A Histochemical Study of Δ^5-3β-Hydroxysteroid Dehydrogenase Activity in the Interstitial Cells of the Mammalian Ovary

Despite numerous investigations on the histochemistry of the ovary, the presence of steroid-converting enzymes has not been studied in the interstitial cells of the mammals ovary[1], or systematically followed comparatively with other ovarian steroidogenic cells during oestrus cycle and pregnancy and in different stages of foetal and reproductive age.

In the present study the distribution of Δ^5-3β-hydroxysteroid dehydrogenase was investigated in suspected steroid secreting structures of the human, rat and guinea-pig ovary, and the histochemical results were compared with some observations performed by electron microscope.

Material and method. The human ovaries were removed almost immediately during accidental or therapeutic interruption of pregnancy from 26 embryos and foetuses and surgically from 10 women in various stages of the menstrual cycle. In addition 40 adult albino female rats and 40 adult guinea-pigs were used. The animals were selected after examination of vaginal smears in each stage of the oestrus cycle and in different days of pregnancy. All the ovaries were removed from animals sacrified by decapitation.

The sections (10–15 μ) washed in a 0.1 M phosphate buffer pH 7.2 were incubated in fresh media at 37 °C for 1 h. For demonstration of Δ^5-3β-hydroxysteroid dehydrogenase (3β-OHD) pregnenolone or dehydroepiandrosterone were used. After incubation the section was washed, fixed in 10% neutral formalin and mounted in glycerol-gelatin[2,3]. In addition, from some ovaries small blocks were removed and used for electron microscopy.

1. During the embryonal and foetal development of the human ovary, a positive reaction for 3β-OHD was found in the following structures: interstitial tissue, theca interna and granulosa cells.

In the interstitial cells the reaction clearly appears only in ovaries under the age of 5 months (in this period a reaction is present also in the 'hilus') and successively its intensity increases in relation to age. In addition a rapid strong reaction was observed in embryos of 6 weeks.

The theca interna and granulosa cells of cavitary follicles showed a moderate enzyme activity only in the last months of the foetal period.

During the menstrual cycle, a positive reaction was found with different intensity in corpus luteum, interstitial tissue, theca interna and granulosa cells.

An intense positivity was observed indistinctly in the corpora lutea and interstitial cells of 5 women some days (probably 6th to 8th) after the ovulation. In 5 women before or during the ovulation a strong activity was exhibited by theca interna and granulosa cells of big cavitary follicles and by interstitial cells – with a moderate difference in the enzymatic activity.

2. The results on the Δ^5-3β-hydroxysteroid dehydrogenase in the rat showed that in all the different phases of the oestrus cycle the interstitial cells are active, with a fluctation of intensity that is approximately the same of that observed in theca interna during proestrus and oestrus and of that observed in corpora lutea and theca interna during the metaestrus and diestrus. During pregnancy, the interstitial cells showed the same intensity of enzymatic activity of the luteal cells in the first quarter of pregnancy; in the second quarter the activity was very weak and, in the third, it completely disappeared.

3. In guinea-pig ovary, the activity of the interstitial cells is very similar to that of theca interna cells during the oestrus cycle (strong in metaestrus, moderate in diestrus and weak in proestrus and oestrus). During the pregnancy the interstitial cells showed the same activity of luteal cells (moderate in the first days, strong when the corpora lutea are developed and finally weak before the involution of corpora lutea).

In addition, it is interesting to note, in all the different ovaries observed, that not every group of interstitial cells or layers of theca interna and luteal cells are active at the same moment and in the same ovary, but only several groups of these cells showed a clear enzymatic activity (Figure 1). These observations are in agreement with the cyclical changes of the interstitial cells and steps of differentiation of the steroidogenic organelles recently revealed by electron microscopy[4–6].

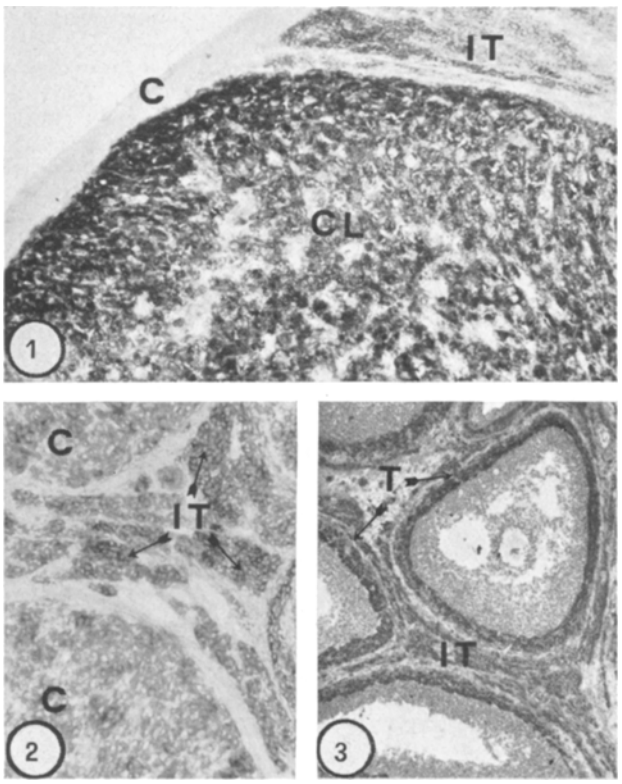

Fig. 1. Human ovary 7 days after ovulation. CL, corpus luteum; IT, interstitial tissue; C, ovarian cortex (3β-OHD + dehydroepiandrosterone).

Fig. 2. Rat ovary (metaestrus). CL, corpora lutea; IT (→), interstitial tissue (3β-OHD + dehydroepiandrosterone).

Fig. 3. Guinea-pig ovary (metaestrus). T, theca interna cells; IT, interstitial tissue (3β-OHA + dehydroepiandrosterone).

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The histochemical results demonstrated: a) that the interstitial cells are very active cells of the mammalian ovary during the foetal life and some phases of the menstrual cycle (human) and during the oestrus cycle and pregnancy (rat and guinea-pig); b) that they have the same steroid converting enzyme present in corpora lutea and theca interna but with a pattern of intensity comparable only to that present in lutein cells.

Recent biochemical data[7] have demonstrated that in the rabbit the interstitial tissue appears to be the site of synthesis of the steroid hormone in ovaries without corpora lutea.

In previous research, MOSSMAN et al.[8] have found that 'interstitial gland cells' are present and profuse in the human ovary from birth to old age. These observations prove that the interstitial tissue may be the most important gland of the human ovary[8].

These results may be very suggestive and interesting if compared with the electron micrographs of the same cells.

In fact in the present study no essential difference in submicroscopic organization of these tissues was observed. In these cells only a different step of differentiation or quantity of steroidogenic organells (mitochondria with villiform cristae, abundant and complicated membranes of smooth endoplasmic reticulum and lipid droplets) are demonstrable. Concluding from the present and some previous studies[4,5], there is strong suggestive evidence that these different ovarian cells may have an equivalent role in mammals in secreting steroid hormones with only a difference of intensity and fluctuation of activity in relation with cyclic changes and steps of differentiation. In fact all these steroidogenic cells (theca interna, luteal cells and interstitial cells) are developed from one ovarian cellular type only and when they are mature secreting cells, may well represent, all together, a large profuse 'interstitium' working with a coordination of cellular activity strictly correlated with the sexual cycle and the eveniences of the pregnancy.

Riassunto. Le cellule interstiziali dell'ovaio presentano spiccata attività enzimatica (3β-OHD) ed organizzazione submicroscopica tipicamente stereidogenica nella donna nel periodo fetale e nel ciclo mestruale; nel ratto e nella cavia nel ciclo estrale e nella gravidanza. Negli stessi mammiferi tale attività è equivalente e coordinata a quella della teca interna e del corpo luteo.

P. MOTTA[9], Z. TAKEVA and V. BOURNEVA

Institute of Morphology, Academy of Sciences, Sofia (Bulgaria), and Institute of Anatomy, University of Rome, Viale Regina Elena 289, Roma (Italy), 7 April 1970.

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Ultrastructure of the 'Onion Bodies' of the Sensory Pore X-Organ of *Paratya tasmaniensis* Reik (Crustacea, Decapoda)

Within the sensory pore X-organ (SPX-organ) or the organ of Bellonci of some of the Decapoda and Stomatopoda there occur stratified concretions referred to as 'onion bodies'[1]. Until now the exact nature of the 'onion bodies' has been in doubt. CARLISLE[2-4] and CARLISLE and KNOWLES[5] considered the 'onion bodies' in *Lysmata seticaudata* and *Pandalus borealis* to be swollen axonic terminations. This interpretation was not acceptable to GABE[1] who, like HANSTRÖM[6], regarded the 'onion bodies' as involved in the elaboration of secretory material. It appears that the nature of the 'onion bodies' may better be revealed by the electron microscope. The following is a preliminary report on the ultrastructure of the 'onion bodies' in the SPX-organ of the freshwater shrimp *Paratya tasmaniensis* Reik.

Material and methods. Specimens of *Paratya tasmaniensis* were collected from the Coal River, near Richmond (Tasmania). Animals were anaesthetized in carbonated water and their eyestalks dissected into chilled 2% OsO_4, made up in phosphate buffer adjusted to pH 7.3 (with or without 5% sucrose). After 1 h in the fixative at 4 °C the distal $^2/_3$ of the eyes were cut off (this was found to be necessary for satisfactory embedding) and the specimens were left in the fixative for 1 further h at room temperature. The specimens were then dehydrated through a graded series of ethanol, cleared in propylene oxide and embedded in Epon 812. Sections of between 1 and 2 μm were cut with glass knives and stained with toluidine blue for the purposes of location and orientation of the 'onion bodies'. Silver to pale gold sections were cut with a diamond knife on an LKB ultratome. Some of the sections were stained with alkaline lead citrate before examination with an AEI EM6 electron microscope operating at 60 kV.

Observations. Under the light microscope the 'onion bodies' appear as stratified concretions and this is in agreement with previous light microscope observations. The electron microscope reveal that the 'onion body' consists of a concentric concretion of either tubular elements (Figure 1) or cisternae (Figure 2); this difference in configuration is possibly due to the different physiological states of the animals[7]. Sparsely scattered amongst the tubular elements or cisternae are lysosome-like bodies.

The tubular elements range in size from 100 to 400 nm in cross-sectional diameter and are arranged in 2 main

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[7] The difference in structure could possibly be due to extreme osmotic sensitivity of the tissue but we are of the opinion that this difference is not due entirely to an osmotic artifact.