HISTOCHEMICAL OBSERVATIONS
ON THE GRANULOSA AND THECA INTERNA
DURING FOLLICULAR DEVELOPMENT AND
CORPUS LUTEUM FORMATION AND REGRESSION
IN THE AMERICAN OPOSSUM

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SUMMARY

A study has been made of the histochemical changes which occur during follicular growth and formation and regression of the corpus luteum in the ovary of the American opossum. The granulosa cells show abundant cytoplasmic RNA. Some lipid bodies consisting of phospholipids are sparsely distributed among the granulosa cells. After ovulation, the granulosa cells undergo 'luteinization' to form the large luteal cells. The most striking histochemical change involved in the differentiation (or luteinization) of the granulosa follicle cell into a luteal cell is the development of abundant diffuse lipoproteins throughout the cytoplasm. Fine lipid granules consisting of phospholipids are also formed in the cytoplasm of luteal cells.

The stromal elements of the theca interna, which contain some sparsely scattered phospholipid granules, do not show any histochemical change during corpus luteum formation.

With the regression of luteal cells, coarse lipid granules consisting of cholesterol and cholesterol esters, triglycerides and some phospholipids accumulate abundantly in the cytoplasm. Some of these regressing luteal cells continue to persist in the ovarian stroma for some time.

INTRODUCTION

Following ovulation the components of ruptured follicles are luteinized in response to luteinizing hormone, and the follicle is transformed into a corpus luteum (see Eckstein, 1962; Amoroso & Finn, 1962). This is the usual event in all mammals, including the monotremes and marsupials. The main function of the corpus luteum is the production of progesterone. However, no attempt has been made to study histochemically the complex cytoplasmic processes which occur during the transformation (or luteinization) of granulosa cells into luteal cells. Luteinization is a descriptive term that has yet to be defined both chemically and histochemically. Microscopical studies have demonstrated an increase in the agranular endoplasmic reticulum coincident with luteinization (Yamada & Ishikawa, 1960; Björkman, 1962;
Green & Maqueo, 1965; Blanchette, 1966a, b). The purpose of the present study was to report some histochemical changes which occur in the granulosa and theca interna both during follicular development and corpus luteum formation and regression in the ovary of the American opossum.

MATERIALS AND METHODS

Ovaries of sexually mature American opossums (Didelphis marsupialis virginiana Kerr) were used throughout the year. Females were maintained in captivity and separated from males. Developing follicles were studied in 27 non-pregnant females. Corpora lutea were studied in 13 pseudopregnant opossums with excessive development of the uterus; sections of the latter showed that this was due to the accumulation of fluid in the hypertrophied mucosa. Regressing corpora lutea from eight females were investigated whose uteri did not show a progestational endometrium. The fixatives and histochemical techniques used have been reported previously (Guraya, 1966; Guraya & Greenwald, 1964a, b).

RESULTS

Histochemistry of developing follicles

From the amount and nature of lipids, the developing follicles in the American opossum ovary can be classified as normal and atretic follicles (Guraya & Greenwald, 1964b). The granulosa cells of normal growing follicles are small (Pl. 1, fig. 1). Sudanophilic lipid bodies consisting of phospholipids are sparsely distributed among the granulosa cells (Pl. 1, fig. 1) (see also Guraya & Greenwald, 1964b). The cytoplasm of the granulosa cells is rich in RNA as determined by the strong positive reaction in the methyl green–pyronin technique followed by a negative reaction in the control sections treated with ribonuclease. In the final stages of follicular development, the granulosa cells form a relatively thin layer, called the membrana granulosa (Pl. 1, fig. 1). The theca interna of the developing follicle consists of a sheath of stromal elements which do not show conspicuous cytological differentiation (Pl. 1, fig. 1). Some sparsely scattered lipid granules are seen which in their histochemical reactions resemble those of the membrana granulosa. They also consist of phospholipids.

Histochemical features of corpus luteum formation

The stage immediately after the rupture of the follicle was not available, the earliest being that at which the corpus luteum was already formed (Pl. 1, fig. 2). In this female the uterus was enlarged considerably, indicating the production of progesterone by the luteal cells. Each corpus luteum was hollow, composed of a luteal cell mass surrounding a central zone of stromal elements. Stromal cells were also present between the luteal cells (Pl. 1, figs. 2–4). Apparently the luteal cells are formed by the hypertrophy or luteinization of granulosa cells. They are distributed in small groups (Pl. 1, fig. 3) and closely associated with the capillary plexuses. As compared with the original granulosa cells (Pl. 1, fig. 1), the luteal cells are hypertrophied and present markedly glandular-looking tissue (Pl. 1, figs. 2–4). Each cell has a large vesicular nucleus. The most striking and significant change during the transformation of
Histochemistry of granulosa and theca interna

Granulosa cells into luteal cells is the development of a diffusely distributed sudanophilic substance throughout the cytoplasm of the latter (Pl. 1, figs. 2–4). The Sudan black-positive reaction of the cytoplasm becomes negative in material fixed in weak Bouin's solution and extracted with hot pyridine, indicating that the cytoplasm of luteal cells develops Sudan black B-positive lipids which are not present in the cytoplasm of granulosa cells except for a small zone (probably the Golgi zone) lying adjacent to the nuclear envelope (Pl. 1, fig. 1). The diffuse lipids of luteal cells seem to be lipoproteins since they react negatively to other histochemical techniques for the demonstration of triglycerides, phospholipids, cholesterol and cholesterol esters (Guraya, 1966). However, the whole of the cytoplasm gave a positive reaction in the periodic acid-Schiff (PAS) test; the distribution of the PAS-positive substance was the same as that of the Sudan black-positive substance. The PAS-positive reaction persisted in the control sections treated for acetylation, indicating that the PAS-positive reaction of the cytoplasm in the luteal cells was mainly due to the diffuse lipid component which, as already described, disappeared after pyridine extractions. Besides the diffuse lipoproteins some fine lipid granules had developed throughout the cytoplasm of luteal cells (Pl. 1, fig. 4). Compared with the lipid bodies of membrana granulosa they stained less intensely with acid haematin, showing the different nature of their phospholipids which also did not need calcium ions and chromation for adequate fixation. However, such a treatment is essential for the proper fixation of the lipid bodies of the membrana granulosa (Guraya & Greenwald, 1964b). No triglycerides, or cholesterol and cholesterol esters demonstrable with histochemical techniques were present in the lipid granules of luteal cells.

The stromal cells, which are apparently derived from the theca interna, showed little cytoplasmic differentiation as judged by their small size (Pl. 1, figs. 3, 4). They did not develop the diffuse lipoproteins formed in the luteal cells of granulosa cell origin. However, the stromal cells showed some sparsely distributed lipid granules also described in the membrana granulosa and theca interna of developing follicles.

Histochemical features of corpus luteum regression

Regression of the corpus luteum was accompanied by the accumulation of coarse, sudanophilic droplets in the cytoplasm of luteal cells (Pl. 2, fig. 5). This indicates the storage of lipids which, since they stained pink in Nile blue, and orange-red in red Sudan dyes, contained triglycerides. The lipids were also Schultz-positive and also contained cholesterol and cholesterol esters. They stained moderately in acid haematin followed by a negative reaction in the control material fixed in weak Bouin's solution and extracted with hot pyridine, thus demonstrating phospholipids in the lipid material in regressing luteal cells. In the final stages of regression, the lipids consisted mainly of triglycerides, cholesterol and cholesterol esters; some pigments developed simultaneously in the lipid droplets as shown by their resistance to fat solvents. The cytoplasmic basophilic substance, due to RNA, was not removed in the control sections treated with ribonuclease. The residual luteal cells filled with coarse lipid granules continue to persist in the ovarian stroma for several months (Pl. 2, fig. 6).
DISCUSSION

The most noticeable change during the transformation of granulosa cells into luteal cells is the development of diffuse lipoproteins throughout the cytoplasm of the latter. The vascularization at ovulation seems to be the key to the development of these diffuse lipoproteins. This cytoplasmic change may be attributed to the effect of luteinizing hormone which is secreted in large amounts during ovulation. The diffuse lipoproteins were not seen in the cytoplasm of granulosa cells during follicular growth. The so-called luteinization of the granulosa cells during the corpus luteum formation was accompanied by the development of diffuse lipoproteins throughout the cytoplasm, a feature which, so far, has been overlooked. Similar diffuse lipoproteins have also been shown to occur throughout the cytoplasm of ovarian interstitial gland cells (Guraya, 1966, 1967a, b). The appearance of diffuse lipoproteins throughout the cytoplasm of hypertrophied steroid-secreting cells may serve as a useful histochemical indicator of the process of luteinization.

What is the physiological significance of diffuse lipoproteins which develop in the steroid-secreting cells? The development of diffuse lipoproteins during luteinization may hold a clue to control mechanisms in steroid production. It is well known that all steroid-secreting gland cells contain abundant agranular endoplasmic reticulum in the cytoplasm (Muta, 1958; Yamada & Ishikawa, 1960; Björkman, 1962; Green & Maqueo, 1965; Christensen, 1965; Christensen & Fawcett, 1961, 1966; Blanchette, 1966a, b), and it can be assumed that the diffuse lipoproteins shown histochemically derive from the agranular endoplasmic reticulum which plays an important role as a site of enzymes involved in steroidogenesis (Christensen, 1965; Christensen & Fawcett, 1966). The scarcity of diffuse lipoproteins or agranular endoplasmic reticulum (Björkman, 1962; Blanchette, 1966a) in the granulosa cells during follicular growth suggests that they are probably not synthesizing much steroid.

The cytoplasm of luteal cells was shown to contain fine secretory lipid granules consisting of phospholipid. They are not seen in the cytoplasm of granulosa cells. Both the fine lipid granules and the diffuse lipoproteins are intimately connected with the synthesis of steroid hormones.

With the regression of the corpus luteum, the luteal cells begin to store coarse lipid granules which consist of cholesterol and cholesterol esters, triglycerides and some phospholipids. Similar lipids are also stored in the regressing corpora lutea of rodents (Guraya, 1964; Guraya & Greenwald, 1965). It is generally believed that when steroidal lipids are accumulated abundantly in steroid-secreting cells, hormone storage is taking place, and when the amount decreases hormone is released (Guraya, 1966). It can now be added that the regressing luteal cells begin to store hormone precursor (steroidal lipids) rather than secrete hormone and that hormone secretion occurs in those luteal cells which show no accumulation of steroidal lipids.

REFERENCES


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DESCRIPTION OF PLATES

**PLATE I**

Fig. 1. Part of large antral follicle of opossum ovary, showing sparsely scattered lipid bodies (L) in the membrana granulosa (MG) and theca interna (TI). Formaldehyde calcium, followed by dichromate calcium, Sudan black B. (× 44.)

Fig. 2. Corpus luteum of pseudopregnancy, showing the development of diffuse sudanophilic lipid in the cytoplasm of luteal cells. Formaldehyde calcium, followed by dichromate calcium, Sudan black B. (× 384.)

Fig. 3. Details of fig. 2 showing diffuse sudanophilic lipid in the cytoplasm of luteal cells (LC). The vesicular nucleus does not show such lipid. Stromal cells (SC) show some lipid granules (L). (× 440.)

Fig. 4. Details of fig. 2, showing diffuse sudanophilic lipids and small lipid granules (arrows) in the cytoplasm of luteal cells (LC). Some lipid granules (L) in stromal cells (SC). (× 990.)

**PLATE 2**

Fig. 5. Part of regressing corpus luteum of opossum ovary, showing accumulation of coarse lipid granules in the cytoplasm of luteal cells. Staining as in fig. 1. (× 225.)

Fig. 6. Remnants of regressing luteal cells in the ovarian stroma. Staining as in fig. 1. (× 225.)