

Postnatal Evolution of Steroidogenic Cells in the Chick Ovary*

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Summary. From hatching to sexual maturity, the left ovary of the chick was studied with cytochemical techniques for the demonstration of steroid synthesis. At the time of hatching, there are in the ovarian medulla cell groups loaded with lipids and cholesterol which give a positive reaction for Δ^5 - 3β -hydroxysteroid dehydrogenase (Δ^5 - 3β -HSDH). Because of the predominant growth of the cortex in the course of the postnatal morphogenesis of the ovary, these cells are shifted to the theca interna of the follicles and the interfollicular spaces (interstitial cells). The cytochemical tests used demonstrate that, at all the stages studied, there is steroid synthesis in these cells. From the 20th day on, granulosa cells show an unspecific enzymatic reaction, which is positive in the control sections incubated without substrate. A strong Δ^5 - 3β -HSDH activity is observed in the granulosa cells of postovulatory follicles. The significance of these findings in relation to the life history of steroid producing cells in the embryonic and postnatal chick ovary is discussed.

Introduction

The production of estrogens, progesterone, and androgens by the adult ovary of the domestic fowl has been demonstrated with the use of different technical approaches (see VAN TIENHOVEN, 1961). Histological and histochemical studies have suggested that the cells responsible for the synthesis of steroids comprise some cells of the theca interna loaded with lipids and cholesterol; groups of cells with similar characteristics present among the follicles in the interstitial tissue (i.e. interstitial cells), and the granulosa cells of growing and postovulatory follicles (CHIEFFI and BOTTE, 1965; WOODS and DOMM, 1966).

In the embryonic ovary of the chick the synthesis of estrogens was demonstrated with biological (WOLFF, 1946) and biochemical methods (GALLIEN and LE FOULGOC, 1957; CEDARD and HAFFEN, 1966; WOLFF, HAFFEN and SCHEIB, 1966). With histochemical methods and electron microscopy it has been shown that the cells responsible for the production of steroids are the interstitial cells situated in the medulla of the embryonic ovary (NARBAITZ and SABATINI, 1963; NARBAITZ and KOLODNY, 1964; NARBAITZ and ADLER, 1966; SCHEIB and HAFFEN, 1967).

The present investigation was started with the purpose of studying, at the cellular level, the localization of steroid synthesis during the postnatal period and in the adult ovary of the domestic fowl. The morphogenetic changes which the interstitial cells of the embryonic medulla undergo to attain their definite distribution in the adult will be described.

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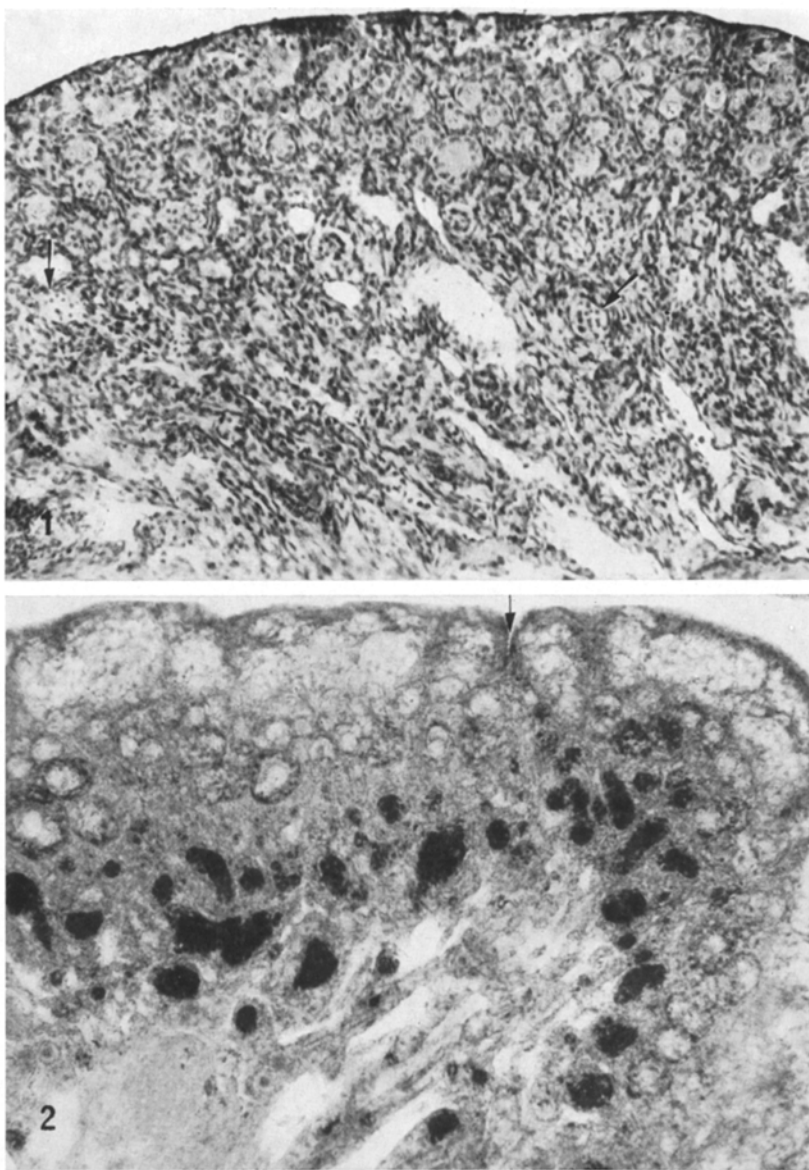


Fig. 1. Microphotograph of a 10th day chick ovary. The cortex and medulla are well defined. With arrows are indicated islets of clear cells in the medulla (H and E). $\times 150$

Fig. 2. 13th day ovary. The cortex has begun the folding process. An invagination of the surface is indicated (arrow). Cell islets loaded with lipids are observed in the medulla (Sudan Black B). $\times 150$

Methods

The ovaries of 34 New Hampshire chickens were used. This total included: 4 adult laying hens and 30 chickens of 2, 10, 13, 20, 30, and 60 days of age.

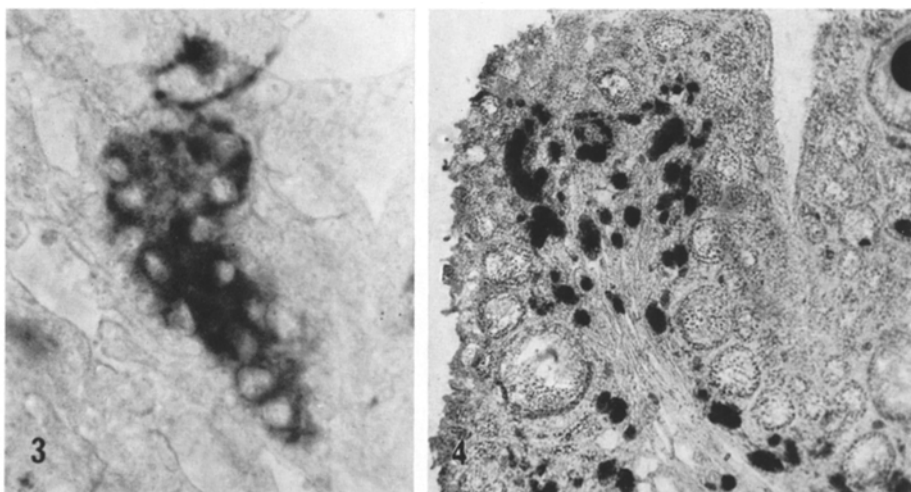


Fig. 3. 13th day ovary. Formazan deposits due to the $\Delta^5\text{-}3\beta\text{-HSDH}$ reaction are observed in the cytoplasm of interstitial cells. $\times 900$

Fig. 4. 30th day ovary. An ovarian fold with the central axis of connective and vascular tissue and a cortex with growing follicles is observed. The steroid-producing cells are migrating toward the cortex ($\Delta^5\text{-}3\beta\text{-HSDH}$ reaction). $\times 100$

Part of the material, fixed in 10 per cent formalin with addition of 1 per cent calcium chloride, was sectioned with a freezing microtome. The sections were subjected to the following histochemical lipid tests: Sudan Black B for lipids in general, birefringence, and Schultz test for cholesterol. Another portion of the ovary was sectioned at 10μ in a cryostat, without previous fixation, and used for the histochemical determination of the $\Delta^5\text{-}3\beta\text{-hydroxy-steroid dehydrogenase}$ ($\Delta^5\text{-}3\beta\text{-HSDH}$) according to the technique of LEVY, DEANE and RUBIN (1959). The substrate, dehydroepiandrosterone (DHA), was dissolved either in propylenglycol or NN'-dimethylformamide. Control sections were run in media: without DHA, without nicotinamide-adenine-dinucleotide (NAD) and DHA, and with addition of 0.02 M iodoacetate without substrate. At all stages histological controls, fixed in Bouin's fluid and stained with hematoxylin-eosin (H and E), were made.

Results

All the ovaries studied, whatever the stage of development, showed the presence of cell islets characterized by their clear cytoplasm, an intense staining with Sudan Black B, positive birefringence and Schultz' test. These cells also showed a positive reaction for the $\Delta^5\text{-}3\beta\text{-HSDH}$. Such cytological and cytochemical characteristics permitted these cell groups to be followed during the postnatal development of the gonad.

A 2 day ovary is essentially similar to that previously described in the last stages of the differentiating chick embryo gonad (NARBAITZ and SABATINI, 1963). The cortical and medullary layers are well defined. The former is occupied by the ovocytes and the second contains, among the connective tissue, islets of interstitial cells, loaded with lipids and cholesterol which give an intensely positive reaction for $\Delta^5\text{-}3\beta\text{-HSDH}$. A 10 day ovary is still similar but the ovocytes appear surrounded by follicular cells forming primary follicles (Fig. 1).

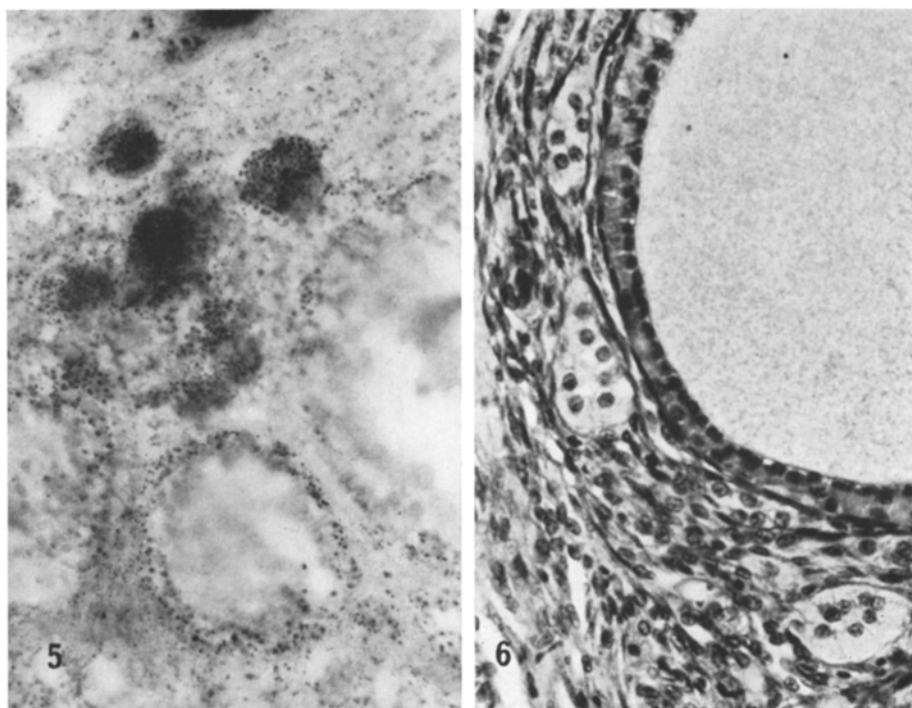


Fig. 5. 30th day ovary. Interstitial cells with a strong enzymatic reaction are observed among the follicles. A lighter and unspecific reaction is observed in the granulosa cells. (Δ^5 - 3β -HSDH reaction). $\times 270$

Fig. 6. 60th day ovary. Islets of interstitial cells are embedded in the theca interna of the follicles (H and E). $\times 450$

From the 13th to the 20th day remarkable morphogenetic changes take place in the ovary. There is a preponderant growth of the cortical layer which results in the formation of folds having an axis of connective and vascular tissue of medullary origin and a superficial layer in which the cortical and medullary components become gradually mixed. Fig. 2 shows the beginning of this process in which the surface starts to invaginate and the interstitial cell islets, loaded with lipids, become displaced toward the cortex. Fig. 3 shows, at high magnification, the strong Δ^5 - 3β -HSDH activity in an islet of interstitial cells.

From the 20th to the 60th day the morphogenetic process continues and the folds of the cortex become very deep by the 30th day. At this time the follicles show considerable growth and the interstitial cells occupy the connective tissue axis, as well as the interfollicular spaces (Fig. 4). During this period the Δ^5 - 3β -HSDH reaction is very strong in the interstitial cells (Figs. 4 and 5) but the follicular cells (i.e. granulosa layer) show a slight unspecific reaction (Fig. 5). In fact, such a reaction appears also in the control sections without substrate and in those incubated with iodoacetate. However the reaction is negative in media without NAD. This unspecific reaction is not affected by the previous extraction

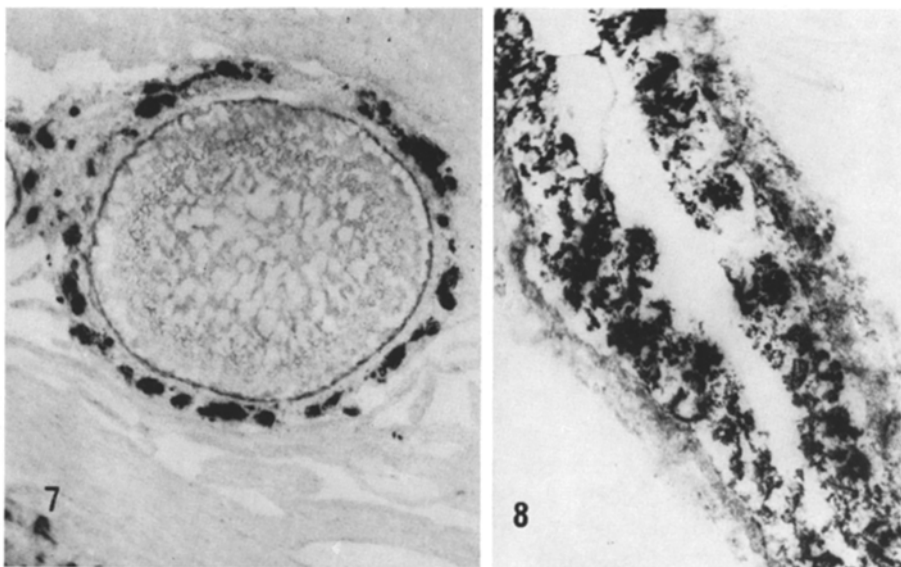


Fig. 7. Adult ovary. A growing follicle with a strong enzymatic reaction is observed. (Δ^5 - 3β -HSDH reaction). $\times 60$

Fig. 8. Collapsed postovulatory follicle. A great increase in thickness of the granulosa layer with a strong positive Δ^5 - 3β -HSDH reaction is observed. $\times 100$

of lipids with acetone for 5 minutes at 4°C or by the substitution of the propyleneglycol for NN' dimethylformamide in the incubation medium.

By 60 days the growing follicles become surrounded by a well defined theca layer and small islets of interstitial tissue become embedded in it (Fig. 6).

In the *adult ovary*, cell groups having the cytological and cytochemical characteristics of interstitial cells are observed within the theca and in the interfollicular connective tissue (Fig. 7). The unspecific reaction of the granulosa cells is still present. In the *postovulatory follicles* the granulosa cell layer greatly increases and shows a very intense Δ^5 - 3β -HSDH reaction (Fig. 8). In the follicles of the control sections the reaction is now negative.

Discussion

Steroid producing cells are cytochemically characterized by the abundance of lipids and cholesterol and more specifically by a positive Δ^5 - 3β -HSDH reaction (JACOBY, 1962). This enzyme oxidizes the Δ^5 - 3β -hydroxysteroids to Δ^4 - 3β ketosteroids, a metabolic step which is involved in the synthesis of practically all steroids having hormonal activity (BAILLE, FERGUSON and HART, 1966). In the chicken ovary, the classical histological studies had postulated that the islets of light cells present in the theca interna, as well as those found in the interstitial tissue, among the follicles, were responsible for steroidogenesis (FELL, 1924; BENOIT, 1926; MARSHALL and COOMBS, 1957).

The present work confirms such histological observations and, with histochemical techniques, demonstrates the steroidogenic activity of such cells. These

have been identified by the large content of lipids and cholesterol and by the positive Δ^5 - 3β -HSDH reaction, and have been followed throughout the postnatal development of the chick ovary. Our findings demonstrate that the islets of interstitial cells found in the medulla of newborn chick ovary become gradually displaced by the morphogenetic growth and folding of the cortical layer, to become embedded among the cells of the theca interna and interfollicular tissue. These results on the postnatal evolution are in agreement with our previous findings in the embryonic ovary of the chick. Since the steroid-producing cell groups of the medulla of the newborn chick ovary originate, in the embryonic ovary, from the primitive sex cords of cortical origin, our findings on the postnatal evolution are in agreement with the classical conception of FELL (1924) about the early history of these hormonal secretory cells.

The unspecific reaction which appears in the granulosa cells after the 20th day of postnatal evolution may be considered to be of enzymatic nature because it does not occur in a medium without NAD. Since this reaction is positive both in NN'-dimethyl-formamide and propylenglycol the possibility that it is due to an alcohol dehydrogenase should be discarded (BAILLE, FERGUSON and HART, 1966). A similar reaction, due to the reduction of -SH groups, was described by ZIMMERMANN and PEARSE (1959) as "nothing dehydrogenase". However in our case the unspecific reaction was not inhibited by iodoacetate.

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