Three-Dimensional Imaging of Blood Vessels in Thyroids from Normal and Levothyroxine Sodium-Treated Rats*

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Summary. Three-dimensional images of the microvascular architecture in the thyroid glands of levothyroxine sodium-treated (for 4 weeks) rats were examined by scanning electron microscopy of corrosion casts made by the injection of methacrylate resin. In the experimental animals, each follicle was surrounded by a clearly defined basket-like capillary network generally independent of the adjacent one in the same manner as in normal animals. However, the capillaries were markedly poor in distribution, conspicuously small in diameter, and their anastomoses were clearly decreased as compared with the normal state. The diameters of the capillaries were 3-8 μm in these cases, while those in normal specimens were 5-15 μm. The capillary bed surrounding each follicle occupied about 25% of the follicular surface area, while that in normal cases occupied about 50%. In conclusion, it became clear that the distribution and morphology of the thyroid capillaries are markedly affected and changed by the functional state of the gland.

This paper reports on three-dimensional images of the microvascular architectures of normal and hypofunctional rat thyroids. The thyroid function itself is suppressed by the thyroxine-treatment owing to the negative feedback mechanism. In the hypofunctional thyroid, it is known that the follicular epithelial cell is attenuated and its cytoorganelles are markedly reduced (FUJITA and SUEMASA, 1968), and that the capillary endothelial fenestrations are reduced in number and population density (ISHIMURA et al., 1976). In a previous paper, we reported changes in the three-dimensional microvascular architecture, such as the dilation and fusion of the blood capillaries, in the hyperfunctional state of this organ (IMADA et al., 1986). All these observations reveal that the structure of blood vessels is reflected by the functional condition of the gland. However, when considering the three-dimensional microvascular architecture of the thyroid gland in the hypofunctional state it is somewhat surprising that no papers have been available except for a short description (KLAK et al., 1983). The present paper was planned to serve towards alleviating this lack of information.

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MATERIALS AND METHODS

Twenty male Wistar rats, 8 weeks of age, weighing 180–200 g, were used for this study. The animals were divided into two groups. Ten animals were fed standard pellets (Oriental Yeast Co., Ltd.) and tap water, while the others were fed standard pellets and tap water containing 0.5% levothyroxine sodium (Thyradin-S, Teikoku-zoki Co., Ltd.) for 4 weeks.

A low viscous solution of methacrylate resin (Mercox, Dainihon Ink and Chemical Co., Ltd.) was used to make corrosion casts of the thyroid glands, according to the procedures by Murakami (1971) and Ohtani and Murakami (1978). Fourteen (7 control and 7 levothyroxine-treated) rats, anesthetized by the intraperitoneal injection of sodium pentobarbital (Nembutal, 50–100 mg/kg), were perfused with Ringer’s solution containing heparin (2 x 10³ units/l) at 37°C via the left ventricle, and then injected with Mercox resin at a rate of 5 ml/min. After the injection, the rats were immersed in hot water (60°C) for 30 min and their thyroids were removed. The excised glands were again immersed in hot water (60°C) for 3 hr, macerated in a 20% NaOH solution for 1–2 days, and washed in H₂O. The specimens were then immersed in a sodium hypochlorite solution (60°C, household bleach) for 8 hr, washed in H₂O, and air-dried. Each corrosion cast was mounted on a metal stub, coated with platinum by use of an Eiko IB-5 ion-coater and observed in a Hitachi S-800 scanning electron microscope (SEM). In order to observe sectional profiles, some casts were frozen in a 30% sucrose solution and sectioned with a cryomicrotome. After washing in H₂O, air-drying and ion-coating, they were observed in the SEM.

Six anesthetized (3 normal and 3 levothyroxine-treated) rats were perfused with a solution of 2.5% glutaraldehyde, buffered with Millonig’s phosphate at pH 7.4, and their thyroid glands removed. One lobe of the thyroid of each animal was minced with a razor blade, and immersed in the same fixative 2 hr. Following this the specimens were postfixed in 1% OsO₄ solution buffered with the same buffer for 1 hr (4°C), and block-stained with 3% uranyl acetate solution for 1 hr. They were dehydrated with graded concentrations of alcohol and embedded in Epoxy resin. Thin sections cut with a MT-1 type ultramicrotome (Sorvall) were doubly stained with uranyl acetate and lead citrate and examined in a Hitachi H-500 transmission electron microscope (TEM).

The other lobes of specimens from six animals were immersed in 10% formalin solution for 24 hr, dehydrated with graded concentration of alcohol and embedded in JB-4 resin (Polysciences Inc.). Semithin sections were cut, stained with toluidine blue or hematoxylin-eosin and observed with the light microscope.

RESULTS

Normal rats

As previously reported (Imada et al., 1986), scanning electron microscopy of vascular corrosion casts of normal thyroids reveals numerous units of basket-like capillary networks densely enclosing each follicle, narrow grooves corresponding to the interfollicular connective tissue, and deep, wide and long fissures corresponding to the interlobular connective tissue. In each unit of basket-like capillary network, the capillary bed
occupies about 50% of the follicular surface area. The diameters of the capillaries (5-15 μm) do not vary greatly (Fig. 1a, 2, 3).

Levothyroxine sodium-treated rats

The thyroid gland after this treatment is by far smaller in size than in normal rats. By light microscopy, all the follicle epithelial cells are excessively lower in height, and the follicle lumina, round or oval in shape, are intensively enlarged as compared with those of the normal rat. Interfollicular connective tissue elements are very thin and the blood capillaries are scantily distributed (Fig. 1b).

Fig. 1. Light micrographs of the rat thyroid in normal and hypofunctional conditions. a. Normal rat. Numerous round or oval follicles (F) surrounded by connective tissue containing capillaries are seen. I interlobular connective tissue. ×340. b. Rat given levothyroxine sodium (for 4 weeks). Follicle lumina (F) are extremely enlarged and follicle epithelial cells are markedly lower in height. Capillaries among the follicles are scantily distributed. ×360
Fig. 2. A scanning electron micrograph of the vascular cast of the normal rat thyroid. Deep, wide and long fissures (arrow) corresponding to the interlobular connective tissue are seen. The lobule consists of numerous basket-like units of capillary networks enclosing the follicle. × 160
By transmission electron microscopy of the thyroids in experimental animals, it is clear that the follicle epithelial cells and their nuclei are flattened, and the follicular lumina are larger in size; the intracellular colloid droplets have disappeared and cyto-organelles such as the elements of rough endoplasmic reticulum and Golgi apparatus are reduced in number and size as compared with those in normal specimens. The

**Fig. 3.** A scanning electron micrograph of the vascular cast of the normal rat thyroid. Many narrow grooves (arrow) corresponding to the interfollicular connective tissue are seen. Each follicle (F) is densely enclosed by a clearly defined basket-like capillary network. a Interlobular artery, v interlobular vein. × 410
capillaries, located in the interfollicle connective tissue, are smaller in diameter and do not indent the follicle epithelial cells.

Scanning electron microscopy of the vascular corrosion casts demonstrates the basket-like capillary network surrounding each follicle in the levothyroxine-treated animals to be as well preserved as that of the normal animals. The basket of capillaries is generally independent of that surrounding the adjacent follicle though their interconnections are sometimes seen as in normal cases. The capillaries covering each

![Fig. 4. A scanning electron micrograph of the vascular cast of the levothyroxine sodium-treated (4 weeks) rat thyroid. Many grooves are seen as in the normal case. The clearly defined structure of the basket-like network covering the thyroid follicle is well preserved but very thin in distribution (as compared with Fig. 2). ×180](image-url)
Fig. 5. A magnification of the three-dimensional image of the vascular cast of the levothyroxine sodium-treated (4 weeks) rat thyroid. The capillaries in the basket-like network are very narrow in diameter, markedly poor in distribution and their anastomoses are clearly decreased (as compared with Fig. 3). F follicle. × 630
follicle in these experimental animals are very narrow in diameter, the most enlarged part measuring about 8 μm in diameter, while the thinnest part about 3 μm. They are much more poorly distributed as compared with those in normal and hyperstimulated animals, and anastomoses of the capillaries with in each basket-like network are also strikingly decreased in number. The capillary bed covers only about 25% of the area of the follicular surface (Fig. 4, 5).

DISCUSSION

With the administration of Thyradin-S (levothyroxine sodium), the levels of thyroxine in the blood are increased, the secretion of TSH (thyroid stimulating hormone) from the anterior pituitary is decreased by negative feedback regulation, and the thyroid function is suppressed by this mechanism (VANDERLAAN and GREER, 1950; SHARRER and ASPER, 1956). Hypophysectomy or thyroxine administration has been known to induce similar hypofunctionally morphological changes in the thyroid gland (YAMADA et al., 1961). As reported by many investigators (DEMPSEY and PETERSON, 1955; WETZEL et al., 1965; SELJELID, 1967; FUJITA and SUEMASA, 1968; SELJELID et al., 1971; BJORKMAN et al., 1974; GALL et al., 1976), the thyroids of hypophysectomized or thyroxine-treated animals become extremely small in size, the follicle lumina are enlarged, and the follicle epithelial cells are attenuated. In addition, it has been clarified through the present three-dimensional observations that by the administration of levothyroxine sodium, the capillary network around each follicle becomes markedly poor in distribution, the diameter of the capillary becomes very narrow (3-8 μm), and the anastomoses of capillaries within each basket-like network are decreased, though the essential structure of the basket covering the thyroid follicle remains well preserved. In the hypophysectomized or thyroxine-treated animals, the thyroid activity lessens and the total protein synthesis is much reduced (RAGHUPATHY et al., 1963; PAVLOVIC-HOURNAC and DELBAUFFE, 1975). We previously reported in the Thyradin-treated mice that the number and population density of the endothelial fenestrations of the thyroid capillaries markedly decrease (ISHIMURA et al., 1976). The present findings in the hypofunctional state of the thyroid are morphologically opposite those of the hyperstimulated thyroids showing the higher distribution (population density) and dilatation of the blood capillary reported by us previously (IMADA et al., 1986). These facts indicate that the structure and development of the microvascular system in the thyroid are highly affected by the functional conditions of the gland.

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