Ultrastructure of the Thyroid Gland in Goitered Coho Salmon
(Oncorhynchus kisutch)¹

J. F. Leatherland, R. Moccia, and R. Sonstegard²

Departments of Zoology and Microbiology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT

Thyroid tissues in coho salmon (Oncorhynchus kisutch) goiters showed marked regional variation in structure and ultrastructure. For the most part the follicles were small, were composed of tall epithelial cells, and contained little or no colloid material. The follicle epithelial cells were essentially similar in fine structure to hyperactive thyroids in mammals and one type of follicle in congenital goiters in humans. A second type of follicle, present in human goiters, was not found in coho salmon. The follicle cells in coho salmon contained dense bodies similar to lysosomes in other species, together with larger organelles which could be colloid droplets. There was also an extensive system of dilated rough endoplasmic reticulum and large areas of Golgi membranes. A marked feature was the presence of homogeneous electron-dense material in the intercellular spaces between adjacent follicle cells.

INTRODUCTION

Coho salmon (Oncorhynchus kisutch) in the Great Lakes suffer epizootics of thyroid hyperplasia (goiter). Field epizootiological studies of Great Lakes coho (17, 21) suggest that extrinsic environmental factors, possibly pollutants, in concert with a low-iodine lake environment, are involved in the etiology of the disease. This paper describes the ultrastructure of the thyroid gland in goitered coho salmon and attempts to compare its fine structure with that in other vertebrates.

MATERIALS AND METHODS

Sexually mature prespawning coho salmon (O. kisutch) were collected from a weir trap on the Credit River in October 1976. Thyroid tissue was excised from the goiters of 10 fish and fixed overnight in ice-cold 5% cacodylate-buffered (pH 7.4) glutaraldehyde, postfixed for 2 hr in ice-cold cacodylate-buffered (pH 7.4) 1% osmium tetroxide, dehydrated in a graded series of acetone, and embedded in Epon resin. Sections were made with glass knives and were mounted on naked copper grids. They were stained with uranyl acetate and lead citrate, and observations were made on a Philips 200 electron microscope.

RESULTS

Goiters in coho salmon took the form of large (up to 2 cm in diameter) red swellings, often multinodular, at the bases of the gill arches. They were often bilateral and in extreme cases prevented the complete closure of the operculum.

The goiter tissue was composed of masses of thyroid follicle cells with only very small amounts of colloid material. In light micrographs (Fig. 1d) the goiters showed marked regional variations in appearance with peripheral areas containing follicles with rather more colloid than those in the central mass of tissue. For the most part the central area (Fig. 1c) was composed of tall columnar epithelial cells which often appeared to lack the follicular arrangement that is typical of normal thyroid tissue. Even when present, the follicle lumina contained little or no colloid material (Fig. 1, a, b, and d).

In electron micrographs the basal cell membrane (Fig. 2a) appeared somewhat convoluted. There was little evidence of interdigitation of the lateral membrane that is found in thyroid epithelial cells of some vertebrates [see review by Pantic (20)]. Opposing lateral membranes were found to be joined by junctional complexes composed of regions similar to the tight junctions, intermediate gap junctions, and desmosome zones found in other epithelial tissues (7) (Figs. 2b, and 3, a and c). The apical membrane was composed of very small numbers of short microvilli projecting into small, colloid-filled spaces (Figs. 2b; 3, a and b; and 4a). In some follicles microvilli project into what seem to be on first examination intercellular spaces (Fig. 4b), although these may in fact be reduced follicular lumina. This could explain the apparent afollicular nature of some parts of the goiter in light micrographs. The nuclei were elliptical or spherical, situated toward the bases of the cells; they had smooth outlines with no marked invagination of the nuclear envelope.

The thyroid epithelial cells possessed an extensive intracytoplasmic membranous system of dilated rough endoplasmic reticulum which extended throughout most regions of the cytoplasm (Figs. 2, 4a, and 5). In certain planes of section, the endoplasmic reticulum took the form of distinct parallel layers (Fig. 2b); the matrix of the endoplasmic reticulum appeared to contain small amounts of a flocculated material. Large mitochondria were scattered through-

¹ The work was supported by grants from the National Research Council of Canada, Environment Canada, and the National Cancer Institute of Canada.
² Research Scholar, National Cancer Institute of Canada.

Received July 25, 1977; accepted October 17, 1977.
out most areas of the cytoplasm commonly in an orientation parallel to the cell axis. Golgi bodies were numerous in the apical region of cytoplasm (Figs. 3a, 4a, and 6). Associated with the Golgi bodies were groups of small clear vesicles, some of which also appeared to associate with cytoplasmic granules in other parts of the cytoplasm (Figs. 3a and 6).

Several types of cytoplasmic inclusions were apparent. Small, electron-dense membrane-bound granules were found in small numbers scattered throughout the apical region of the cells (Figs. 3a, 5b, and 6). In addition, larger membrane-bound inclusions were present. Some of these contained a granular matrix (Figs. 3a, and 5), whereas others contained a more finely granulated, more homogeneous material (Fig. 5a). The contents of the lumina in some follicles were similar to the granular matrix of the large cytoplasmic inclusions described above (Figs. 2b and 3a), while in other follicles the lumen contained small amounts of a dispersed amorphous material (Figs. 3b and 4b). Bundles of microfibrils were found most commonly associated with desmosomes and in regions adjacent to the apical membrane (Fig. 7).

The intercellular spaces between neighboring follicle cells were commonly filled with a fine layer of homogeneous electron-dense material (Fig. 4a).

DISCUSSION

In most teleost fishes the thyroid gland is composed of small numbers of follicles scattered throughout the lower jaw region. This dispersed arrangement of the follicles makes them difficult subjects for study by the electron microscope since they are difficult to locate. Consequently, descriptions of teleost thyroid fine structure are few compared with those for other classes of vertebrates (8, 10, 20, 22). Thus, samples of normal thyroid for comparative electron microscopic study are difficult to obtain. Moreover, since the thyroid in salmonids is markedly seasonal in its apparent activity [see Sonstegard and Leatherland (21) for literature review], it is essential to use normal fish in a comparable stage of their life cycle so that comparisons between normal and goitered can have significance. In both 1976 and 1977, all migrating Lake Ontario coho salmon had either hyperplastic or goitered thyroids; consequently, normal tissue was not available for this study. In light microscope studies of coho salmon on the Pacific coast of North America, the thyroid epithelial cell height increased during the early stages of anadromous migration but decreased before the fish reached the spawning areas (4). In coho from Lake Ontario, there is a progressive thyroid hyperplasia which does not decrease as the anadromous migration proceeds (21). Thus, the thyroid hyperplasia in Great Lakes coho does not appear to be correlated with spawning per se. However, since the first appearance of goiters is concomitant with the start of the anadromous migration (21), it is likely that the stimulus promoting thyroid hyperplasia and ultimate goiter is in some way related to greater needs of thyroid hormones during the early stages of the anadromous migration (21), possibly involving the mobilization of metabolites in these fasting fish.

The general appearance of the thyroid follicle cells in the goitered coho salmon used in this study was essentially similar to that in nongoitered tetrapods (5, 12, 20) and resembled the type 2C cells of the lamprey endoderm (1, 11).

The massive formations of distended endoplasmic reticulum and large goiters in the follicle cells suggest a high rate of thyroglobulin synthesis presumably stimulated by elevated levels of TSH.3 These cells resemble those of the thyroid epithelium in TSH-stimulated mammals (12, 20). However, serum thyroxine and triiodothyronine levels are low in these goitered fish (17, 21), suggesting that the fish have an impaired ability to produce and/or secrete the thyroid hormones.

The identification of cytoplasmic inclusions in mammalian thyroid follicle cells is a matter of debate. In particular, there are discrepancies regarding the identification of thyroglobulin (colloid) droplets that are to be secreted into the lumen and those that are to be released. Similarly, there are discrepancies in the identification of lysosomes in thyroid follicle cells in mammals (14, 15). Since the fish thyroid tissue used in this study was in a pathological state and since normal tissue was not available for study (see above), interpretation of the observations described here must be made with some reservation. However, most of the organelles evident in the coho epithelial cells appear to have their counterparts in mammalian thyroid cells. The smaller dense granules at the cell apices resemble the "dense bodies" in TSH-injected rats (20) and are similar to the organelles considered to be lysosomes in TSH-injected dogs (19) and in human goiter cells (14). The larger cytoplasmic inclusion could similarly be interpreted as either colloid droplets or lysosomes, although the appearance of the contents of these globules was very similar to the appearance of the colloid in the follicular lumina. The large droplets containing the more homogeneous matrix were similar in appearance to lipofuscin droplets found in mammalian tissue (14, 15).

The well-marked junctional complexes of adjacent follicular cells in goitered coho thyroid were similar to those found in mammalian thyroid (6, 17, 20) and in other epithelial cells (7) and emphasized the requirement of a functionally tight epithelium in order for the gland to function. In goitered O. kisutch the apical cell membrane of the follicle cells was similar to that in several nongoitered teleosts (8–10, 22), in that it was composed of only very few microvilli compared with other vertebrates (19).

The cause or nature of the accumulation of electron-dense material in the intercellular spaces between adjacent follicular cells is not known. This phenomenon was not described in studies of the normal thyroid gland in other vertebrates (6, 9, 12, 15, 18, 20, 23) or in goitered humans (14).

Ketelbant-Balasse et al. (14) described 2 types of follicles in a congenital human thyroid goiter, 1 made up of cells containing lysosomes, very similar to those found in this study. The second type of cells contained large accumulations of lipofuscin droplets in addition to lysosomes; these cells were not found in coho salmon goiters. Klinck et al. (15) reports that normal human thyroid shows a marked

---

3The abbreviation used is: TSH, thyroid-stimulating hormone.
variability in structure from region to region and from subject to subject. Although there was some variation in thyroid structure in different regions of the goiter, the coho thyroid appeared more uniform in structure than did human thyroid. Thus, although thyroid tissue in goitered coho salmon had many of the characteristics of thyroids in other vertebrates, they lacked some of the characteristics of thyroid tissue in congenital human goiters.

The cause of the goiters in Great Lakes coho is not known. Field epizootiological studies of Great Lakes coho suggest that extrinsic environmental factors (possibly pollutants) are involved in the etiology of the goiters (17, 21). The Great Lakes have been polluted with a vast array of chemicals that either alone or in concert may act as goitogens exacerbating the development of goiters in the low-iodine environment of the Great Lakes. Organochlorines are suspect (e.g., polychlorinated biphenyl, DDT, dieldrin, mirex) in that they have widespread distribution in the Great Lakes and are known to alter thyroid activity in fish (16, 24), birds (19), and mammals (2).

ACKNOWLEDGMENTS

We wish to thank Lucy Lin for her technical assistance.

REFERENCES

Fig. 1. a to c, sections of paraffin-embedded thyroid tissue from a goitred coho salmon showing the different appearance of the glandular tissue in different regions of the same section. Paraffin section; H & E. × 250. d, Epon-embedded material of tissue similar to that shown in a to c. Note the uneven dispersal of azure ll-stained material in the follicle lumen (L). × 410.
Fig. 2. a, basal region of an epithelial cell showing the basal membrane (M). Note the vesiculated appearance of the cell cytoplasm and the droplet containing a granular matrix. × 4,000. b, apex of follicular cell showing microvilli projecting into a dense luminal matrix (L). Note the marked endoplasmic reticulum of the cells and fibrillar organelles (arrows) adjacent to the lateral membrane. Note also the junctional complexes between lateral membranes of adjacent epithelial cells. × 4,800.
Fig. 3. a, apical region of epithelial cells. Note the "dense bodies" (d) in the apical cytoplasm. Note also, the small vesicles (large arrows) associated with the Golgi body and also with one of the "dense bodies." Note also the space between adjacent cells (small arrows). L, follicle lumen. × 9,250. b, cells surrounding small follicle lumen (L). × 17,500. c, junctional complex adjacent to the follicle lumen (L). × 13,000.
Fig. 4. a, horizontal section through a group of follicle cells showing the extensive vesiculation of the cytoplasm and the pronounced Golgi profiles (G). Note the electron-dense nature of material in intercellular spaces (arrows). × 7,500. b, microvilli projecting into a small follicle lumen (L). Note the indented appearance of the apical membrane (arrows). × 12,900.
Fig. 5. a, colloid-like droplets (C) in cytoplasm of adjacent epithelial cells. Note the homogeneous appearance of the matrix in one compared with the granular matrix of the second. × 8,000. b, basal region of the cytoplasm of an epithelial cell showing a colloid droplet filled with a granular matrix. Note several "dense bodies" (d) closely associated with the colloid droplet (C). × 8,000.
Fig. 6. Apical region of epithelial cell cytoplasm containing moderate numbers of dense bodies (d) and marked Golgi profiles (G). × 10,300.
Fig. 7. Part of a degenerating epithelial cell (DC). Note the bundles of microfibrils in various parts of the cytoplasm (arrows) particularly adjacent to the lateral cell membrane. × 13,000.
Ultrastructure of the Thyroid Gland in Goitered Coho Salmon (*Oncorhynchus kisutch*)

J. F. Leatherland, R. Moccia and R. Sonstegard

*Cancer Res* 1978;38:149-158.

**Updated version**
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/38/1/149

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.