NOTE

Relationship between the Follicular Cells and Marginal Layer Cells of the Anterior Pituitary

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Synopsis

In the anterior pituitaries of adult rats of both sexes, follicular cells resemble marginal layer cells in fine structure. Numerous occurrences of cilia were electronmicroscopically confirmed on the free surface of both cells. The absence of secretory granules, the poor development of endoplasmic reticula and abundant free ribosomes and polysomes are the other coincidental cytological properties between both cells showing the immaturity. Thus, the two cells seem to be intimately related. The follicular cells may play an important role in the mitotic activity of the gland. Cilia and microvilli on the free surface of follicular cells are not easily changed by some experimental conditions.

Pituitary marginal layer in the adult rat consists of the simple columnar cells surfacing the pituitary cavity developing from the Rathke's pouch. Its free surface is equipped with the microvilli and cilia (Romeis, 1940). Since the rat anterior pituitary develops through the proliferation of the cell-cords growing out from the thick marginal layer (anterior wall of Rathke's pouch) during the embryonic period (Yoshimura *et al.*, 1970), there is a possibility that the anterior wall of Rathke's pouch may be a primordium of the cell-cords, of which some constituents may thereafter differentiate into the granulated cells but some others keep their original property as the agranular follicular cells. The finding demonstrated here that there is an abundance of cilia and microvilli in the pseudolumina among a cluster of follicular cells of the adult rats may suggest a correlation with this developmental possibility.

Materials and Methods

Widespread cytological investigations have been made in our laboratory using the pituitary glands of many Wistar-Imamichi rats under the normal and experimental conditions. Among the numerous observations, we shall report here selectively only the two cases in which the equipment of cilia was clearly demonstrated in the pseudolumina among a cluster of follicular cells. Those cases are: 1) normal Wistar-Imamichi female rats, 60-day-old, diestrous. 2) male rats, 60-day-old, given an *iv* injection with TRH (1 μ g/ml/rat) daily for 3 days.

Electronmicroscopy:

Pituitary tissues from each group were fixed in 2% osmic acid in veronal acetate buffer (pH 7.3) for 2 hr. After fixation, the tissues were dehydrated in ascending ethanol and embedded in Epon. The thin sections were made with Porter-Blum ultratome, and stained with saturated uranyl acetate and with lead citrate (Reynolds, 1963). The sections were examined with the JEM7 and 100B type electron-microscopes.

Findings

Marginal layer cells (Fig. 1)

Marginal layer constantly consists of the simple columnar cells in the adult rats. A small number of short microvilli sporadically arise on their free surface. The epithelial cells are not equivalently columnar in shape, occasionally representing the irregular contour. The two adjacent cells are connected with each other with the tight junction (terminal bar); the basal side of the cells adheres to the underlying connective tissues. These cells always lack the secretory granules regardless of the sex and age-difference, being scanty of delicate tubular endoplasmic reticula but rich in free ribosomes and polysomes (Fig. 1). Some of the cells have many cilia on their

free surface, but some others have not. Microvilli usually appear sporadically on the free surface of the superficial cilia-rich cells.

Follicular cells (Figs. 2 and 3)

Several numbers of follicular cells constitute an islet-like cluster scattered at the deep area of the gland. They are immature cells, being small in size and irregular in shape. The distinct boundary between them is smoothly or straightly extended, but sometimes bends densely, interdigitating each other. This interdigitation may lead to the initial formation of microvilli. The neighbouring cells are tightly connected with *Zona occludens* (double arrows in Fig. 2). The wide pseudolumina or confluences of the interspace among the cells are equipped with the microvilli and cilia (arrow). The pseudolumina are often filled

Fig. 1. Marginal layer cells from a normal female rat, 60-day-old, dioestrous. $\times 8,000$.

Fig. 2. Follicular cells from a male rat injected with TRH for 3 days. $\times 10,000$. Arrow shows the pseudolumen. Double arrows show tight junction.

Fig. 3. High magnification of the pseudolumen filled with microvilli and cilia among a mass of follicular cells. $\times 45,000$.

with them. The follicular cells contain numerous free ribosomes and polysomes, and occasionally a few delicate tubular endoplasmic reticula instead of the secretory granules. The Golgi apparatus is not prominent. The incidence of pseudolumen equipped with microvilli is high, while that equipped with cilia is low. In other words, a great majority of follicular cells belong to the microvilli type, while a limited number of them belong to the cilia type. The incidence of the latter cannot be easily affected under any experimental conditions.

An abundance of cross-sections of cilia are demonstrated on high magnification (Fig. 3). They contain two central filaments surrounded by nine double ones, and the central fiber is arranged straightly in parallel to a pair of outer fibers (doublets) on their longitudinal sections. Microvilli are arranged toward the various directions concomitantly with cilia filling the pseudolumen.

Discussion

It was found for the first time by Barnes (1961) that certain chromophils in the mouse anterior pituitary were ciliated. Each pituitary cilium is associated with a basal body and a proximal centriole, either projecting singly into a large intercellular space or being deeply embedded in the cytoplasm of acidophils or the other cells. The existence of numerous cilia was detected by Dingeman and Feltkamp (1972) in the space among follicular cells kept in pituitary grafts from the male mice transplanted into the kidney capsules of the female mice. However, a great majority of cilia have not been demonstrated in the intercellular cavity among the follicular cells of the rat pituitary. Non-granulated epithelial cells of the pars tuberalis of the rat pituitary project the microvilli and cilia into the intercellular space (Oota and Kurosumi, 1966). They resemble in fine structure the follicular

cells of the anterior lobe. The marginal zone of the pars intermedia of the rat pituitary is also equipped with microvilli and cilia (Kobayashi, 1968).

The fine structural similarities between the marginal layer cells and follicular cells of the pars anterior are as follows: 1) equipment of numerous cilia and microvilli on the free surface, 2) agranularity of the cells, 3) abundant occurrence of ribosomes and polysomes. These similarities may account for the intimate relationship in development: The marginal layer and follicular cells may have the same developmental origin, that is, the anterior wall of Rathke's pouch. The marginal layer cells of the pars anterior and pars intermedia, and nongranulated cells of the pars tuberalis, are homologous in fine structure with the striking similarity to the follicular cells, as evidenced by the presence of cilia and microvilli. Mitotic figures are detectable in the follicular cells in all the cases examined, because they keep the immaturity even during the adulthood.

Ohtsuka *et al.* (1971) cultured the pellets of dispersed various types of cells obtained from the pituitaries of old rats, and examined the incorporation of ^3H -thymidine into the nuclear DNA *in vitro*. Since the incorporation by the chromophobes and ambiguous cells was highest, but that by the chromophils was almost negligible, the chromophobes were regarded as the cells performing the high mitotic activity. Recently, Shioda (1975) injected ^3H -thymidine into the immature rats after intravenous perfusion with TRH. He found autoradiographically the fact that the labelled chromophobes could differentiate non-specifically into the labelled chromophils in 5 or 7 days. This autoradiographic finding *in vivo* is not contradictory to the *in vitro* finding by Ohtsuka *et al.* (1971) and Yoshimura *et al.* (1974) who observed light and electronmicroscopically that a mass of dispersed chromophobes in culture might

transform into the acidophils or basophils in connection with CRF, TRH and LRH.

In reference to the fine structural similarity between marginal and follicular cells, it may be speculated that the follicular cells are primitive or undifferentiated cells which continue to perform the mitotic activity without granulation and cytodifferentiation. This interpretation may be applicable to a concept of "renewal cell system" which will be presented in our following paper.

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