THE SUBMICROSCOPIC STRUCTURE OF THYROID EPITHELIUM UNDER THE INFLUENCE OF THYROXIN

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An electron-microscopic study of thyroid epithelium under experimentally varied conditions of secretory activity, i.e., essentially with different rates of discharge of active hormone into the bloodstream, is necessary to establish connections between the various stages in the secretory process and the actual cellular structures involved. This particular research is concerned with an investigation into the ultrastructure of thyroid gland cells in the rat, under conditions of gradual saturation of the animal body with thyroxin introduced from without. Excess of exogenous thyroxin in the blood leads to a diminution in the release of the hormone from the follicles of the thyroid into the bloodstream and a decrease in the rate of synthesis of the specific protein by the thyroid cells.

At the level of light microscopy, the cytomorphological and cytochemical characteristics of thyroid epithelium under conditions of reduced endocrine activity have been given in a series of works [2, 3, 4, 6, 8, 9]. Electron-microscopic investigations into the inactive state of thyroid epithelium are restricted to information about particular cell components of the follicular epithelium of hypophysectomized rats [5, 10]. Literature on the normal submicroscopic organization of thyroid epithelium is less restricted [5, 10, 18, 25, 27].

EXPERIMENTAL

White rats (males), weighing 80-100 g at the beginning of the experiment, were given thyroxin in their drinking water over several weeks. The daily production of the hormone in the young rat is from 5-7 micrograms of thyroxin [7]. On the basis of this amount and taking into account our method of administering thyroxin, we gradually increased the amount of thyroxin in the drinking water to the equivalent of 20 micrograms per day for each animal. This dose appeared to be sufficient to evoke obvious stages in the histological appearance of the organ in a relatively short time while preserving the integrity of the majority of the follicles. Prolonged administration of thyroxin may lead to degeneration of the follicles and the formation of colloid cysts [26].

The glands were excised from the animals at various times throughout the experiment; from the 1st to the 7th day after the commencement of exogenous thyroxin treatment of the rats. Glands taken from 3 animals were fixed at each interval.

As controls representing the general trend in morphological changes in the organ, we took part of the gland of each animal and studied it under the light microscope after the usual treatment of fixation, sectioning and staining. For the electron microscopic investigations, fragments of gland were fixed in 1% OsO_4 solution at a pH of 7.4 and were embedded in a mixture of methacrylates. Sections were prepared with an LKV ultramicrotome, type 4811A. To increase the content of the sections we used uranyl acetate and lead monoxide followed by staining with other substances. The sections were examined in an electron-microscope UÉMV-100.

RESULTS

By the end of the experiment the action of thyroxin had lead to near uniformity of structure among the follicles. The latter were filled with a dense colloid; the epithelial cell parallel to the base. Both cells and nuclei had suffered some reduction in their dimensions. The organ had become less completely filled with blood.

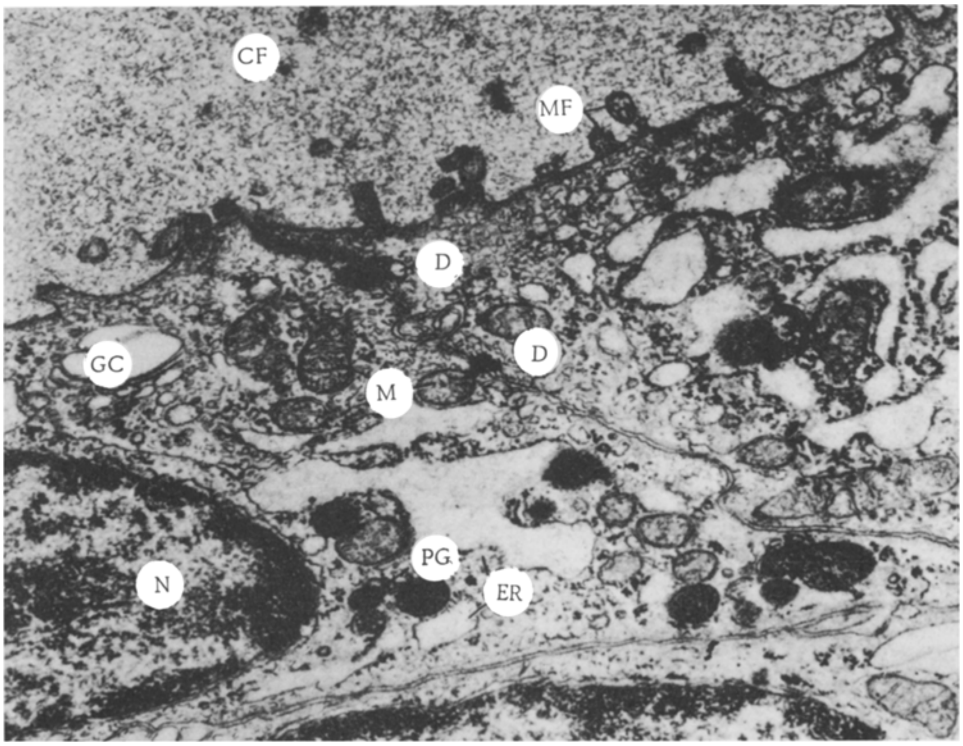

Fig. 1. Cells of follicles after 6-day treatment with thyroxin. N) Nucleus; M) mitochondria; GC) Golgi complex; ER) endoplasmic reticulum; PG) dense "peroxidase" granules; MF) microfibrillae; D) desmosomes; CF) cavity of follicle. Magnification 30,000 ×.

Observations under the electron-microscope revealed definite changes in the ultrastructure of the thyroid cells. Normally they are convex and covered with a great number of microfibrillae, but under the influence of thyroxin they lose their convexity. The number of microfibrillae also undergoes a decrease, and those remaining become short and blunt (Figs. 1 and 2MF). If the microfibrillae are a factor in the resorption of material from the lumen of the follicle, as has been suggested, then a decrease in their number would appear to result in a diminution of resorption. The loss of convexity by the apical surface of the epithelial cells can apparently be attributed to the influence of mechanical pressure on the surface by the colloid. The characteristic vesicles which are visible in the microfibrillae and apical cytoplasm in normal, active glands [27] disappear with the reduction in endocrine activity. This confirms the view [27] that the presence of these structures is related to the resorption of material into the lumen.

Under the influence of thyroxin, the small (diameter 50-150 mμ), dense granules of the apical layer of the cytoplasm disappear. In normal cells, the accumulation and solution of these granules precedes the active resorption of material from the lumen of the follicle. It would appear that the contents of the granules are secreted into the cavity and this is connected in some way with the resorption of material from the follicular lumen.

The integrity of the junctions between the lateral walls of the thyroid cells, which is maintained by the numerous and characteristic desmosomes [13] and digitate processes, is preserved throughout the course of the experiment (cf. Figs. 1 and 2, D). As in normal cells, the basal membrane forms numerous folds (Fig. 2, BF).

Both normal cells and those subjected to the influence of thyroxin usually contain in their cytoplasm large granules of moderate density and these granules are considered to represent an intracellular colloid [12].

It is the opinion of certain authors [22] that the drops of colloid formed within the cytoplasm of the epithelial cells must be later secreted into the lumen of the follicle. The hypothesis has also been expressed [1, 19, 20] that the appearance of colloid droplets within the cell reflects the resorption of this material from the lumen. In recent years, publications have appeared which suggest that the whole process of thyroid hormone formation must be carried out within the cell itself [12, 23, 24] and that the hormone, at any rate in part, must be secreted directly into the

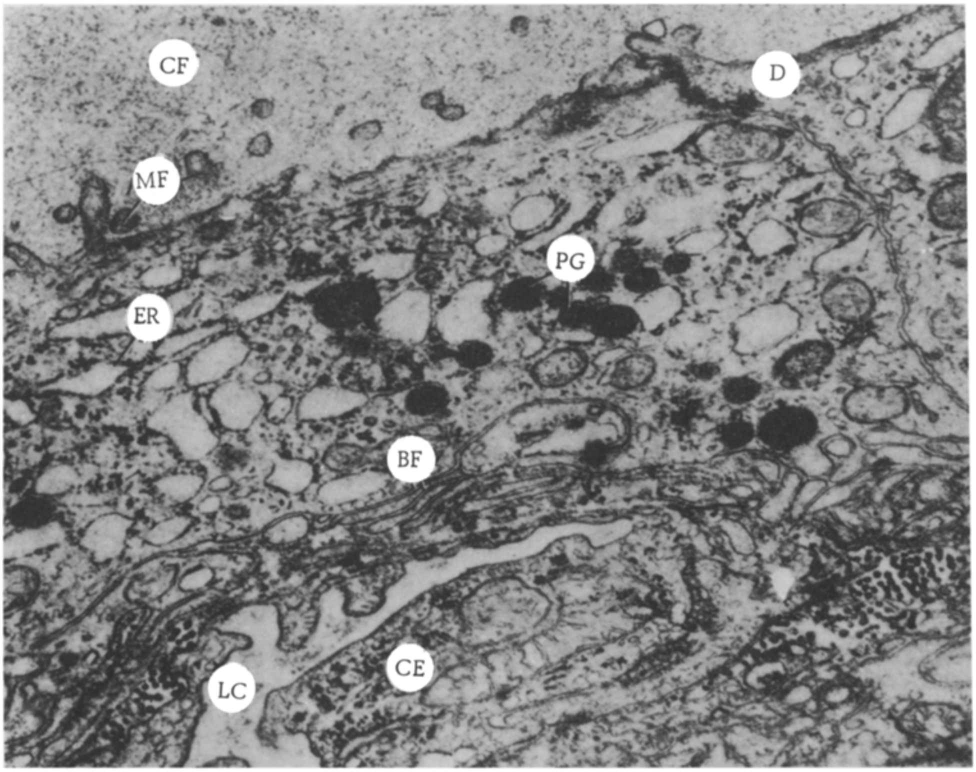

Fig. 2. Cells of follicle and blood capillary after 6-day treatment with thyroxin. BF) Basal folds of cell membrane; CE) cytoplasm of endothelial cells; LC) lumen of capillary. Magnification 30,000 ×. Other symbols as in Fig. 1.

bloodstream. In the light of these facts, the appearance of colloid droplets in the cytoplasm appears to reflect some disturbance in the breakdown mechanism for synthesized protein, which in normal cells is well adjusted to the needs of the cell and takes place prior to the extrusion of the cell contents both into the follicle and directly into the bloodstream.

The other type of cytoplasmic granules—large, dense, rounded granules of diameter 0.15-0.80 μ, with a clearly defined surface membrane—are only encountered in inactivated glands. In the latter, medium sized granules predominate to a greater extent than in the cells of normal glands and they may form small accumulations (cf. Figs. 1 and 2, PG). An increase in the number of this type of granule has been noted in older cells [14]. At the present time it is not easy to guess their true nature. Some authors [16] consider that the large dense granules contain peroxidase. Others [15] link these structures with the Golgi apparatus and consider it probable that they contain glycoprotein. We cannot completely exclude the possibility of this type of granule being associated with the endoplasmic mitochondria, and it seems to us highly likely that these granules are of an enzymatic nature.

Throughout the process of thyroxin saturation the mitochondria retain their usual structure, although they more often assume a rounded shape than in normal cells and are distributed throughout the whole of the cytoplasm (cf. Figs. 1 and 2, M). Under the light microscope under similar conditions, the number of mitochondria has been found to undergo reduction, and fragmentation of the threads and granules have been observed [6, 21]. Accumulations of mitochondria near the basal folds of the cells have also been noted [27].

Lipid granules are encountered more rarely than in normal cells and when they do occur they appear less dense and are mainly situated in the basal parts of the cell. According to data obtained by using the light microscope [9], the number of lipid granules in the thyroid cells increases in the activated glands.

The Golgi complex, is more clearly defined in cells with a reduced endocrine activity than in normal cells; it assumes a position above the nucleus; cisterna and vacuoles are the predominant elements of its structure. The vesicular component of the Golgi complex is only faintly expressed (Fig. 1, GC).

One very noticeable feature of the thyroid gland under conditions of diminished secretory activity is the structural change in the endoplasmic reticulum. In the normal, and particularly the activated gland in rats, these cells are characterized by having a reticulate form of cytoplasm [10, 25, 27]; the cavities of the endoplasmic reticulum have very diffuse contents and the matrix encompassing the other organelles is very slender. In thyroid cells exhibiting decreased secretory activity, the cavities of the endoplasmic reticulum (see Figs. 1 and 2, ER) become reduced in size and the greater part of their fluid content is lost, leaving only narrow, parallel vacuoles, isolated from each other. The granules component on the surface of the membrane and in the cytoplasmic matrix is quite well developed.

The diminution in size of the cavities of the endoplasmic reticulum under the described conditions suggests that these cavities both contain and conduct to the base of the cells the soluble products of iodothyroglobulin proteolysis. As a result of the contraction in size of the endoplasmic reticulum the cells become more compact and are somewhat smaller. When examined under the light microscope, after staining by Brachet's method, the cells appear to be more intensely basophilic than are normal thyroid cells [8].

It is important to compare changes in the endoplasmic reticulum and the density of the granular components of the cytoplasmic matrix under conditions of reinforcement and stimulation of the specific secretory function, with quantitative changes in the proteins and nucleic acids of the cytoplasm. These changes should also be related to the nucleus, a structure which undergoes marked changes under the conditions of our experiment. The nuclei of the stimulated thyroid cells decrease in size and become denser in appearance. Osmophilic material shows a particularly dense concentration along the internal nuclear membrane where the unstained regions are confined to narrow strips, evidently corresponding to the sites of the nuclear pores (Fig. 1, N). The surface of the nucleus becomes more uneven. According to data obtained under the light microscope [4] the amount of DNA in nuclei under the conditions of the experiment corresponds to that associated with the diploid complement of chromosomes.

No definite changes in the structure of the basal membranes of the follicular cells or of the capillary cells have been noticed. The blood vessels, however, show some reduction, and their reduced surfaces lie very close to the follicles. The cytoplasmic margins of the endothelial cells adjacent to the lumen of the vessels become wrinkled (Fig. 2, CE).

Thus, the injection of thyroxin is accompanied by changes in the ultrastructure of the thyroid cells. These changes are associated with a general condition of the cells which results in a definite drop in the output of active hormone elements into the bloodstream and in this sense diminishes the specific hormonal activity of the thyroid glands. The decreased output of hormone from the thyroid lumina into the blood stream is accompanied by the disappearance of micropinocytotic vesicles and small apical granules and by a decrease in size of the cavities of the endoplasmic reticulum. Synthesis of specific proteins undoubtedly continued to some extent throughout the period of our experiments. This is not only suggested by the accumulation of a colloid in the follicular cavities of the thyroid (a reflection of the exocrine aspect of the gland's activity) but by the ultrastructural condition of the granular endoplasmic reticulum in those cells mainly responsible for specific protein synthesis. In order to evaluate the extent to which specific protein synthesis is reduced under these conditions, it will be necessary to carry out further research on the quantitative determination of total and specific proteins, nucleoproteins and enzymes in the thyroid cells and the follicular cavities and to determine the dimensional changes which occur in the follicular cavities, the thyroid cells and their nuclei.

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