An Electron Microscopic Study on Follicular Formation and TSH Sensitivity of the Fetal Rat Thyroid Gland in Organ Culture

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ABSTRACT. Thyroids of 15-day rat fetuses prior to culture had no follicles. After 2 days in culture, regardless of added TSH in the medium, thyroids had small, colloid-storing follicles. In the follicular cells, the rER and Golgi apparatus were somewhat well developed as compared with the thyroids prior to culture. Thyroids of 17-day fetuses prior to culture were first inlaid with follicles. With TSH, 2-day cultures of 17-day thyroids showed a more marked dilation of rER and a further development of Golgi apparatus than those without TSH. These results indicate electron microscopically that TSH has no effect on the first formation of follicles as well as on the initial development of follicular cells, but that TSH can promote the further development of follicular cells themselves of once-developed follicles.—**KEY WORDS:** rat fetus, thyroid gland, TSH sensitivity.

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The thyroid anlage first appears as an invaginated thickening of the endoderm in the ventral midline of the pharyngeal gut. This thickening forms a small bilobed diverticulum that descends along the front of the pharynx to reach its definitive position around the trachea, where it begins to differentiate histologically as well as functionally. Ultrastructurally, thyroid cells are already united side by side with each other prior to the first appearance of the primitive follicular lumen [2, 9]. In fetal rats, follicles first appear on day 15 [7], 17 [1, 4, 11], or 18 [12] of gestation. The apical surface of each thyroid cell facing the primitive follicular lumen is studded with protruding microvilli, and Golgi apparatus in each cell is usually situated between the nucleus and the apical surface of the cell [5].

Removal of the fetal rat pituitary gland by surgical decapitation retards the growth of the thyroid, an effect which is prevented by the injection of TSH into the hypophysectomized fetus [6, 10]. Unilateral thyroidectomy causes a significant compensatory enlargement of the remaining lobe of the gland [3]. These results suggest that the pituitary-thyroid system in the rat begins to function before birth.

According to our previous experiment *in vitro* [13], TSH is not required for the first formation of follicles, but the follicles first formed on day 17 become sensitive to TSH for their subsequent development. However, our previous study dealt only with the formation of follicles, without referring to the development of follicular cells themselves. Accordingly, the present study was designed to examine ultrastructural changes in follicular cells of the fetal rat thyroid glands which were transplanted with added TSH to organ culture for 2 days.

Wistar rats were used. They were given commercial diet (Labo MR Breeder) and water, both *ad libitum*. Females were placed with males overnight and examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was detected was designated as day 0 of gestation. Thyroids of 15- to 17-day fetuses were removed. Each thyroid was cultured for 2 days with or without 0.1 IU/ml TSH (Sigma) according to the procedure for culture described previously [13].

After incubation, the cultures were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.4, at 4°C for 2 hrs, and then postfixed in 1% osmium tetroxide in 0.2 M cacodylate buffer, pH 7.4, at 4°C for 1 hr. Following rapid dehydration with ethanol, each culture was embedded in Spurr Resin (TABB Laboratories Equipment Ltd.). Thin sections were stained with uranyl acetate and lead citrate, and examined with a JEM-100 CX electron microscope.

Thyroids of 15-day fetuses prior to culture consisted of irregularly arranged cell cords without follicle formation. The cords were composed of clusters of closely packed cells. In the central region of each cluster, however, adjacent cell membranes were complexly interdigitated each other. Very narrow intercellular spaces formed by such complex interdigitation of cell membranes contained a slight accumulation of substances with a high electron density. The cell junction was observed to be already accomplished by some zonulae adherens and desmosomes from place to place (Fig. 1). The cytoplasm was rich in polysomes, but poor in rough endoplasmic reticulum (rER). Golgi apparatus was not well developed. After 2 days in culture without TSH, thyroids became cell masses inset with small follicles which were made up of a few cells containing colloid with a high electron density (Fig. 2). Numerous microvilli protruded into the follicular lumen. Adjacent cell membranes at the apical side were in close contact with each other by a desmosome or a zonula adherens. In the follicular cells, rER appeared to be increased in amount and Golgi apparatus was somewhat well developed, as compared with the thyroid prior to culture. TSH, when added in culture, did not alter such ultrastructures of thyroids *in vitro*.

Thyroids of 16-day fetuses prior to culture mostly consisted of radially arranged cell groups. The interdigitation of cell membranes in the central region of each cell group became more complex. The follicular lumen was not yet formed, but the intercellular spaces appeared to be slightly expanded and were filled with substances with a high electron density. After 2 days in culture without TSH, 16-day thyroids had more developed follicles with

Fig. 1. Electron micrograph of a 15-day thyroid prior to culture, showing cell-membrane infoldings, finger-like protrusions, zonulae adherens (arrow) and desmosomes (D). Intercellular spaces contain tiny electron dense substances (arrowhead). N: nucleus, R: rough endoplasmic reticulum (rER), M: mitochondrion, $\times 26,000$.

Fig. 2. Electron micrograph of a 2-day culture of a 15-day thyroid without TSH, showing a follicular lumen filled with colloid, microvilli (arrowhead) and junctional complexes (arrow). N: nucleus, $\times 8,400$.

Fig. 3. Electron micrograph of a 2-day culture of a 16-day thyroid without TSH, showing developed rER (R) and Golgi apparatus (G), and mitochondrion (M). Such features are also the same in the cultures in the presence of TSH. N: nucleus, $\times 26,000$.

Fig. 4. Electron micrograph of a 17-day thyroid prior to culture, showing a follicular lumen filled with colloid with a high electron density and microvillus (arrow). N: nucleus, R: rER, G: Golgi apparatus, M: Mitochondrion, Z: zonula adherens, $\times 26,000$.

Fig. 5. Electron micrograph of a 2-day culture of a 17-day thyroid without TSH, showing developed rER (R) and Golgi apparatus (G), and mitochondrion (M). N: nucleus, $\times 20,000$.

Fig. 6. Electron micrograph of a 2-day culture of a 17-day thyroid in the presence of TSH, showing further developed rER (R) and Golgi apparatus (G), compared with those shown in Fig. 5. N: nucleus, M: mitochondrion, $\times 20,000$.

stored colloid than had 15-day thyroids. Golgi apparatus was observed to be situated at the supranuclear cytoplasm, and rER developed relatively more than that in 15-day thyroids (Fig. 3). However, TSH, when added in culture, did not alter the fashion of follicle formation and the development of cell organelles.

Thyroids of 17-day fetuses prior to culture were first inlaid with small, colloid-storing follicles (Fig. 4). Many microvilli protruded from the apical surface of cells into the follicular lumen. Close to the follicular lumen, zonulae adherens and desmosomes were observed at the lateral side of cells. However, rER developed still poorly. The 2-day cultures without TSH had well-developed follicles (Fig. 5). Most of cell masses or groups in thyroids developed into follicles. A majority of these follicles stored colloid with a high electron density. The rER and Golgi apparatus were developed in a fashion comparable to that observed before incubation (Fig. 4). TSH, when added in culture, induced a marked dilation of rER and a better development of Golgi apparatus (Fig. 6).

An earlier study revealed that in organ culture fetal rat thyroid glands were able to develop follicles to store colloid in a TSH-free medium [8]. In our previous study [13], 2-day cultures of 14-day fetal thyroids failed to develop follicles, regardless of TSH added in the culture medium. However, 2-day cultures of 15- and 16-day thyroids formed small colloid-containing follicles, although no follicle was present prior to culture. Therefore, these observations suggest that the initial formation of thyroid follicles is independent of TSH.

In the present study, we observed the fashion of follicle formation by electron microscopy. Then, thyroids of 15-day fetuses prior to culture did not exactly form follicles, although in the central region of cell clusters, adjacent cell membranes were interdigitated each other. These interdigitations were likely destined to form microvilli after the formation of follicular lumen. Moreover, some zonulae adherens and desmosomes were present at the apical end of the lateral cell membranes. According to ontogenic studies, the zonula occludens [2] or the zonula adherens [9] is present between adjacent cells before the primitive follicular lumen appears. Therefore, 15-day thyroids seem just starting to form follicles. In the present study, added TSH did not later the fashion of follicular formation, leading to the conclusion that TSH has no effect on the follicular formation at the electron microscop-

pic level as well.

In our previous study, once-developed follicles seemed to be able to respond to TSH in such a way that the follicular epithelial cell was higher and the follicular diameter was larger in TSH-treated thyroids than in thyroids without TSH. Similarly, in the present electron microscopic observation, TSH clearly promoted the development of the ultrastructure of follicular epithelial cells, in particular the development of rER and Golgi apparatus. In general, removal of the pituitary gland in adult animals induces lowering of follicular cells and shrinkage of rER and Golgi apparatus, whereas a long-term TSH-treatment makes follicular cells higher [5]. Considering such a nature of thyroids, it is evident that added TSH in 17-day thyroid cultures activated the function of epithelial cells, the sign of such TSH effect being evidenced by the expansion of rER and well development of Golgi apparatus. Thus, the present electron microscopic study also supports the view that TSH can promote the development of epithelial cells themselves of once-developed follicles.

REFERENCES

1. Calvert, R. 1972. *Am. J. Anat.* 133: 269-290.
2. Calvert, R. and Pusterla, A. 1973. *Gen. Comp. Endocr.* 20: 584-597.
3. Eguchi, Y. and Morikawa, Y. 1966. *Anat. Rec.* 156: 415-422.
4. Feldman, J. D., Vazquez, J. J., and Kurtz, S. M. 1961. *J. Biophys. Biochem. Cytol.* 11: 365-383.
5. Fujita, H. 1975. *Int. Rev. Cytol.* 40: 197-280.
6. Hwang, U. K. and Wells, L. J. 1959. *Anat. Rec.* 134: 125-141.
7. Ishikawa, K. 1975. *Okaj. Fol. Anat. Jpn.* 41: 295-311.
8. Jost, A. and Martinet, C. 1975. *Arch. Anat. Hist. Embr.* 58: 89-94.
9. Rémy, L., Michel-Bechet, M., Athouel-Haon, A.-M., Magre, S., Cataldo, C., and Jost, A. 1980. *Arch. Anat. Micro.* 69: 91-108.
10. Sethre, A. E. and Wells, L. J. 1951. *Endocrinology* 49: 369-373.
11. Stockel, M. E. and Porte, A. 1970. *Z. Zellforsch. Mikrosk. Anat.* 106: 251-268.
12. Strum, J. M., Wicken, J., Stranbury, J. R., and Karnovsky, M. J. 1971. *J. Cell Biol.* 51: 162-175.
13. Yamamoto, M., Takizawa, T., Arishima, K., and Eguchi, Y. 1986. *Anat. Rec.* 215: 361-364.