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Electron Microscopy of Cytodifferentiation and its Subcellular Steroidogenic Sites in the Granulosa Cell of the Human Ovary*)

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ABSTRACT

The cytodifferentiation and subcellular steroidogenic sites in the granulosa cell of the developing follicle and *in vitro* effect of estradiol-17β (E_2) on the granulosa cell of the preovulatory follicle in the human ovary were investigated using the electron microscopic cytochemistry.

The follicular cell in the primordial follicle showed an elongated nucleus, rough endoplasmic reticulum, Golgi apparatus, rod-shaped mitochondria with lamellar cristae, free ribosomes and a few lipid droplets. In the secondary follicle, the granulosa cell derived from the follicular cell had a round nucleus, rough endoplasmic reticulum, lipid droplets, Golgi apparatus, microfilament, mitochondria with lamellar or tubular cristae and a small amount of smooth endoplasmic reticulum. Especially, the granulosa cell in the preovulatory follicle considered to be a transitional form to the steroid-secreting cell was characteristic of rough endoplasmic reticulum, lipid droplets, mitochondria with lamellar or tubular cristae and moderately well developed smooth endoplasmic reticulum. On the other hand, the granulosa cell in the postovulatory follicle showing a typical steroid-secreting activity had abundant lipid droplets, round mitochondrial with tubular or vesicular cristae, well developed smooth endoplasmic reticulum and lysosomes.

The hallmarks of the cytodifferentiation of the granulosa cell were i) an appearance of lipid droplets, ii) a structural change of mitochondrial cristae from lamellar to tubular configuration and iii) an appearance and development of smooth endoplasmic reticulum.

Reaction products for 3β-hydroxysteroid dehydrogenase (3β-HSD) activity were localized on tubular or lamellar cristae and inner membrane of the mitochondria, and on the membrane of smooth endoplasmic reticulum in the granulosa cell of the preovulatory as well as of the postovulatory follicle.

The granulosa cell of the preovulatory follicle incubated in the medium containing E_2 showed a structural change of mitochondrial cristae from lamellar to tubular configuration, and an appearance and development of smooth endoplasmic reticulum.

From these facts, it is suggested that the granulosa cell in the preovulatory follicle has already a steroid-secreting activity and luteinizes abruptly, and E_2 contained in the culture medium could stimulate the functional differentiation of the granulosa cell in the prevulatory follicle affected with gonadotrophin.

INTRODUCTION

It is well known that granulosa cells and theca cells become corpus lutein cells after the

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ovulation. The fine structural change of granulosa cells at this stage, which have a receptor of estradiol-17β (E_2) as a target, is an interesting problem for the functional morphology of steroidogenesis. Though many investigators reported the ultrastructural change of luteinization of the granulosa cell in the manmalian ovary (Björkman 1962[4]; Blanchette 1966[5]; Weakley 1966[16]; Bjersing 1967[3]; Byskov 1967[6]), little is known about the functional differentiation of the early granulosa cell at the preovulatory as well as ovulatory stage of the human ovary.

The purpose of this study was planed to investigate the morphological changes and the localization of 3β-dydroxysteroid dehydrogenase (3β-HSD) activity in the granulosa cell of the developing follicle and the effect of E_2 on the functional differentiation of the granulosa cell in cultured human preovulatory follicle affected with gonadotrophin.

MATERIALS AND METHODS

Ten human ovaries in the follicular phase and in the postovulatory phase were removed by wedge resection. The specimens were fixed for 2 h with 2.5% glutaraledhyde solution adjusted to pH 7.4 with 0.1 M cacodylate buffer, refixed for 1 h with 2% O_SO_4 adjusted to pH 7.4 with 0.1 M cacodylate buffer. A portion of the unfixed or fixed materials were incubated for 15 min at room temperature using the ferricyanide technique of Benkoël et al. (1976)[1]. The incubation medium consisted of 1.2 mg dehydroepiandrosterone in 1.5 ml of dimethylformamide, 12.5 ml of 0.1 M cacodylate buffer, pH 7.4, 2 ml of 0.1 M sodium citrate, 2 ml of 15 mM potassium ferricyanide solution, 2 ml of 90 mM copper sulfate solution, 2 g sucrose and 7.2 mg N. A. D. After incubation, the specimens were washed with 0.1 M cacodylate buffer adjusted to pH 7.4 and 0.2 M sucrose at 4°C, fixed with 1% glutaraldehyde solution adjusted to pH 7.4 with 0.1 M cacodylate buffer and postfixed for 1 h with 2% O_SO_4 adjusted to pH 7.4 with 0.1 M cacodylate buffer.

The preovulatory follicle was incubated with McCoy's 5a medium containing 10^{-8}M E_2 (Sigma) at 37°C for 3 h in a mixure of 95% air and 5% CO_2. These materials were then dehydrated in ethanol and embedded in Poly Bed 812. Sections cut on a Porter-Blum MT-1 ultramicrotome, stained with Raynold's lead solution and coated with carbon were examined with Hitachi HU-12A type and Hitachi H-300 type electron microscopes. Control incubation was done respectively by omitting the substrate or E_2 from the medium.

For light microscopy, thick sections were stained with 0.5% toluidine blue solution.

RESULTS

Light microscopy

The primordial follicle was composed of one layer of the flattened follicular cells (pre-granulosa cells) and an oocyte. Among these follicles were connectiue tissue cells with elongated nuclei (Fig. 1).

The secondary follicle showed several layers of granulosa cells, cuboidal or columnar in shape, and somewhat eosinophic cytoplasm (Fig. 1).

Though numerous granulosa cells in the preovulatory follicle have an oval nucleus and relatively basophilic (Fig. 2), they become eosinophilic and rich in abundant lipid droplets at the postovulatory stage (Fig. 3).

Electron Microscopy

Granulosa cells in the primordial and secondary follicle

The flattened follicular cells (pre-granulosa cells) with elongated nuclei had rough endoplasmic reticulum, Golgi apparatus, elongated mitochondria with lamellar cristae and free ribosomes. Some of them had already a few lipid droplets in their cytoplasms (Fig. 4). The granulosa cells in the secondary follicle showed oval or round nuclei, rough endoplasmic reticulum, mitochondria with lamellar or tubular cristae, Golgi apparatus, a few lipid droplets, free ribosomes and microfilaments (Fig. 5). Mitotic figures were also frequently recognized.

Granulosa cells in the preovulatory and postovulatory follicle

In the preovulatory follicle, the granulosa cells, considered to be a transitional form to the steroid-secreting cells, contained round nuclei, rough endoplasmic reticulum, Golgi apparatus, mitochondria with lamellar or tubular cristae, moderately developed smooth endoplasmic reticulum, lipid droplets and abundant free ribosomes (Fig. 6).

In the postovulatory follicle, they showed a typical steroidsecreting appearance having round nuclei, Golgi apparatus, lipid droplets, round

mitochondria with tubular or vesicular cristae, smooth endoplasmic reticulum and lysosomes (Fig. 7).

Ultracytochemical localization of 3β-hydroxysteroid dehydrogenase (3β-HSD) activity

Reaction products for 3β-HSD activity were localized on tubular or lamellar cristae and inner membrance of the mitochondria, and on the membrame of smooth endoplasmic reticulum in the granulosa cell of the preovulatory as well as of the postovulatory follicle (Figs. 8, 9 and 10). No reaction products were recognized in these cell types incubated in the control medium without substrates.

Effect of estradiol-17β (E_2) on the cultured granulosa cell

The granulosa cells in the control section incubated without E_2 were quite similar in appearance to those in the preovulatory follicle (Fig. 11).

On the other hand, the granulosa cells incubated with E_2 revealed a structural change of mitochondrial cristae from lamellar to tubular configuration and an appearance and development of smooth endoplasmic reticulum (Figs. 12 and 13).

DISCUSSION

The fine structural change of granulosa cells between preovulatory and postovulatory follicle is most characteristic (Mestwert et al. 1977[11], 1979[12]). In primordial and secondary follicle, the granulosa cells had a moderate development in the cell organelles with rough endoplasmic reticulum, moderasely developed Golgi apparatus, mainly rod-shaped mitochondria with lamellar cristae, free ribosomes. Mitotic figures of the granulosa cells have been frequently seen.

In our observations, follicular cells (pre-granulosa cells) in the primordial follicle had already a few lipid droplets. The lipid droplets increase in number in the granulosa cells, as the follicle maturates. In the preovulatory follicle, granulosa cells show lipid droplets, mitochondria with lamellar or tubular cristae, smooth endoplasmic reticulum and lysosomes. The structural change from lamellar to tubular configuration in the mitochondrial cristae is known to be an early indicator for steroidogenesis (Dimino et al. 1979[9]; Hiura et al. 1981[10]). In the granulosa cells in the postovulatory follicle, these characteristic structures become more prominent. The cell has abundant lipid droplets, round mitochondria with tubular or vesicular cristae and well developed smooth endoplasmic reticulum.

From these facts, it is suggested that the granulosa cells might have already a steroidogenesis at the preovulatory stage. Clinically it is known that the slight elevation of the plasma progesterone occurs just before the ovulation.

According to Samuel et al. (1951)[14], this enzyme 3β-HSD plays an important role in the biosynthesis of the active steroid hormone. The ultracytochemical technique for 3β-HSD is very useful to clarify whether or not these granulosa cells in transitional form in the preovulatory follicle have a steroidogenic activity. Reaction products of copper ferrocyanide for 3β-HSD activity were localized on tubular or lamellar cristae and inner membranes of the mitochondria, on the membranes of smooth endoplasmic reticulum in the granulosa cells of the preovulatory as well as postovulatory follicle.

These facts also mean that the granulosa cells in both preovulatory and postovulatory follicles have a steroidogenic activity.

It has been reported that the cultured granulosa cells showed "luteinization" and differentiation into the steroid-secreting cells without any hormonal stimulation (Crip and Channing 1972[7]; Suzuki et al. 1976[15]). However, Goldenberg et al. (1972)[9] and Nakano et al. (1977)[13] emphasized that E_2 is necessary to induce the proliferation of the granulosa cells under the interaction of gonadotrophic hormone and Bernand (1975)[2] also recognized that E_2 stimulates progesterone secretion by rat granulosa cell in culture.

In our experiment, the granulosa cells from the preovulatory follicle cultured in the medium containing E_2 showed the following ultrastructural changes: mitochondrial changes from lamellar to tubular in cristae, and appearance and development of smooth endoplasmic reticulum. This fact suggests that E_2 might influence not only the proliferation but also the functional differentiation (luteinization) of the granulosa cells of the preovulatory follecle exposed to some extemt to gonadotrophin.

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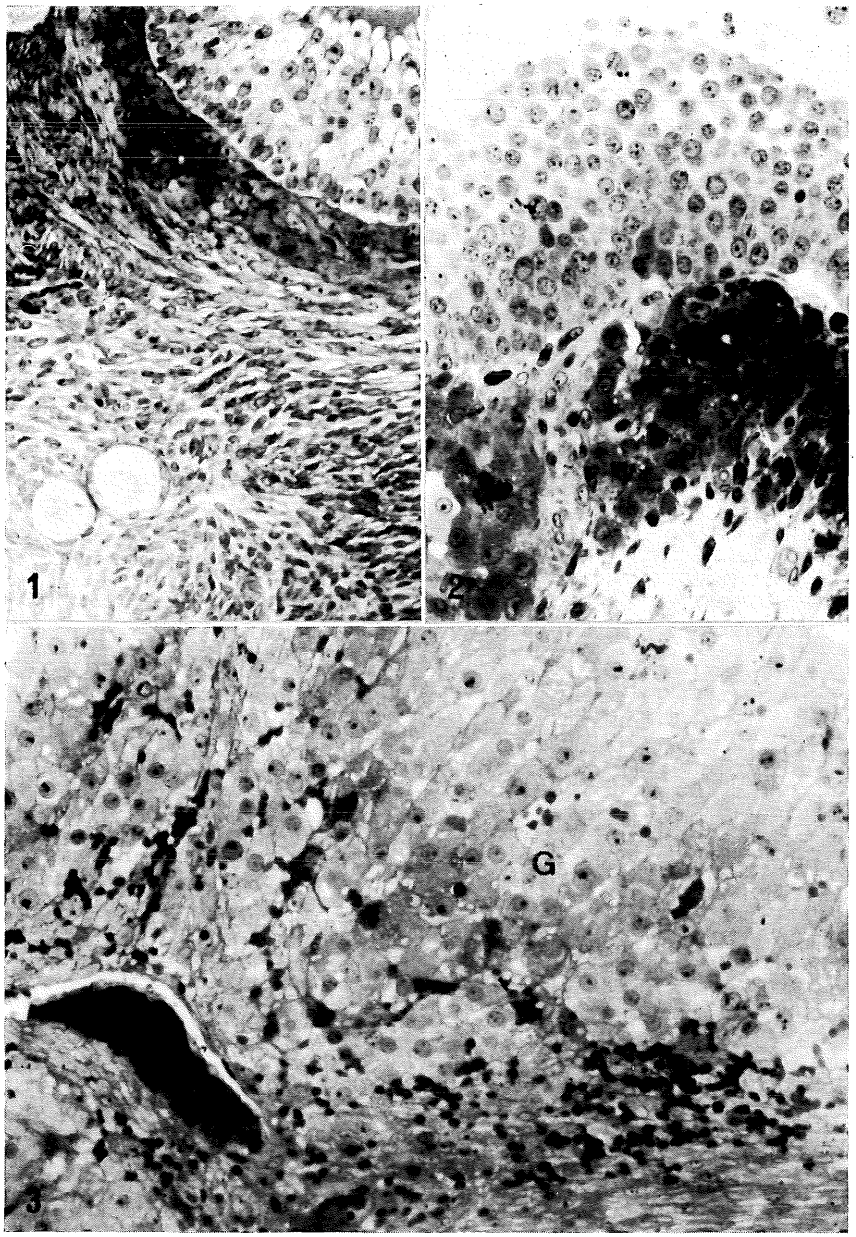
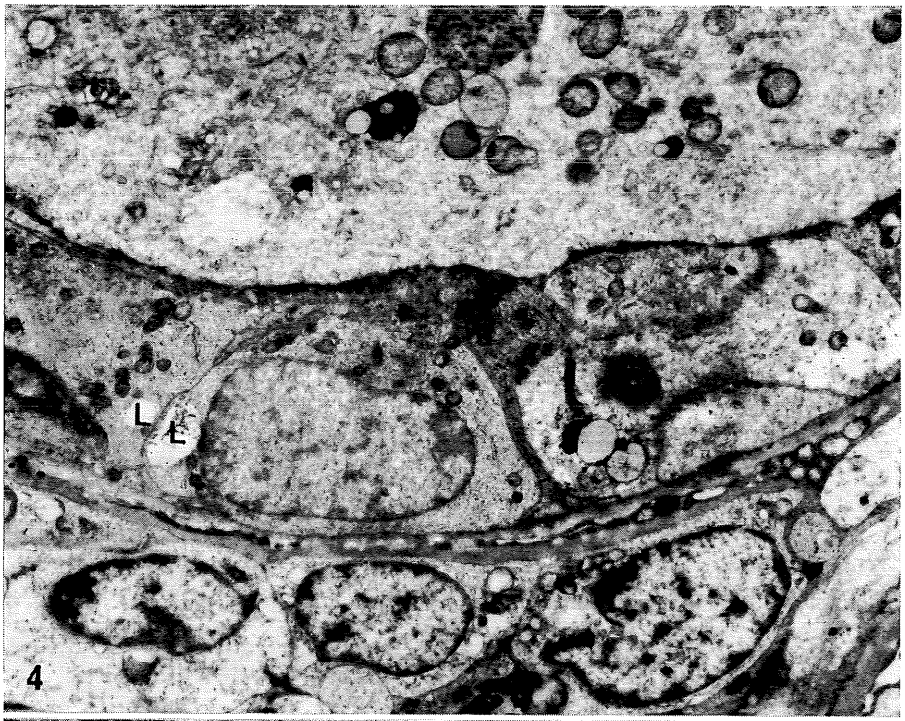


Fig. 1. Two primordial follicles composed of one layer of flattened follicular cells (left lower), and the secondary follicle with several layers of the granulosa cells (upper). ×530

Fig. 2. The preovulatory follicle with multi-layers of the granulosa cells (upper) and hypertrophied theca interna (lower). ×280

Fig. 3. The postovulatory follicle with numerous granulosa lutein cells (G) containing round nuclei and abundant lipid droplets. ×220



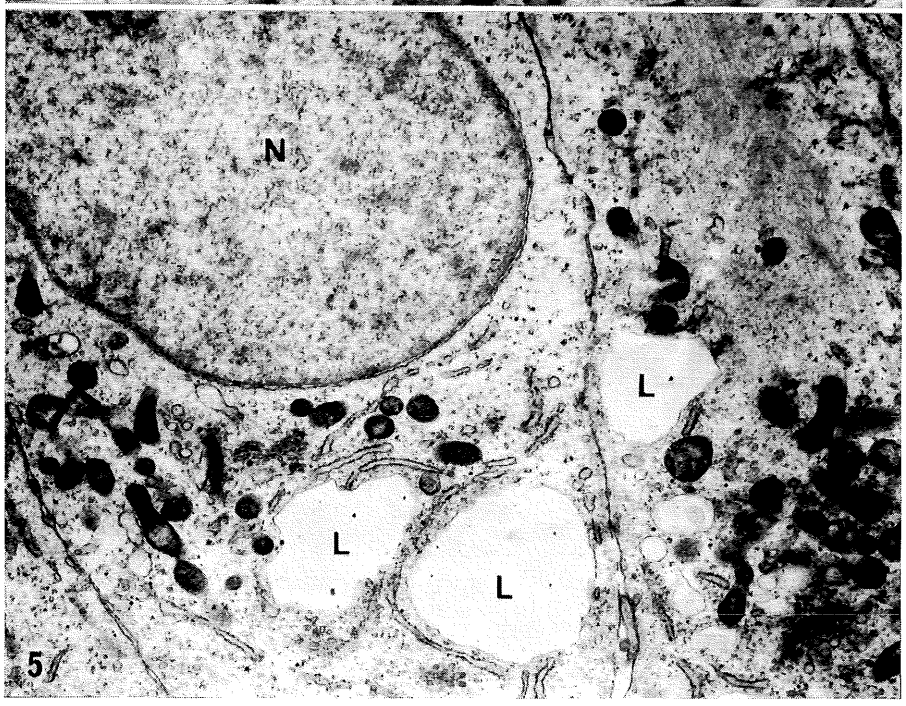


Fig. 4. The primordial follicle consisting of one layer of follicular cells. Some of which have a few lipid droplets (L) in the cytoplasm. ×6,000

Fig. 5. Parts of granulosa cells in the secondary follicle. Lipid droplet (L), microfilaments, poorly developed rough endoplasmic reticulum and mitochondria with lamellar cristae are seen. N: nucleus ×8,700

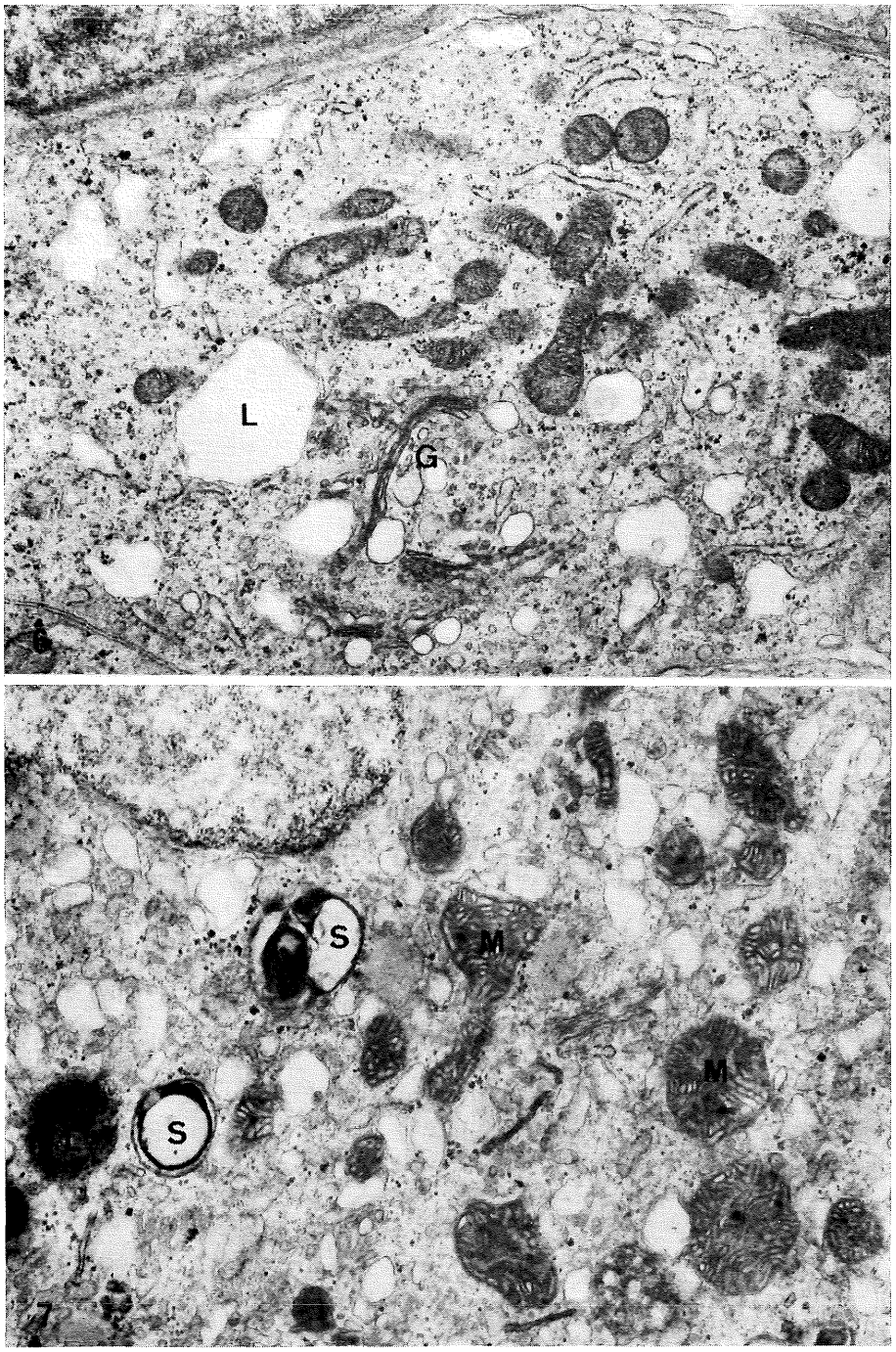
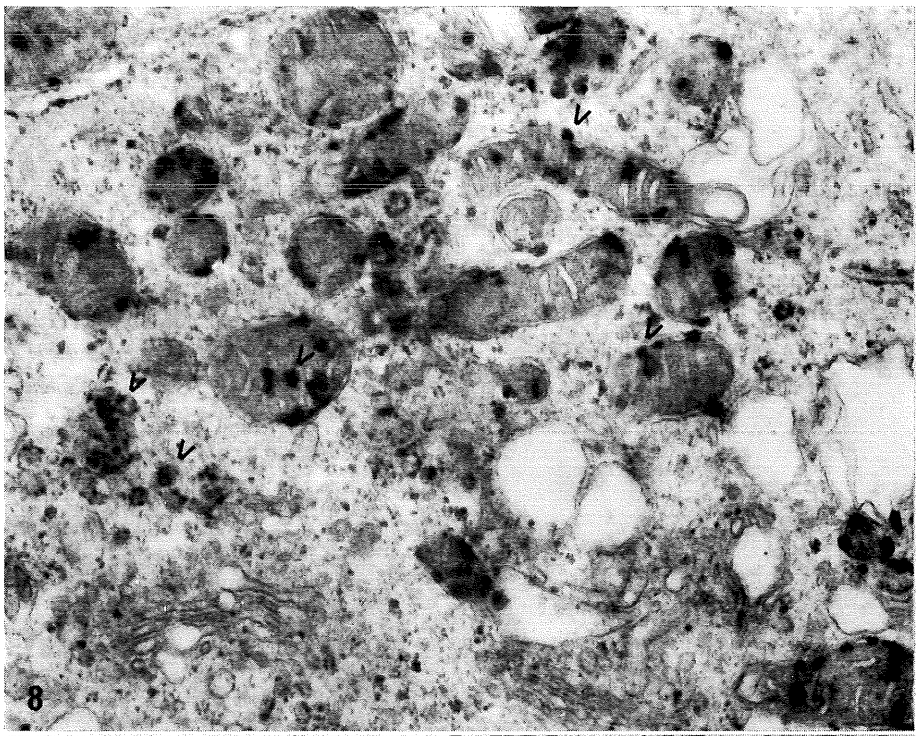


Fig. 6. A part of the granulosa cell which might be a transitional form to the steroid-secreting cell in the prevulatory follicle. Lipid droplets (L), rough endoplasmic reticulum, smooth endoplasmic reticulum, Golgi apparatus (G), mitochondria with lamellar or tubular cristae and free ribosomes are seen. ×15,000

Fig. 7. A part of the granulosa cell in the postovulatory follicle. Lipid droplets (L), round mitochondria with tubular cristae (M), smooth endoplasmic reticulum and lysosome (S) are seen. ×15,000



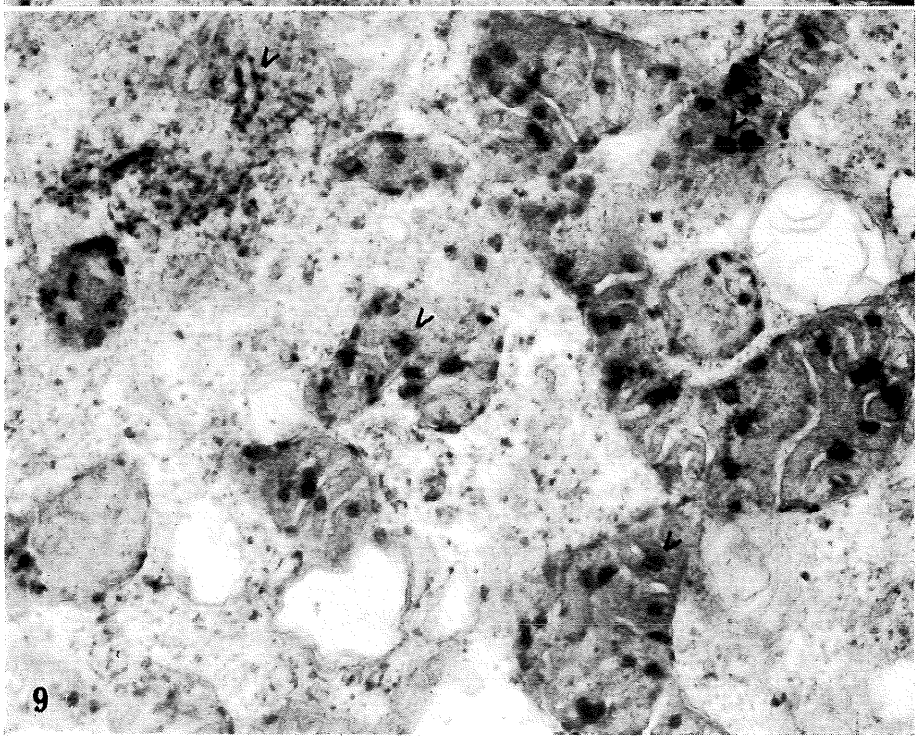
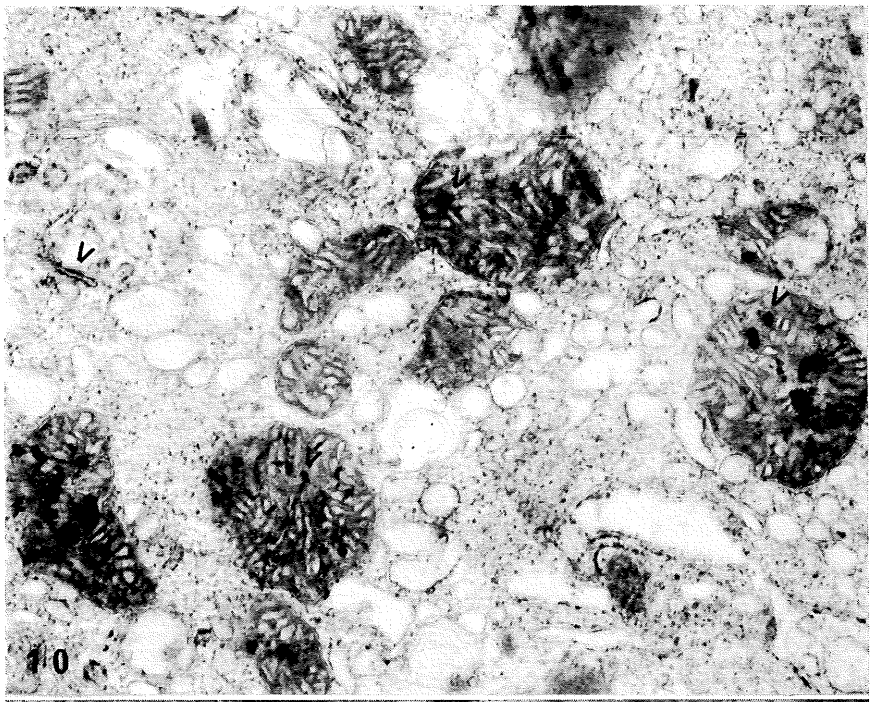


Fig. 8. 3β-dydroxysteroid dehydrogenase activity in the granulosa cell of the preovulatory follcle. Reaction products (v) are localized on tubular or lamellar cristae and inner membrane of the mitochondria and on the membranes of smooth endoplasmic reticulum. ×28,800

Fig. 9. 3β-hydroxysteroid dehydrogenase activity in the granulosa cell of the preovulatory follicle. Reaction products (v) are localized on lamellar cristae and inner membrane of the mitochondria, and on the membranes of smooth endoplasmic reticulum. ×30,000



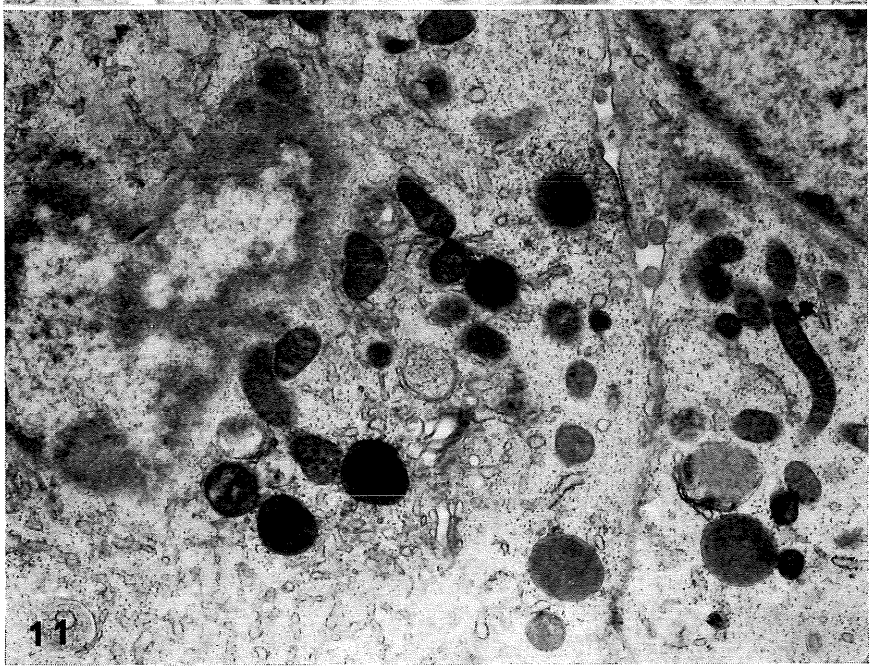


Fig. 10. 3β-hydroxysteroid dehydrogenase activity in the granulosa cell of the postovulatory follicle. Reaction products (v) are localized on tubular cristae anld inner membrane of the mitochondria, and on the membrane of smooth endoplasmi cetriculum. $\times 23,000$

Fig. 11. A part of the granulosa cell of the preovulatory follicle incubated in the control medium. Lipid droplets, microfilaments, mitochondria with lamellar or tubular cristae, rough endoplasmic reticulum are seen. $\times 10,200$

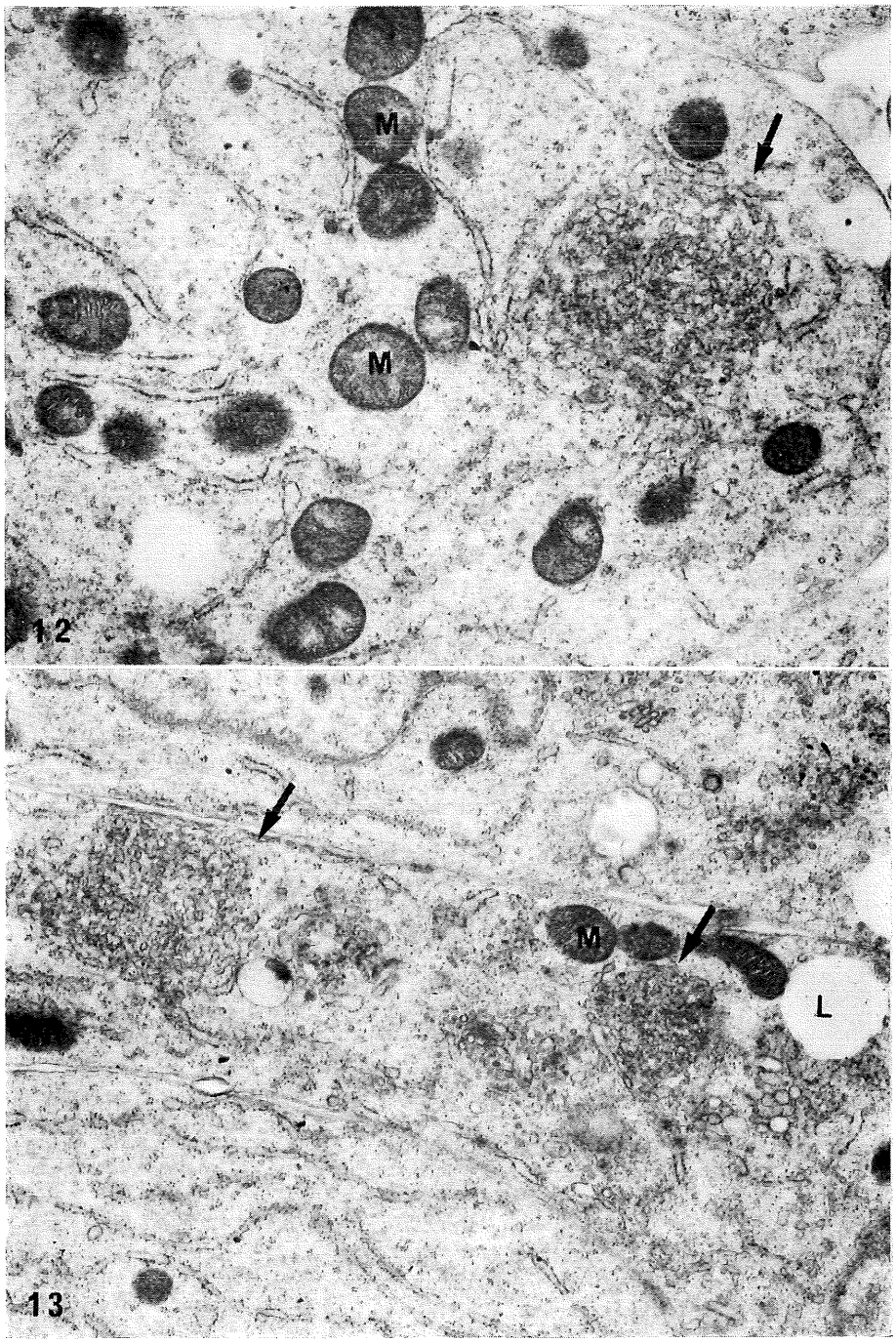


Fig. 12. Parts of the granulosa cells of the preovulatory follicle incubated in the medium containing E_2. Rough endoplasmic reticulum, round mitochondria with tubular cristae (M) and well developed smooth endoplasmic reticulum (arrow) are seen. ×16,000

Fig. 13. Parts of the granulosa cell of the preovulatory follicle incubated in the medium containing E_2. Lipid droplets (L), mitochondria with lamellar or tubular cristae (M), well developed smooth endoplasmic reticulum (arrow) are seen. ×12,000