

Histoenzymological differences in the envelope of white and stages of rapidly growing yellow follicles in the ovary of crow (*Corvus splendens*)

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Abstract. Histoenzymological differences in the localization and intensities of succinate and lactate dehydrogenases, tetrazolium reductases and steroid dehydrogenases have been studied in the follicular envelope of white and stages of yellow follicles of the crow ovary. The activities of these enzymes in the thecal gland cells show a progressive increase during follicular growth. The granulosa layer of white follicle is devoid of Δ^5 - 3β -hydroxysteroid dehydrogenase which appear and show a gradual increase in the rapidly growing yellow follicles along with other enzymes.

Keywords. Ovarian follicles; dehydrogenases; *Corvus splendens*.

1. Introduction

Related to the deposition of yolk and secretion of steroid hormones during rapid growth and maturation of avian follicle the follicular envelope undergoes marked morphological, histochemical and biochemical changes (Perry *et al* 1978; Guraya 1978; Gilbert 1979). These studies have been carried out mainly on the chick ovary. Our knowledge about the histophysiology of follicular envelope in seasonally breeding birds is very meagre as the rapidly growing vitellogenic follicles occur only during the reproductive phase. However, Chalana and Guraya (1980) have described morphological and histochemical changes in the follicle wall of early growing follicles in the crow ovary. This study was initiated to determine the histoenzymological differences related to metabolic and steroidogenic features in the follicular wall of white and rapidly growing yellow follicles in the crow ovary.

2. Materials and methods

Female specimens of crow (*Corvus splendens*), obtained by shooting during breeding months were used for study (July–September). The fully developed ovaries of 10 birds were immediately removed and the large vitellogenic follicles of variable dimensions (table 1) were separated from the rest of the ovarian tissues. The follicles were then immediately mounted on discs and placed under the heat extraction chamber of cryocut to speed up the freezing process. Sections (12–14 μm) were cut in a cryostat at 20°C. The frozen sections were placed on coverslips, thawed and kept at room temperature for 10–15 min before incubation in different media for localization of NADH and NADPH-tetrazolium reductases, lactate and succinate dehydrogenases and Δ^5 - 3β -hydroxysteroid dehydrogenase (table 1). For localization of these enzymes, the tetrazolium salt procedures were followed (Lojda *et al* 1979). Simultaneously, the control sections were also stained by omitting the substrate in each case.

Table 1. Histochemical localization of enzymes in the wall of white and rapidly growing follicles.

Enzyme	Yellow follicles																			
	White follicles				Stage 1 (4 mm)*				Stage 2 (7 mm)				Stage 3 (10 mm)				Stage 4 (15 mm)			
	GC	TC	GC	TC	GC	TC	GC	TC	GC	TC	GC	TC	GC	TC	GC	TC	GC	TC		
NADH-tetrazolium reductase	b	c	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d		
NADH-tetrazolium reductase	a	b	b	c	b	c	c	d	c	d	c	d	c	d	c	d	c	d		
Succinate- dehydrogenase	b	a	b	b	b	c	c	c	c	c	c	c	c	c	a	b	a	b		
Lactate dehydrogenase	a	a	c	b	c	b	c	b	c	b	c	b	c	b	a	a	a	a		
Δ^5 - 3β -hydroxy- steroid dehydrogenase	0	b	b	c	b	c	b	c	b	c	c	c	c	c	c	c	c	b		

GC, granulosa cells; TC, thecal gland cells; 0, negative reaction; a, weak reaction; b, moderate reaction; c, strong reaction; d, very strong reaction. * Figures in parentheses indicate average diameter of ten follicles selected for localization of enzymes.

3. Results

The observations on the histochemical reactions in the wall of white and rapidly growing follicles are summarized in table 1.

3.1 *Tetrazolium reductases*

Both NADH and NADPH-dependent tetrazolium reductases show similar sites of positive reactions (granulosa and thecal gland cells) in follicles of all stages. However, NADPH-tetrazolium reductase shows relatively lesser activity (table 1). The wall of rapidly growing yellow follicles shows increasing enzyme activities as compared to that of white follicles. Thecal gland cells in the theca interna of stages 1 and 2 follicles contain relatively more enzyme activity than the granulosa cells (figures 1, 2). The thecal gland cells in follicles of stages 3 and 4 are mainly present in the theca externa but they continue to show almost similar staining intensity for both the tetrazolium reductases as observed in stages 1 and 2 which is also slightly more than the granulosa cells. In addition, a continuous zone staining intensely for these enzymes is observed in the theca interna (figure 3). The zone of the theca interna present between the intensely stained theca interna and granulosa layer shows weak to moderate reaction in early stages (figures 1, 2) but the enzyme activity disappears completely in stage 3 follicles. As compared to white follicles, marked increase occurs in enzyme activities in the follicular epithelium of yellow follicles which show no conspicuous difference among stages 1 to 4 follicles.

3.2 *Lactate and succinate dehydrogenases*

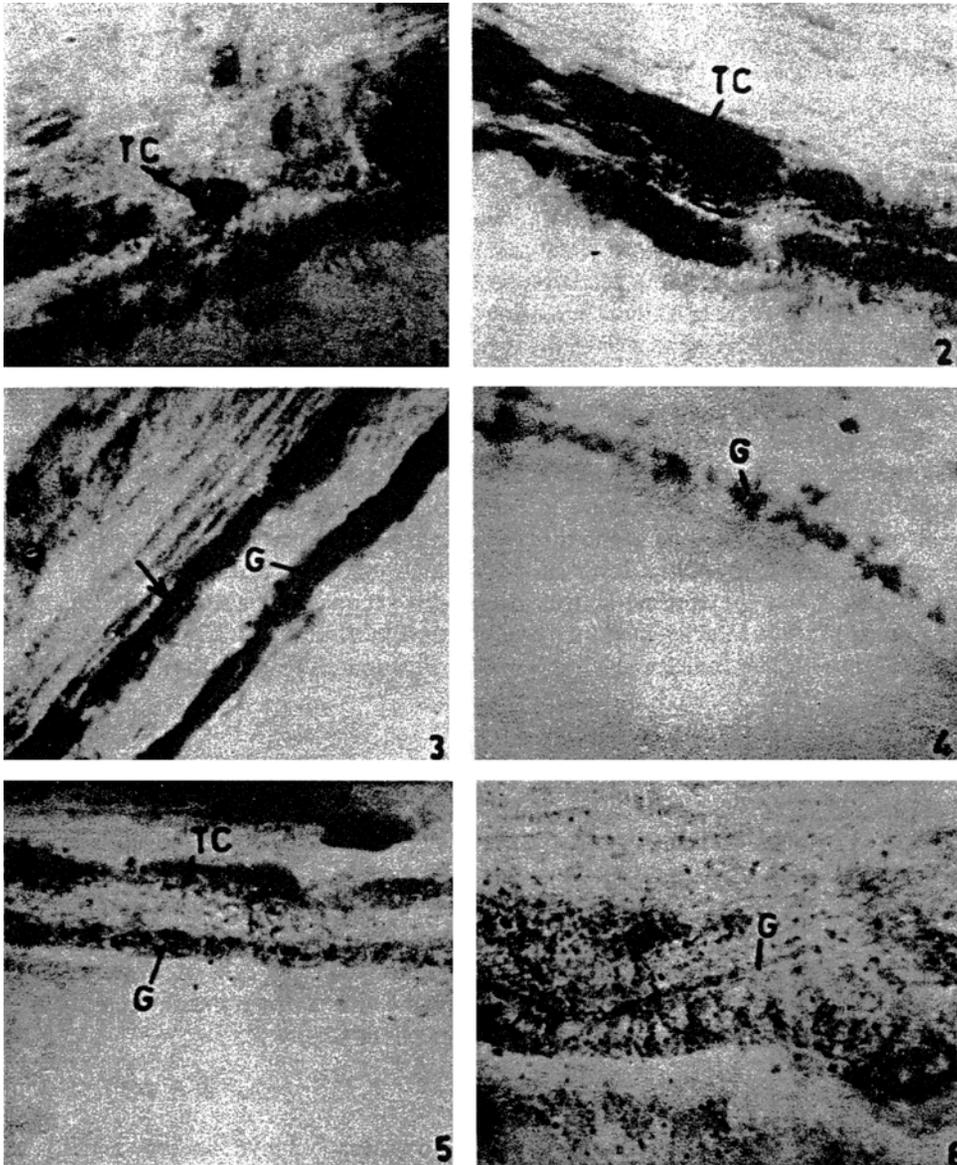
Lactate and succinate-dehydrogenase activities in granulosa and thecal gland cells as compared to white follicles (figure 4) show a marked increase during rapid follicular growth phase up to stage 3. The granulosa cells show more lactate dehydrogenase activity than the thecal gland cells of stages 1 to 3 but no difference has been observed for succinate dehydrogenase (table 1). A decrease of lactate and succinate dehydrogenase activities is seen in theca as well as in granulosa cells of largest follicle (stage 4). In stages 3 and 4, a zone of theca interna which shows tetrazolium reductase activities also reacts intensely for both these enzymes (figure 5).

3.3 *Steroid dehydrogenases*

Using pregnenolone as substrate, Δ^5 - 3β hydroxysteroid dehydrogenase activity is observed in the granulosa and thecal gland cells of rapidly growing follicles (figure 6). In contrast, the granulosa cells of the white follicles do not show enzyme activity.

4. Discussion

A comparison of the histochemical characteristics of the wall of white and large vitellogenic follicles has revealed conspicuous changes in the activities of NADH- and NADPH-tetrazolium reductases, succinate and lactate dehydrogenase and Δ^5 - 3β -hydroxysteroid dehydrogenase. Increased activity of both the tetrazolium reductases in the granulosa and thecal cells of the rapidly growing follicle indicates enhanced rate of reoxidation of NADH and NADPH coenzymes (Lojda *et al* 1979). The granulosa and thecal



Figures 1-6 ($\times 400$). 1-3. Stages of follicle showing NADH tetrazolium reductase activity in granulosa cells (GC) and thecal cells (TC) 1. stage 1. 2. stage 2. 3. stage 4. Note a continuous zone (arrow) in the theca interna having intense enzyme activity. 4. Section of the large white follicle showing succinate dehydrogenase activity in the GC. 5. stage 3 follicle showing lactate dehydrogenase activity in the GC and TC. 6. stage 2 follicle showing Δ^5 - 3β -hydroxysteroid dehydrogenase activity in the GC.

cells of hierarchical follicles in the chick ovary are the well known sites of steroid synthesis and secretion (Huang and Nalbandov 1979; Huang *et al* 1979; Hammond *et al* 1980). The increase in tetrazolium reductases in the wall of rapidly growing follicles of the crow ovary may also be associated with the increased rate of steroid biosynthesis

during this phase of growth. This is also supported by the increased activity of Δ^5 - 3β -hydroxysteroid dehydrogenase in the wall of rapidly growing follicles. The wall of early growing follicles in chick have been found to contain Δ^5 - 3β -hydroxysteroid dehydrogenase activity (Chieffi and Botte 1965; Boucek *et al* 1966) but in the crow follicle, especially in the granulosa cells, the enzyme activity appears only during rapid growth phase of the follicle, suggesting its increased steroidogenic activity. Although the extent of contribution of follicles to the plasma steroid levels has not been determined in the wild bird species, the present histochemical study has shown that similar to chick, in the rapid growth phase, the follicles in the crow ovary are also involved in steroid biosynthesis.

In addition to steroidogenesis, the follicular envelope during the rapid growth phase is also involved actively in the transport of yolk precursors (Jordanov and Boyadjieva 1974). The increase in metabolic enzymes such as during this phase of the follicular growth may be associated with the increased rate of energy consumption.

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