Fine Structural Differentiation of Steroid-Producing Cells in the Ovary of Immature Rat

First appearance of estrogen in the peripheral blood of female rats coincides with cavitation of the follicles, epitheloid transformation of thecal cells, and with the first evidence of the 3β-hydroxysteroid dehydrogenase (3β-OH-SDH) activity in the ovary[1,2]. In this study we compared above all the character of cells displaying positive 3β-OH-SDH reaction with the well-known picture of steroid-producing cells[3]. Identification of cell types participating in steroid biosynthesis in the immature rat ovary was also considered important.

Ovaries of 45 Wistar rats aged from 5 to 20 days after birth were examined. A portion of each ovary was used for electron microscopy (fixed in Karnovsky's mixture, and postfixed in OsO_4), and the rest investigated histochemically for the 3β-OH-SDH activity (according to Allen with dehydroepiandrosterone as substrate). Ultrathin sections from Epon 812 were examined in a Tesla BS 242D and JEM 7A electron microscope. Histological structure of the ovaries was verified in a light microscope, 2 μ thick sections from Epon 812 were stained with toluidine blue.

At the age of 5 to 9 days, the ovary contains a great amount of primary follicles with increasing number of growing follicles hitherto without the antra. Interfollicular (interstitial) spaces contain only young indifferent fibroblasts. 3β-OH-SDH activity is absent up to the 8th day, on the 9th day the first isolated formazan deposits appear in the periphery of follicles. At the age of 10 days numerous growing follicles with beginning antrum formation, and initiating thecal epitheloid transformation beneath the basal membrane were observed. Simultaneously, 3β-OH-SDH activity was demonstrated outside follicles in all of the ovaries examined. Though the exact localization is impossible, most of the formazan clumps occur outside the basal membrane. Between the 11th and 15th day they can be found in the peripheral zone of the theca.

At 10 days of age single thecal cells display the distinct evidence of activation presented first by the development of the Golgi complex, from the periphery of which the smooth endoplasmic reticulum vesicles are spreading diffusely into the cytoplasm. An increase in number of the free ribosomal component, and a dilatation of ergastoplasmic formations was found. Large rod-like mitochondria with well developed tubular cristae appear in the cells. The beginning of steroidogenic transformation is also associated with the development of lysosomes.

In 11- to 15-day-old rats the differentiation of thecal cells is proceeding, and after the 15th day the peripheral zone of the theca shows fully developed typical steroid-producing cells. The cells are of polygonal shape, the nuclei are round with conspicuous nucleoli. The cytoplasm is packed with smooth-walled vesicles, and acquires a foamy appearance (Figure 3). Tubular cristae within the mitochondria are noticeable. Lipid droplets are a further common finding in these cells, even with the light microscope (Figure 1). No essential changes could be observed between the 16th and 20th day. In spite of certain variability in results it is possible to remark a further thickening of the theca and, therefore, a greater extent of the above-mentioned findings. As in the preceding results the changes in thecal cells are increasing in the direction of the peripheral zone. The 3β-OH-SDH activity could not be demonstrated outside the thecal area (Figure 2).

Following the 12th day, a segregation of small cell groups with steroidogenic features from the thecal surface into the interfollicular space was observed. The other cells in these spaces appear as histochemically non-reactive, without submicroscopical evidence of steroid biosynthesis. Submicroscopically they show mostly the features of young fibroblasts.

[1] J. Presl, J. Jirásek, J. Horský and M. Henzl, J. Endocrin. *31*, 293 (1965).
[2] J. Presl, J. Herzmann and J. Horský, J. Endocrin. *45*, 611 (1969).
[3] A.K. Christensen and S.W. Gillim, in *The gonads* (Ed. K.W. McKerns; Appleton-Century-Crofts, New York 1969), p. 415.

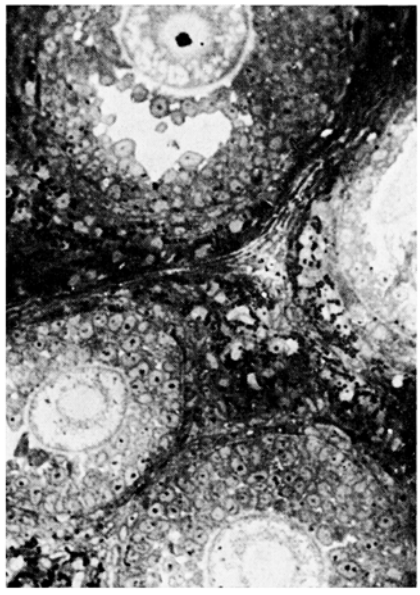

Fig. 1. Follicles in the immature rat ovary at the age of 15 days. Accumulation of the lipid droplets in the peripheral thecal zone (toluidine blue).

Fig. 2. 3β-hydroxysteroid dehydrogenase activity in the same ovary as in Figure 1.

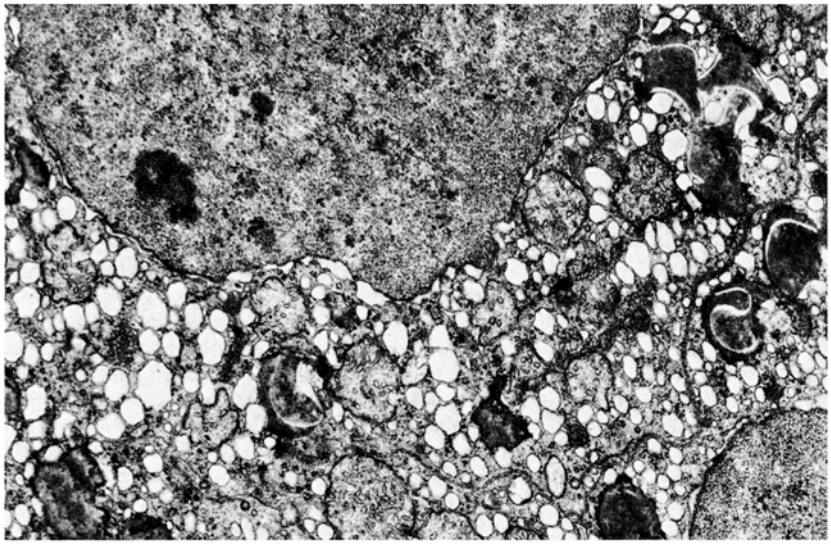

Fig. 3. Detail from the two adjacent steroid-secreting cells in the same ovary as in Figure 1. Mitochondria with tubular cristae, vesicular features of the agranular endoplasmic reticulum, irregular lipid droplets.

The results confirmed explicitly the local and temporal coincidence of the positive 3β-OH-SDH reaction with the typical submicroscopical manifestation of steroid biosynthesis. In addition to the peripheral zone of thecal cells which begins to react on and after the 10th day, small groups of cells with the identical submicroscopical features and enzymatic reactivity appeared in the interfollicular spaces following the 12th day. The development of these cell groups by segregation from the theca coincides with the described development of the so-called primary, histochemically reactive interstitial tissue[1,3]. Cytodifferentiation of the initially indifferent thecal cells, which in the beginning are indistinguishable from young fibroblasts, proceeds invariably in the direction of the peripheral thecal zone, and the fully developed steroid-producing cells are found close to the capillaries. Therefore, it is suggested that the stimuli for the cellular steroidogenic transformation proceed from the blood vessels rather than from the follicle. The hypothesis of the development of the interstitial tissue cells from the follicular cells[1,3] or from the interfollicular spaces pericapillary undifferentiated stromal cells[2] could not be confirmed[4–6].

Zusammenfassung. Steroid-produzierende Zellen wurden in der Theca folliculi der geschlechtsunreifen Ratten vom 10. Lebenstag an elektronenmikroskopisch gefunden.

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5 E.G. RENNELS, Am. J. Anat. *88*, 63 (1951).
6 H.-E. STEGNER, in *Gonadotrophins and Ovarian Development* (Eds. W. R. BUTT, A. C. CROOKE, M. RYLE; Livingston, Edinburgh-London 1970), p. 132.

New Chromosomic Base Number for the Himalayan Genus *Meriandra* Benth. (Labiatae)

The genus *Meriandra* is represented by 2 species in the Indian subcontinent (HOOKER[1]) and 1 species namely *Meriandra bengalensis* is cultivated for its use in medicine. *Meriandra strobilifera* Benth. is a small woolly caespitose perennial shrub, commonly found on limestone outcrops in the West-Himalayas within an altitudinal range of 1200–2000 m.

A population (GILL 7578) of *M. strobilifera* from Naldehra (1200 m.) in the West-Himalayas has been found to have a haploid chromosome number of 9 (Figure). A consistant gametophytic number of 9 has been obtained from several other populations from Solan-Kalka hills. The species is self compatible and self pollinating. Chromosome counts were made from microsporocytes following the method already described in a previous paper (GILL[2]). Voucher specimens are deposited at the Herbarium of Panjab University, Chandigarh (India).

Résumé. Nouveau nombre de base pour le genre du Himalayas *Meriandra* Benth. (labiées).

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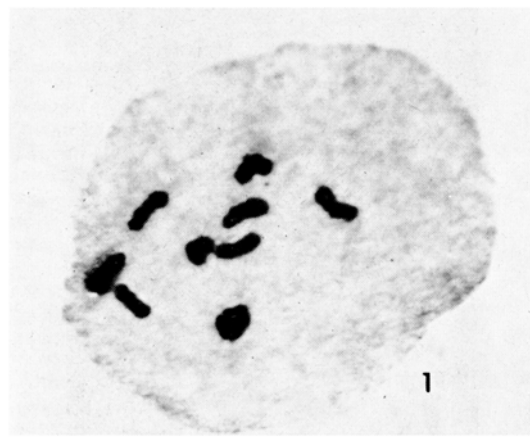

Meriandra strobilifera ($n = 9$), first metaphase. ×1350.

1 J. D. HOOKER, *The Flora of British India* (Ed. L. Reeve and Co. Ltd., London 1885), vol. 4.
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