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Hydroxysteroid dehydrogenases in the ovary of larva, pupa and adult eri silkworm, *Philosamia ricini* (Hutt.)—A histochemical study

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Abstract. Histochemical analysis of the various developmental stages of *Philosamia ricini* showed the presence of Δ^5-3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, 11β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase in the lamellar epithelial cells, precursors of oocytes, nurse cells and follicle epithelial cells of the germarium of larva and pupa, oocyte-nurse cells complex, follicle epithelial cells of immature and mature follicles and intermediate cells of pupa suggesting that these tissues are able to metabolize Δ^5-3β-hydroxysteroids, 17β-hydroxysteroids and 11β-hydroxysteroids to corresponding ketosteroids.

Keywords. Eri silkworm; ovary; vitellogenesis; hydroxysteroid dehydrogenases.

1. Introduction

Steroids other than ecdysones are known to occur in the ovaries of invertebrates (Gottfried *et al* 1967; Carreau and Drosdowsky 1977; Schoenmakers and Voogt 1980). *In vivo* studies have shown that the ovary of the locust, *Schistocerca gregaria*, is capable of transforming vertebrate steroids (Dube and Lemonde 1970). Recently the presence of estradiol in the ovary of *Bombyx mori* and its role in the ovarian development have been studied (Ohnishi *et al* 1985; Ogiso and Ohnishi 1986; Ogiso *et al* 1986). In the present investigation histochemical technique has been employed to demonstrate the presence (or absence) of the enzymes involved in the metabolism of steroids in the ovaries of larva, pupa and adult *Philosamia ricini*. In addition the distribution of glucose-6-phosphate dehydrogenase (G-6-PDH), in the ovary of this silkworm has also been studied.

2. Materials and methods

The larva, pupa and adult *P. ricini* were obtained from the laboratory bred stock. For histological study the ovaries of respective stages were fixed in Bouin's fluid for 20–25 h. The tissues were processed in alcohol grades and embedded in paraffin wax and 5 μm sections were cut and stained with haematoxylin and eosin.

For histochemical studies, the larva, pupa and adult were decapitated and the ovaries were removed immediately and frozen over dry ice vapour at $-50°C$ and sectioned in a cryostat at $-20°C$. The histochemical methods employed are similar to those employed for the steroid hormone producing tissues of vertebrates. For the histochemical demonstration of Δ^5-3β-hydroxysteroid dehydrogenase (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

Table I. Activity of HSDH in the ovary of larva, pupa and adult *P. ricini*.

Developmental stages and stages of follicles in *P. ricini*	Enzymes and the substrates*	Intensity of reaction**					
		Lamellar epithelium	Precursor cells in germarium	Nurse cells	Follicle epithelial cells	Oocyte	Intermediate cells
IV and V instar larvae							
1st and 3rd day of each instar in 1st and 2nd stage follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione	+ + +	Activity could not be ascertained***				
Pupae							
1st, 3rd, 6th and 9th day, in 3rd stage follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydrodyandrostenedione)	+ + +	+ +	−	+ +	−	+ +
4th, 5th and 6th stage follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	+ + +	+ +	+ +	+ +	−	+ + +
12th and 15th day, in 7th and 8th stage follicles	Δ^5-3β-HSDH (DHA and pregnenolone) 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	+ + +	+ +	+ + +	+ + +	+ + + (Peripheral region only)	+ + +
9th stage follicle	Δ^5-3β-HSDH (DHA	+ + + +	+ +	+ +	+ + +	+ + +	+ +
	pregnenolone),	+ + +	+ +	+ +	+ + +	+ + +	+ +
	17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	+ + +	+ +	+ +	+ + +	+ + +	+ +

Adult moths, in immature follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	Tissue absent	+ + +	+ + +	+ + +	+ + +	Cells absent
Adult moths, in mature follicles (stage 10 follicles)	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	Tissue absent	In mature follicles nurse cells (5) and follicle epithelial cells (6) are not found. It contains only the mature oocyte (7) surrounded by chorion.			+ + +	Cells absent
Atretic follicles	Δ^5-3β-HSDH (DHA and pregnenolone), 17β-HSDH (testosterone and 17β-estradiol) and 11β-HSDH (11β-hydroxyandrostenedione)	Immature follicles consisting of nurse cells (5), follicle epithelial cells (6) and oocyte (7) undergo atresia	+ + +	+ + +	+ + +	+ + +	Cells absent

*All the chemicals are of sigma grade, obtained from Sigma Chemical Co., USA.

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

***In the IV and V instar larvae the presence of formazan granules could not be confirmed in the precursor cells, nurse cells, follicle epithelial cells and oocytes of the germarium due to their compact nature and scanty cytoplasm.

described earlier (Baillie *et al* 1966; Hurkadli *et al* 1988a). After incubation the sections were washed in distilled water fixed in 10% neutral formalin for 30 min and mounted in glycerol gelly. Parallel sections incubated in the media lacking the respective substrates served as controls. A few sections were also incubated in a medium containing dehydroepiandrosterone (DHA) and isoxozol, an inhibitor of Δ^5-3β-HSDH activity as a specific control for Δ^5-3β-HSDH activity.

3. Results and discussion

The ovary of *P. ricini* is polytrophic meroistic type. Histologically the development of the ovary leading to the formation of mature egg can be broadly divided into 4 distinct phases with 10 follicle stages (Hurkadli *et al* 1988b).

Table 1 summarises the results on histochemical reaction for HSDH in the developing and adult ovary of *P. ricini.*

During larval stage of *P. ricini* a moderate Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the lamellar epithelial cells of the ovarian cap and a few formazan granules were present in the matrix cells. From fourth day of pupation a weak Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the precursors of oocyte, nurse and follicle epithelial cells of stages 1 and 2 follicles in the germarium. The reaction was not noticed in the oocyte-nurse cells complex and in ovariolar sheath. A weak reaction was observed in the intermediate cell layer, in the follicle epithelial cells surrounding oocyte-nurse cells complex and in the ovariolar sheath (figure 1). Parallel consecutive frozen sections were rapidly stained with haematoxylin and eosin and observed under the microscope to identify the cells that showed formazan granules in the sections used for histochemical study.

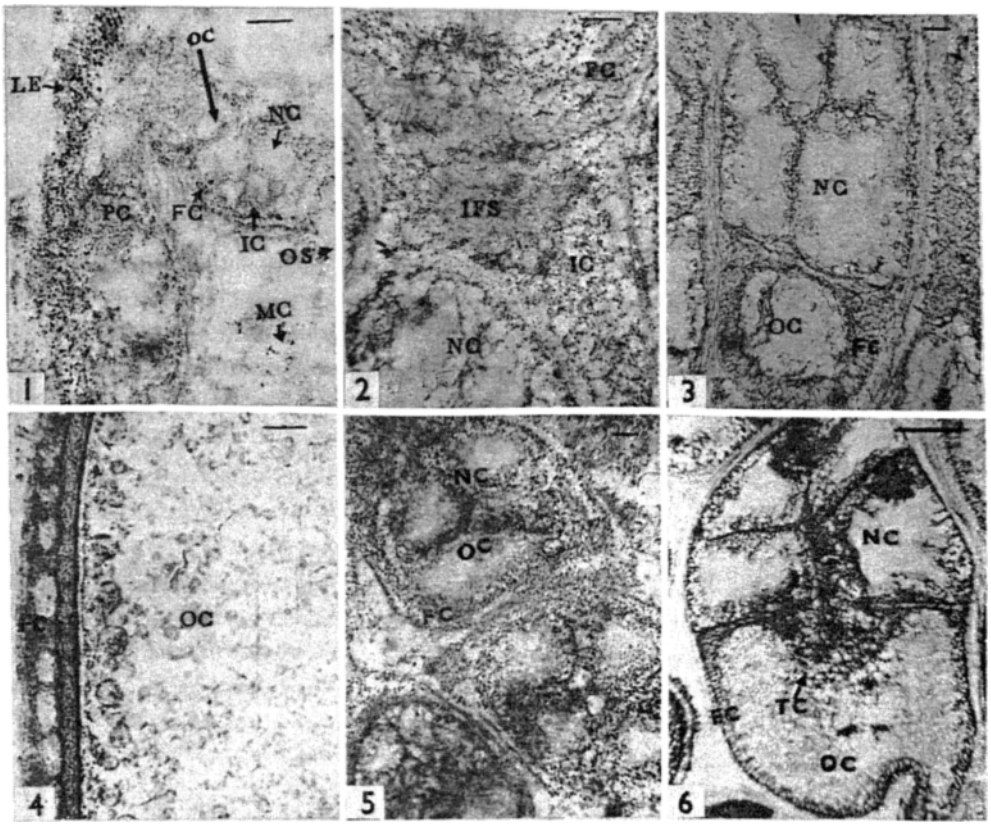
During 4th-6th follicle growth stages, a weak Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the follicle epithelial cells and nurse cells. Intermediate cells showed a moderate activity for all the 3 HSDH. The activity of these enzymes were absent in the ooplasm and in the interfollicle stalk cells (figure 2).

In the 7th and 8th stage vitellogenic follicles a moderate Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the follicle epithelial cells, nurse cells, intermediate cells and in the peripheral region of the ooplasm (figure 3). In the 9th vitellogenic stage follicle, the enzymes activity were spread all over in the ooplasm and slightly reduced in the nurse cells. With Δ^5-3β-HSDH activity, the substrate DHA was relatively better utilized than pregnenolone in the 9th vitellogenic stage.

In stage 10 follicles a moderate Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity was observed in the ooplasm and degenerating follicle epithelial cells (figure 4). The activity of these enzymes were also seen in the phagocytic follicle epithelial cells, degenerating nurse cells and ooplasm in the atretic follicles.

The intensity and distribution of 17β-HSDH activity with substrates, testosterone and 17β-estradiol were equally well utilized by the various components of the ovary during larval, pupal and adult stages.

An intense G-6-PDH activity in the lamellar epithelial cells and a moderate activity in the matrix cells of the ovarian cap of larva of *P. ricini* was observed. Some of the precursor cells in the germarium also showed a moderate G-6-PDH activity. In the 2 and 3 stage follicles, an intense G-6-PDH activity was observed in



Figures 1–6. 1. Δ^5-3β-HSDH activity in the lameller epithelium (LE) and matrix cells (MC) of the ovarian cap, in some of the precursors cells (PC), follicle epithelial cells (FC) and intermediate cells (IC) of the stage 3 follicle in fresh frozen section of the ovary of fourth day old pupa of *P. ricini*. Note the absence of formazan granules in the oocyte (OC) and nurse cells (NC), DHA was used as the substrate. 2. Δ^5-3β-HSDH activity in the FC, IC and NC of the fifth stage follicle in the fresh frozen section of the ovary of 9-day old pupa of *P. ricini*. Note the absence of formazan granules in the interfollicle stalk cells (IFC). Pregnenolone was used as substrate. 3. Δ^5-3β-HSDH activity in the FC, peripheral region of the ooplasm of the OC and in the cytoplasm of the NC of the seventh stage early vitellogenic follicle in fresh frozen section of the ovary of *P. ricini*. DHA was used as substrate. 4. Δ^5-3β-HSDH activity in the FC after the formation of chorion and in the ooplasm of the OC in the mature egg (stage 10 follicle) of the 15-day old pupa of *P. ricini*. DHA was used as the substrate. 5. G-6-PDH activity in the FC and NC of the stage 4 follicle, in fresh frozen section of the ovary of 3-day old pupa of *P. ricini*. 6. G-6-PDH activity in the NC, FC, trophic cord (TC) and ooplasm of the OC of stage 7 follicle in the fresh frozen section of the ovary of 12-day old pupa of *P. ricini*.
The scale line indicates 40 μm.

the follicle epithelial cells, nurse cells (figure 5). Nurse and follicle epithelial cells, interfollicle stalk cells in 5 and 6 stage follicles continued to show an intense G-6-PDH activity, while intermediate cells showed a moderate activity. In the vitellogenic follicles an intense G-6-PDH activity was observed along the tropic cord and in the peripheral region of the ooplasm (figure 6). An intense activity of this enzyme was seen in the peripheral region and a moderate activity in the

remaining part of the mature egg. The atretic follicles of *P. ricini* showed an intense G-6-PDH activity.

The *in vitro* and *in vivo* conversion of DHA to androstenedione, pregnenolone to progesterone indicate the presence of Δ^5-3β-HSDH activity in the ovaries of mollusc, *Sepia officianalis* (Carreau and Drosdowsky 1977), star fish, *Asterias rubens* (Schoenmakers and Voogt 1980) and insects, *S. gregaria* (Dube and Lemonde 1970), *B. mori* and *Antheraea mylitta* (Hurkadli *et al* 1988c). In the present investigation, a moderate Δ^5-3β-HSDH activity obtained in the lamellar epithelial cells of ovarian cap of larval and pupal stages and a weak activity obtained in the follicle epithelial cells of third stage follicle and a moderate activity in the follicle epithelial cells, nurse cells and intermediate cells during 4th, 5th and 6th follicle growing stages may indicate the ability of these tissues to metabolize DHA to androstenedione and pregnenolone to progesterone. The absence of Δ^5-3β-HSDH activity in the oocyte-nurse cells complex and ovariolar sheath in stage 3 follicles may indicate that they are not able to metabolize DHA and pregnenolone during this stage. The activity in traces obtained in the matrix cells during larval and pupal period may indicate its ability to metabolize these steroids to some extent.

After the initiation of vitellogenesis oocyte showed Δ^5-3β-HSDH activity in the peripheral region of the ooplasm. This observation indicates that oocyte attains ability to metabolize DHA and pregnenolone only after the commencement of vitellogenesis but not prior to it. The absence of Δ^5-3β-HSDH activity in the interfollicle stalk cells suggest that they do not have the capacity to metabolize these steroids. The presence of Δ^5-3β-HSDH activity in the precursors cells in the germarium, the immature and mature oocytes in the ovarioles of adult and in the atretic follicles of *P. ricini* suggest their ability to metabolize DHA and pregnenolone.

The gonadal and non-gonadal tissues of the cricket, *G. domesticus* are known to transform testosterone to androstenedione (Lehoux and Sandor 1969). In the ovary of *B. mori* estradiol was extracted, identified and estimated by radioimmunoassay and chromatography-mass spectrometry (Ohnishi *et al* 1985) and its role in the ovarian maturation has also been studied (Ogiso and Ohnishi 1986) 17β-HSDH has been histochemically demonstrated in the ovary of *B. mori* and *A. mylitta* (Hurkadli *et al* 1988c).

The presence of 17β-HSDH activity in the various developing and adult ovarian components of *P. ricini* indicate their ability to bring about the interconversions of testosterone to androstenediol and 17β-estradiol to estrone.

11β-HSDH is known to transform 11β-hydroxysteroids to 11-ketosteroids. Amongst insects, prothoracic glands of water beetle, *D. marginalis* is known to produce 11-deoxycorticosterone (Schildknecht *et al* 1966). The presence of 11β-HSDH has been shown in the ovary of *B. mori* and *A. mylitta* (Hurkadli *et al* 1988c). The presence of 11β-HSDH in the ovaries of larva, pupa and adult *P. ricini* and atretic follicles indicate their ability to metabolize 11β-hydroxysteroids.

G-6-PDH is involved in generating reduced nicotinamide adenine dinucleotide phosphate (NADPH) in steroid synthesizing or metabolizing tissues of vertebrates (Mc Kerns 1968). The intensity of G-6-PDH reaction was more than that of Δ^5-3β-HSDH, 17β-HSDH and 11β-HSDH activity. G-6-PDH was also noticed in the interfollicle stalk cells, where the activity of HSDH was not present. From the present investigation it may be inferred that G-6-PDH has wider distribution

compared to HSDH. Further its presence in the ovarian cells that also possess steroid converting enzymes provides an additional evidence that the ovary of *P. ricini* has the potential to convert hydroxysteroids to ketosteroids. The study also confirms the findings of earlier workers (Lehoux and Sandor 1970; Schoenmakers and Voogt 1980; Ohnishi *et al* 1985; Ogiso and Ohnishi 1986; Hurkadli *et al* 1988c) that the so called vertebrate steroids are not the exclusive features of vertebrate gonads but also found in many invertebrates.

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