demonstrated in the testis of these insects (Lehoux and Sandor 1970) and in the testis of two hemipterans, *Graphosoma italicum* and *Eurydema ventralis* (Trandaburu and Tasca 1976). To the best of our knowledge there is no report on the histochemical demonstration of 17β-HSDH and 11β-HSDH in the testis of insects.

In the present investigation an attempt has been made to present histochemical evidence for steroid metabolizing potential of the testis of eri silkworm, *Philosamia ricini* during IV and V instar larval period, pupal period and in the adult before and after mating by demonstrating the presence of Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity. In addition, the histochemical localization of glucose-6-phosphate dehydrogenase (G-6-PDH) in the testis of this silkworm, is also carried out.

2. Materials and methods

The larva, pupa and adult *P. ricini* reared in the laboratory were used in the present investigation. For histochemical study the animals from respective stages of larva,

pupa and adult were decapitated, the testes were removed and immediately frozen over dry ice vapour at −50°C and sectioned in a cryostat at −20°C. For the histochemical demonstration of Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity the frozen sections were incubated in the media containing different substrates (table 1) prepared according to the procedure described by Baillie *et al* (1966) and Saidapur and Nadkarni (1972). For the localization of G-6-PDH activity, the frozen sections were incubated for 15 min at 37°C in the medium prepared according to the methods described by Bara (1965a, b). After incubation the sections were washed in distilled water, fixed in 10% neutral formalin for 30 min and mounted in glycerol jelly. Parallel sections incubated in the media lacking the substrates served as controls. A few sections incubated in the medium containing dehydroepiandrosterone (DHA) co-enzyme, nitro blue tetrazolium (NBT) and cyanoketone/isoxozol, as specific control of Δ^5-3β-HSDH activity.

3. Results and discussion

The results on the histochemical reaction for the HSDH, indicated by the deposition of blue diformazan granules in the cytoplasm of different cells types of the testis of larva, pupa and adult *P. ricini* are summerised in table 1.

A weak Δ^5-3β-HSDH activity was observed only in the cells of the peritoneal sheath and epithelial layer of the testis (figure 1) of IV and V instar larva of *P. ricini*. Of the two substrates used, pregnenolone was somewhat better utilized than DHA.

On the first day of pupation the spermatogoneal cells showed activity in traces, the primary and secondary spermatocytes did not show any activity (figure 2). From third day of pupation and onwards upto 12th day of pupation the cells of peritoneal sheath and epithelial layer showed moderate activity with DHA as the substrate and on 15th day of pupation the activity was intense with both the substrates (figure 3). The spermatogoneal cells showed a weak activity from 3rd day pupation to the remaining period of the pupal life. The primary and secondary spermatocytes, spermatids and spermatozoan bundles at the respective developmental stages of pupa showed a gradual increase in the intensity activity from traces to moderate (table 1).

A fairly intense Δ^5-3β-HSDH activity was observed in the cells of the peritoneal sheath and epithelial layer, spermatogonial cells, primary and secondary spermatocytes, spermatids and spermatozoan bundles of the testis of adult *P. ricini* before mating (figure 4).

The distribution of 17β-HSDH activity with testosterone and 17β-estradiol in the cells of the peritoneal sheath and epithelial layer of the testis of IV and V instar larvae was similar to that of Δ^5-3β-HSDH activity in the corresponding instars.

There was a gradual increase of 17β-HSDH activity from moderate to intense in the cells of peritoneal sheath, epithelial layer and spermatogoneal cells from 3rd day of pupation upto 9th day of pupation. On 12th and 15th days of pupation the activity was reduced to moderate with both the substrates.

On the 3rd day of pupation, the 17β-HSDH activity was in traces in primary and secondary spermatocytes and there was no activity in the spermatids. On 6th day of pupation the activity was moderate in primary and secondary spermatocytes and in spermatids. In the spermatozoan bundles the activity was in traces. On 9th, 12th and 15th day of pupation the activity was moderate in primary and secondary spermatocytes, spermatids and spermatozoan bundles (figure 5). There was relatively

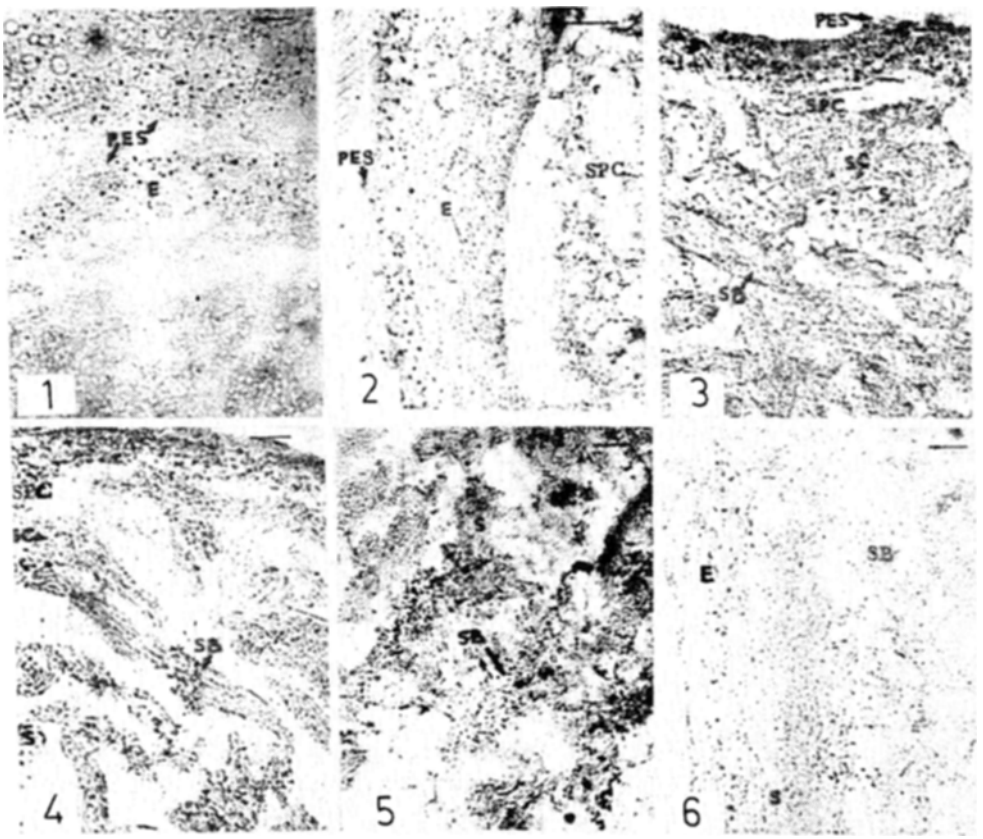
Table 1. Activity of hydroxysteroid dehydrogenases in the testis of larva, pupa and adult *P. ricini*.

Developmental stages	Enzymes and the substrates[a]	Intensity of reaction[b]			
		Peritoneal sheath and epithelial cells	Spermatogonial cells	Primary and secondary spermatocytes	Spermatids and spermatozoan bundles
IV instar larva	Δ^5-*3β-HSDH*				
1st and 3rd day	DHA	+ +	−	Not yet formed	Not yet formed
	Pregnenolone	+ + +	−	-do-	-do-
	17β-HSDH				
	Testosterone	+ +	−	-do-	-do-
	17β-estradiol	+ +			
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ +	−	-do-	-do
V instar larva					
1st day	Δ^5-*3β-HSDH*				
	DHA	+ +	−	Only Primary	-do
	Pregnenolone	+ + +	−	spermatocytes	-do
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ +	−	−	-do-
	17β-HSDH				
	Testosterone	+ +	−	−	-do-
	17β-estradiol	+ +	−	−	-do-
3rd day	Δ^5-*3β-HSDH*			Primary and	
	DHA	+ +	−	secondary spermatocytes	Not yet formed
	Pregnenolone	+ + +	−	−	
	17β-HSDH				
	Testosterone	+ +	−	−	-do-
	17β-estradiol	+ +	−	−	
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ +	−	−	-do-
Pupa					
1st day	Δ^5-*3β-HSDH*				
	DHA	+ +	±	−	-do-
	Pregnenolone	+ + +	±	−	
	17β-HSDH				
	Testosterone	+ +	±	−	-do-
	17β-estradiol	+ +	±	−	
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ +	±	−	-do-
3rd day	Δ^5-*3β-HSDH*				
	DHA	+ + +	+ +	±	-do-
	Pregnenolone	+ + + +	+ +	±	
	17β-HSDH				
	Testosterone	+ + +	+ +	+	-do-
	17β-estradiol	+ + +	+ +	±	
	11β-HSDH				
	11β-hydroxyandro-stenedione	+ + +	+ +	±	-do-

Table 1. (*Contd.*)

Developmental stages	Enzymes and the substrates[a]	Intensity of reaction[b]			
		Peritoneal sheath and epithelial cells	Spermatogonial cells	Primary and secondary spermatocytes	Spermatids and spermatozoan bundles
6th day	*Δ^5-3β-HSDH*				
	DHA	+ + +	+ +	+ +	+
	Pregnenolone	+ + + +	+ +	+ +	+
	17β-HSDH				
	Testosterone	+ + +	+ +	+ +	+ +
	17β-estradiol	+ + +	+ +	+ +	+
	11β-HSDH				
	11β-hydroxyandrostenedione	+ + +	+ +	+ +	+
9th day	*Δ^5-3β-HSDH*				
	DHA	+ + +	+ +	+ + +	+ + +
	Pregnenolone	+ + + +	+ +	+ + +	+ + +
	17β-HSDH				
	Testosterone	+ + +	+ + +	+ + +	+ + + +
	17β-estradiol	+ + +	+ + +	+ + +	+ + +
	11β-HSDH				
	11β-hydroxyandrostenedione	+ + +	+ + +	+ + +	+ + +
12th day and 15th day	*Δ^5-3β-HSDH*				
	DHA	+ + +	+ +	+ + +	+ + +
	Pregnenolone	+ + + +	+ +	+ + +	+ + +
	17β-HSDH				
	Testosterone	+ + +	+ +	+ + +	+ + + +
	17β-estradiol	+ + +	+ +	+ + +	+ + +
	11β-HSDH				
	11β-hydroxyandrostenedione	+ + +	+ +	+ + +	+ + +
Adult Before mating	*Δ^5-3β-HSDH*				
	DHA	+ + +	+ + +	+ + +	+ + +
	Pregnenolone	+ + + +	+ + +	+ + +	+ + +
	17β-HSDH				
	Testosterone	+ + +	+ + +	+ + +	+ + + +
	17β-estradiol	+ + +	+ + +	+ + +	+ + +
	11β-HSDH				
	11β-hydroxyandrostenedione	+ + +	+ + +	+ + +	+ + +
After mating	*Δ^5-3β-HSDH*				
	DHA	+ +	+ +	+ +	+ + +
	Pregnenolone	+ + +	+ +	+ +	+ + +
	11β-HSDH				
	Testosterone	+ +	+ +	+ +	+ + +
	17β-estradiol	+ +	+ +	+ +	+ +
	11β-HSDH				
	11β-hydroxyandrostenedione	+ +	+ +	+ +	+ + +
	Control	−	−	−	−

[a]All the chemicals are of Sigma grade, obtained from Sigma Co., USA.
[b]Intensity of reaction is graded from minimum (+) to intense (+ + + +) activity, (−) denotes absence of reaction and (±) denotes trace activity.



Figures 1–6. 1. Δ^5-3β-HSDH activity (dark granules) in the cells of the peritoneal sheath (PES) and epithelial layer (E), in the fresh frozen section of the testis of IV instar larva of *P. ricini* with DHA as the substrate. 2. Δ^5-3β-HSDH activity in the cells of the peritoneal sheath (PES), epithelial layer (E) and the spermatogoneal cells (SPC) in the fresh frozen section of the testis of one day old pupa of *P. ricini* with pregnenolone as substrate. 3. Δ^5-3β-HSDH activity in the cells of the peritoneal sheath (PES), epithelial layer (E), spermatogoneal cells (SPC), spermatids (S) and spermatozoan bundle (SB), in fresh frozen section of the testis of 15 day old pupa of *P. ricini* with pregnenolone as substrate. 4. Fairly intense Δ^5-3β-HSDH activity in the epithelial layer (E), spermatogonial cells (SPC), spermatids (S) and spermatozoan bundles (SB) in fresh frozen section of the testis of adult *P. ricini* before mating, with DHA as substrate. 5. 17β-HSDH activity in the spermatids (S) and spermatozoan bundles (SB) in fresh frozen section of the testis of 12 day old pupa of *P. ricini* with 17β-estradiol as substrate. 6. A weak 17β-HSDH activity in the epithelial layer (E), spermatids (S) and spermatozoan bundles (SB) in fresh frozen section of the testis of adult *P. ricini* after mating with testosterone as the substrate.

The scale in the figures indicate 40 μm.

more diformazan granules in the sperm bundles when testosterone was used as the substrate, indicating that the spermatozoan bundles seemed to have utilized testosterone better than 17β-estradiol.

In the testis of adult *P. ricini* the spermatozoan bundles utilized testosterone better than 17β-estradiol. The reaction in spermatogonial cells, primary and secondary spermatocytes, spermatids and spermatozoan bundle of adult after mating was reduced to moderate activity (figure 6).

The intensity and distribution of 11β-HSDH activity with 11β-hydroxyandrostenedione as the substrate, in the various components of the testis of IV and V instar larvae and on the day of investigation during the pupal period (1st, 3rd, 6th, 9th, 12th and 15th day) and in the adults, before and after mating in *P. ricini* (table 1) was similar to that of 17β-HSDH activity in the corresponding periods.

An intense G-6-PDH activity was observed in all the components of the testis in larva, pupa and adult of *P. ricini*. After mating a slight reduction in the enzyme activity was observed in all the testicular components except the cells of peritoneal sheath and epithelium of the testis.

The control sections incubated in the medium lacking either the substrate or the co-enzyme did not give any positive reaction but some times a very weak reaction was observed. The control sections treated with cyanoketone or isoxozol prior to incubation and incubated in normal medium containing DHA, did not give any histochemical reaction, thus indicating the specificity of Δ^5-3β-HSDH activity.

The steroid biosynthesis is not an exclusive prerogative of vertebrates, since steroid biosynthesis is wide spread throughout the animal kingdom (Barrington 1968). The histochemical demonstration of Δ^5-3β-HSDH activity is generally taken as an evidence, albeit indirect, that the particular tissue is capable of steroid metabolism or biosynthesis (Lehoux and Sandor 1970). *In vitro* and histochemical studies by Lehoux and Sandor (1970) have shown the occurrence of Δ^5-3β-HSDH in the testis of cockroaches, *G. portentosa*, *B. fumigata* and the cricket, *G. domesticus*. However, there is no detailed information as to which of the testicular components showed Δ^5-3β-HSDH activity. Recently Trandaburu and Tasca (1976) have histochemically demonstrated the presence of Δ^5-3β-HSDH activity in the spermatids and spermatozoan of two heteropteran species, *G. italicum* and *E. ventralis*. In the present investigation the cells of the peritoneal sheath and epithelium are the first testicular components to possess the Δ^5-3β-HSDH activity.

The occurrence of Δ^5-3β-HSDH in the spermatids and spermatozoan bundles in the testis of this insect is in conformity with that of *G. italicum* and *C. ventralis* (Trandaburu and Tasca 1976). The reduction of Δ^5-3β-HSDH activity in all the components of the adult after mating indicate the reduced metabolic rate of steroid hormones. In the present investigation occurrence of Δ^5-3β-HSDH in the testicular components during larval, pupal and adult stages, it may be suggested that the testis of developing and adult *P. ricini* possess the enzyme or enzymes necessary to convert exogenous pregnenolone to progesterone and DHA to androstenedione.

17β-HSDH catalyses the interconversion of androstenedione testosterone and 17β-estradiol-estrone in the gonads of vertebrates (Baillie *et al* 1966). 17β-HSDH enzyme system has been isolated from the various nonreproductive tissues and testis of *G. domesticus* (Lehoux and Sandor 1969) and the ovary and testis of *S. gregaria* (Dube and Lemonde 1970). In these studies the enzyme activity was shown to be highest in the male and female gonads on tissue weight basis. 17β-HSDH activity has also been histochemically demonstrated in the ovary of *Bombyx mori* and *Antheraea mylitta* (Hurkadli *et al* 1988).

In the present investigation a weak 17β-HSDH activity in the various components of the testis of IV and V instar larva of *P. ricini* indicates a weak metabolism of sex steroids. The activity of this enzyme was gradually extended to the spermatogonial cells, primary and secondary spermatocytes, spermatids and spermatozoan bundles of the testis during pupal period. At the same time even the intensity of reaction was also increased. These results indicate the increased quantity of 17β-HSDH activity

being present in these tissues during latter half of pupal period. This in turn may result in the increased rate of sex steroid metabolism. In the last few days of pupal period, the activity of this enzyme was maximum which may be correlated to the increased metabolism of testosterone and 17β-estradiol by spermatozoans during that period. 17β-HSDH activity found in the various components of the testis of adult after mating is reduced which may be due to cessation of spermatogenic activity of the testis. Our histochemical findings, that the testis of *P. ricini* contains 17β-HSDH activity is in conformity with the biochemical investigations of other workers (Lehoux and Sandor 1969, 1970; Ohnishi 1985). The present results indicate that the testis of larva, pupa and adult *P. ricini* possesses 17β-HSDH activity which can bring about the interconversions of testosterone-androstenediol and 17β-estradiol-estrone. It may be suggested that the testis of *P. ricini* has the capacity to metabolize the sex steroids.

The occurrence of corticosterone and cortisol in the haemolymph of the cricket *G. domesticus* has been reported (Lehoux and Sandor 1970). The ovaries and pyloric caeca of the star fish, *A. rubens* are known to produce *11*-desoxycortosterone (Schoenmakers and Voogt 1980). 11β-HSDH activity has been histochemically demonstrated in the ovaries of *B. mori* and *A. mylitta* (Hurkadli *et al* 1988). In the present investigation 11β-HSDH activity has been demonstrated in the testis of larva, pupa and adult *P. ricini* and this indicates the ability of the testis to metabolize 11β-hydroxysteroids.

G-6-PDH provides energy needed for hydroxylation during steroidogenesis or steroid metabolism in steroidogenic tissues of vertebrates (McKerns 1968). G-6-PDH activity indicates the presence of NADPH generating system in the testis of developing and adult *P. ricini.*

In conclusion the present investigation indicates that the testis of the larva, pupa and adult *P. ricini* has the necessary steroid converting enzymes that can convert the exogenous hydroxysteroids to ketosteroids.

Acknowledgements

The authors are grateful to WHO, Geneva for the gift of fine chemicals. One of the authors (HKH) is thankful to the University Grants Commission, New Delhi for a fellowship.

References

Baillie A H, Ferguson A and Hart D McK 1966 *Developments in steroid histochemistry* (New York: Academic Press)

Bara G 1965a Histochemical localization of Δ^5-3β-hydroxysteroid dehydrogenases in the ovaries of a teleost fish, *Scomber scomber* (L); *Gen. Comp. Endocrinol.* **5** 284–296

Bara G 1965b G-6-phosphate dehydrogenase activity in the ovaries of *Scomber scomber* (1); *Experientia* **21** 638–640

Barrington E J W 1968 Phylogenetic perspectives in vertebrate endocrinology; in *Perspectives in vertebrate endocrinology* (eds) E J W Barrington and C G Jorgensen (New York: Academic Press) pp 1–46

Dube J and Lemonde A 1970 Transformations des Steriodes par la femelle adulte d'un insecte orthopterè, *Schistocerca gregaria* Forskal: *Gen. Comp. Endocrinol.* **15** 158–164

Hurkadli H K, Hooli M A and Nadkarni V B 1988 Hydroxysteroid dehydrogenases in the ovaries of silkworm, *Bombyx mori* and *Antheraea mylitta* (Lep.)—A histochemical study; *Sericologia* (in press)

Lehoux J A, Sondor T, Lanthier A and Lusis O 1968 Metabolism of exogenous progesterone by insect tissue preparations *in vitro*; *Gen. Comp. Endocrinol.* **11** 481–488

Lehoux J G and Sandor T 1969 Conversion of testosterone Δ^4-androstene-3, 17-dione by house cricket, *Gryllus domesticus*, male gonad preparations *in vitro*; *Endocrinology* **84** 652–657

Lehoux J G and Sandor T 1970 The occurrence of steroid metabolizing enzyme systems in invertebrates; *Rev. Steroids* **16** 141–171

McKerns K W 1968 Mechanism of ACTH regulation of the adrenal cortex: in *Functions of adrenal cortex* (ed.) K W McKerns (New York: Appleton Century Crofts) **1** 4.[illegible]–537

Ohnishi E, Ogiso M, Wakabyashi K and Fujimoto N 1985 Identification of estradiol in the ovaries of silkworm, *Bombyx mori*; *Gen. Comp. Endocrinol.* **60** 35–38

Sandor T, Sonea S and Mehdi A Z 1975 The possible role of steroids in evolution; *Am. Zool.* (*Suppl.* 1) **15** 227–253

Saidapur S K and Nadkarni V B 1972 Δ^5-3β-hydroxysteroid dehydrogenases and glucose-6-phosphate dehydrogenases in the testis of toad, *Bufo melanosticus* (Schneider); *Indian J. Exp. Biol.* **10** 425–527

Schoenmakers H J N and Voogt P A 1980 *In vitro* biosynthesis of steroids from progesterone by ovaries and pyloric caeca of star fish; *Gen. Comp. Endocrinol.* **41** 408–416

Thompson M J, Kaplanis J N, Robbins W E and Svoboda J A 1973 Metabolism of steroids in insects; *Adv. Lipid Res.* **11** 219–265

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