The Epizootiology and Pathogenesis of Thyroid Hyperplasia in Coho Salmon (Oncorhynchus kisutch) in Lake Ontario

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SUMMARY

The thyroid glands of coho salmon collected at different stages of their anadromous migration exhibited progressive and extensive hyperplasia and hypertrophy. The incidence of overt nodule formation rose from 5% in fish collected in August to 24% in fish collected in October. The histological picture of the goiters was similar to that found in thiourea-treated teleosts and thiouracil-treated mammals. There was a concomitant, significant decrease in serum thyroxine and triiodothyronine values between September and October (thyroxine, 1.0 ± 0.3 μg/100 ml and 0.4 μg/100 ml in September and October, respectively; triiodothyronine, 400.3 ± 51.6 ng/100 ml and 80.2 ng/100 ml in September and October, respectively) and marked hypertrophy and hyperplasia of thyrrotrophs. These data indicate a progressive hypothyroid condition which, although it may be linked to iodide deficiency, may well be enhanced by other environmental factors. The evidence for involvement of other factors is discussed.

INTRODUCTION

Several authors have described thyroid hyperplasia and goiters in fish from the Great Lakes Basin (6, 16, 17, 23-25). Most authors consider the goiters to be endemic and, in some cases, have shown that they can be reduced by iodine administration or by treatment with thyroid preparations (21, 23-25). Black and Simpson (6), however, reported evidence of invasiveness and the presence of thyroid follicles in intravascular foci in Lake Erie coho salmon, which may suggest that they are carcinomas. The literature of thyroid hyperplasia and carcinomas in fish has been excellently reviewed by Wellings (41).

In a pilot study of Lake Ontario coho salmon in 1974, 21 salmon were captured on October 26, in a weir on the Credit River during their spawning run from Lake Ontario. Of these, 9 (42.9%) exhibited distinct growths (goiters) on the gill arches, and all had indications of thyroid neoplasia as evidenced by diffuse swellings at the base of the gill arches. Earlier (May 25), a large catch of coho salmon (308) of the same age group in which the goiters were found were captured in Lake Ontario; no overt goiters were detected in necropsies performed on these fish. In this paper, we report investigations conducted during the 1975 spawning runs, which were instigated in order to elucidate the epizootiology and pathogenesis of the thyroid condition. Evidence is presented which suggests that the thyroid hyperplasia is caused by extrinsic environmental factors in addition to iodide deficiency. The potential utility of monitoring fish and their associated neoplasms as indicator organisms for the early detection of environmental carcinogens is explored with particular reference to coho salmon, a species which, because of its position in the food web, is of considerable environmental relevance and may reflect the health of the whole lake ecosystem.

MATERIALS AND METHODS

Specimen Collection. Coho salmon were collected by gill net (5-inch stretch mesh) off the mouth of the Credit River in Lake Ontario on August 18, 1975 and September 5, 1975. The fish of the August collection were actively feeding as evidenced by gut examinations and angler success, whereas, in the fish of the September collection, the guts were totally devoid of food, and angler success was low. A 3rd collection was made on October 24, 1975. The fish were collected by snagging below a dam on the Credit River. Coho salmon smolts were obtained from hatchery stock which were reared in the laboratory at Guelph in well water until sacrificed.

Histology. Tissues for light microscopy were excised from freshly killed fish and placed in Bouin’s fixative or Bouin’s Hollande sublimate (pituitary). Pituitaries for examination by electron microscopy were fixed in 5% glutaraldehyde, postfixed in osmium, and embedded, using the procedures described previously (22). Sections of pituitary material were stained with 1 of the following procedures: Gabe’s aldehyde fuchsin, Alcian blue-periodic acid-Schiff orange G, Herlant’s tetraphrome, or Cleveland-Wolfe trichrome.

Serum Sampling. The tail of each fish was severed, and blood samples were collected in sterile plastic tubes. The blood was allowed to clot overnight at 4°C; the serum was pipetted into plastic serum storage vials and was stored frozen at −40°C until analysis. Serum samples were suitably diluted for measurement of Ca\(^2+\), Na\(^+\), and K\(^+\) concentrations on a Unicam Model 1950 atomic absorption spectrophotometer. Thyroxine and T\(_3\) concentrations were mea-

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\(^3\) The abbreviations used are: T\(_3\), triiodothyronine; PCB, polychlorinated biphenyls.
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The Seralute procedure was slightly modified according to Modification Sheet No. 1-75-D, to achieve maximal sensitivity of the assay.

**Statistics.** The data were compared either by 1-way analysis of variance or by the t test. Individual means of the analysis of variance data were compared by least significant difference.

**RESULTS**

**Gross Pathology of Goiter.** The presence of goiters was determined by retracting the operculum of each fish and examining the base of the gill arches for evidence of swellings or nodules. Fish with 1 or more distinct nodules of >1 cm diameter were recorded as having overt goiter (Fig. 1). The goiters were firm to the touch and were light pink. Fish exhibiting large, multinodular goiters were commonly only one-half as large as the nongoitered specimens.

In the August collection, 52 salmon were captured, of which 3 exhibited overt goiters (5%). In the September collection, 68 coho were captured, of which 12 exhibited overt goiters (17.6%), and in the 3rd collection of 51 fish, made on October 24, 12 exhibited overt goiters (24%), while the remaining 39 animals exhibited gross thyroid hyperplasia as evidenced by diffuse swellings at the base of the gill arches. At the time of the October collection, the coho were "ripe" and could readily be spawned manually.

**Thyroid Histology.** The thyroid gland in coho salmon is, as in most teleosts, scattered throughout the region of the lower jaw, principally in those regions adjacent to the ventral aorta and the bases of the gill arches. In nongoitered fish (including the smolts) (Figs. 2 and 3), the thyroid was composed of a relatively small number of follicles with squamous or cuboidal epithelial cells. The colloid was homogeneous with little or no vacuolation. In the extreme goitrous condition (Figs. 4 to 7), the follicular appearance and colloid were no longer apparent. The epithelial cells were markedly hypertrophic and evident as tall columnar cells. The epithelial cells were commonly arranged into what appeared to be tubules with the follicle lumen forming the empty cavity of the tubule (Fig. 4). The nodular goiters showed a marked variation in appearance of the thyroid tissue in different regions. In some regions the gland contained small islets of apparently inactive follicles; some areas had the nodules with the tubular form described above, and other areas appeared to be in stages intermediate between the 2 extremes. There was no evidence of inflammation of the tissue, no marked invasion of leukocytes, and no sign of encapsulation of the nodules. There was a marked variability in the apparent vascularization of the nodules. Some areas, commonly on the periphery of the nodules, appeared well vascularized, whereas the more central regions were poorly invested with capillaries. Some of the central regions of the nodules were evidently necrotic, although it was not clear whether this was a fixation artifact.

In the nongoitered coho of the August collection, the thyroid was composed of relatively small numbers of follicles with low cuboidal or moderately hypertrophied epithelial cells. The colloid was plentiful and homogeneous with little or no vacuolation. The nongoitered coho of the September collection exhibited a thickening of the epithelial cells, some of which were columnar. Colloid was markedly reduced as compared with the earlier collection. The nongoitered fish of the October collection all exhibited tall columnar thyroid epithelial cells and were all largely depleted of colloid substance.

The thyroids of goitered specimens of all 3 collections were similar to those described previously. No evidence of metastasis was found in histopathological examinations of liver, spleen, and kidney samples.

Because of the great variability both within and between specimens, it was necessary to derive a method of evaluation of thyroid histological appearance (a "thyroid index"). Three histological criteria were chosen: the follicle appearance (whether follicles were evident), the colloid content, and the epithelial cell appearance.

The follicle appearance and colloid content of the gland were assessed separately on a 1 to 5 scale, 1 representing the follicular form and homogeneous colloid of a nonstimulated thyroid and 5 representing the nonfollicular form and colloid-depleted thyroid evident in the nodules of goitered fish. The epithelial cell appearance was evaluated on a scale of 1 to 6; Stage 1 represents the low profile cuboidal cells of the nonstimulated thyroid, Stage 2 represents cuboidal cells, Stage 3 represents slightly hyperplastic cuboidal cells, Stage 4 represents a mixture of hyperplastic cuboidal cells and columnar cells, and Stages 5 and 6 represent columnar epithelial cells. In most cases, because of the regional differences in appearance within the gland, the evaluation was made on overall appearance of the largest components.

The mean values for each of the criteria in the adult coho are shown in Chart 1. All 3 criteria show an increase in the September 1975 and October 1975 collections as compared with the fish collected from Lake Ontario in August 1975. The follicle appearance, colloid content, and epithelial cell appearance values for all the coho smolts were 1.

**Pituitary Histology.** The structure of the pituitaries of the coho used in these studies is essentially similar to that described in other salmonid fishes (2, 7, 9, 10, 22, 27, 29.
Concentration of ions and thyroid hormones in the serum of coho salmon collected from several sources. Measurements of thyroxine and T3 in the smolts were made on pooled serum from 2 to 3 fish.

<table>
<thead>
<tr>
<th>Concentration of serum constituents</th>
<th>Laboratory-reared smolts, March 1976</th>
<th>Lake Ontario collection, September 1975</th>
<th>Credit River collection, October 1975</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine (µg/100 ml)</td>
<td>0.5 ± 0.1* (5)</td>
<td>1.0 ± 0.3 (11)</td>
<td>0.4 ± 0.1 (16)</td>
</tr>
<tr>
<td>T3 (ng/100 ml)</td>
<td>37.2 ± 5.9 (5)</td>
<td>400.3 ± 51.6 (11)</td>
<td>80.2 ± 14.7 (17)</td>
</tr>
<tr>
<td>Ca²⁺ (mg/100 ml)</td>
<td>21.3 ± 2.3 (13)</td>
<td>21.9 ± 3.3 (17)</td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mEq/l)</td>
<td>148.6 ± 1.8 (13)</td>
<td>149.5 ± 3.0 (17)</td>
<td></td>
</tr>
<tr>
<td>K⁺ (mEq/l)</td>
<td>3.1 ± 0.8 (13)</td>
<td>1.6 ± 0.4 (17)</td>
<td></td>
</tr>
</tbody>
</table>

*a Mean ± S.E.
*b Numbers in parentheses, number of measurements.
the rostral and proximal zones, each of which contains a functionally different cell types. Thus, the teleost pituitary is separated into 2 distinct zones, the distal and the proximal pars distalis, close to the neurohypophysis, and correspond in location and staining characteristics to inactive thyrotrophs (2, 27).

Serum Levels of Thyroid Hormones. Serum thyroxine and T3 concentrations were measured in smolts and adult fish of the September and October 1975 collections; mean values are shown in Table 1. Thyroxine and T3 levels were significantly higher in the September adults as compared with both the smolt and October adult groups (p < 0.05 for smolt thyroxine; p < 0.01 for October adult thyroxine; p < 0.001 for smolt and October adult T3). There were no significant differences between the serum T3 or thyroxine levels in the smolt and October adult coho.

There was a significant (p < 0.001) positive correlation between T3 and thyroxine levels in the adult fish (Chart 3); however, there was no significant correlation between the thyroid index (see "Materials and Methods") and serum thyroxine (Chart 4) or serum T3 concentrations.

Serum Ions. Serum levels of Ca++, Na+, and K+ were measured in adult fish collected in September and October 1975 in order to determine the ionic status of the fish; the means of the data ± S.E. are shown in Table 1. There were no significant differences between the concentrations of any of the ions in the 2 groups.

DISCUSSION

The exact role(s) of the thyroid gland in teleosts is still in some doubt. Several authors describe a marked seasonal variation in the activity of the thyroid in teleosts, the periods of greatest apparent activity being associated with the reproduction period (4, 8, 14, 15, 26, 37-39). In addition, since in anadromous salmonids the thyroid appears most active at the onset of their spawning migration (concomitant with the movement into freshwater) (8, 14, 35, 39), it has been suggested that the thyroid is a regulator of osmotic and/or homeostasis. Hoar (15-17) suggests that the apparent decrease in thyroid activity of anadromous species may reflect a response to iodide deficiency (associated with the entry into freshwater from the iodide-rich sea) rather than a true increase in thyroid hormone secretion. Thus, although the gland appears hyperactive, the fish may be in a hypothyroid state. The reports that the thyroid hyperplasia regresses during the anadromous migration of Atlantic (35) and coho (8) salmon also argue against an osmotic (or) regulatory function for the salmonid thyroid.

The evidence from the studies reported here, namely, that even in landlocked salmon the thyroid is apparently active at the time of their prespawning migration and that even severely hypothyroid fish appear to be able to regulate serum ions (see Table 1), also suggests that the increase in apparent thyroid activity concomitant with the anadromous migration is not ipso facto suggestive of an osmotic (or) regulatory function for the thyroid. The apparent increase in activity in these coho appears to be the response to a hypothyroid condition but does suggest that at this time of the life cycle the fish require levels of thyroid hormones that are greater than at other times (cf. Table 1, the euthyroid smolt stage).

The teleost pars distalis is separated into 2 distinct zones, the rostral and proximal zones, each of which contains functionally different cell types. Thus, the teleost pituitary has advantages over that of most other vertebrates for histophysiological studies (36). Nonetheless, there is a great deal of confusion in the published literature regarding the identification of thyrotrophs in salmonid fishes. The cells identified as thyrotrophs in trout (2, 32) have also been termed adrenocorticotropic hormone (7). Similar cells were identified as gonadotrophs in coho and kokanee salmon (10). The cells termed thyrotrophs in this report were morphologically identical to hyperactive thyrotrophs in goiterogenous-treated fish (19, 31) and thyroidectomized mammals (3, 12).

The iodine concentration in the Great Lakes is 20 to 60 times lower than in sea water (42). Freshwater fish indigenous to the Great Lakes Basin apparently have adapted to this condition, but this adaptation may be less successful for newly introduced anadromous species such as coho salmon. The seasonal requirement of thyroid hormone in concert with the low iodine freshwater environment probably accounts, in part, for the rapid appearance of goiters in the fish studied in this investigation. These coho demonstrated several symptoms of severe hypothyroidism, namely, low serum thyroxine and serum T3 levels, hyperplastic and hypertrophic thyrotrophs, and large nodular nodules.
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solved (1, 13). Furthermore, it is of interest that hypothroidism has been associated with breast cancer in postmenopausal women (28).

These studies raise significant questions regarding the effects of constant low or chronic levels of environmental pollutants on animal health. There are, in the literature, growing data to suggest many endocrine parallels between teleosts and mammals in the regulation of endocrine functions. Fish, by the process of biological magnification, are exposed to environmental insults often higher than those to which other animals are exposed. It is apparent that monitoring the endocrine disorders and neoplasia in these animals has obvious merit.

ACKNOWLEDGMENTS

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REFERENCES

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Fig. 1. Nodular (overt) goiters (arrows) at the base of the gill arches in an adult coho salmon.

Fig. 2. Thyroid tissue in a nongoitered adult coho collected in August 1975. Note the low epithelial cell height and the homogeneous appearance of the colloid. Paraffin section. H & E, × 175.

Fig. 3. Similar region in coho salmon smolt. Paraffin section. H & E, × 175.
Figs. 4 to 7. Thyroid goiter of a salmon collected in October. The 4 micrographs were taken of different regions of the same section of the goiter; note the markedly variable appearance of the goiter tissue.

Fig. 4. Elongated or tubular appearance of the follicles. × 70.

Fig. 5. Afollicular region. × 175.

Figs. 6 and 7. Stages intermediate between those of Figs. 4 and 5. Paraffin section. H & E, × 175.
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Fig. 8. Proximal pars distalis region of the adenohypophysis in a coho salmon smolt showing the small, Alcian blue-stained thyrotroph cells (arrows) scattered in the dorsal region of the gland adjacent to the neurohypophysis (NH). Paraffin section, Alcian blue-periodic acid-Schiff-orange G, x 175.

Fig. 9. Region similar to that shown in Fig. 8, in an adult, goitered coho salmon showing the enlarged and numerous thyrotrophs (T) occupying the central regions of the lobules. Somatotrophs (A) are seen in the peripheral parts of the lobules adjacent to the neurohypophysis (NH). Paraffin section. Cleveland-Wolfe trichrome, x 175.

Fig. 10. Region similar to that shown in Fig. 9, showing the prominent thyrotrophs (T) occupying the central regions of the lobule and invading the spaces between the lobules. Globular gonadotrophs (G) can also be seen in the region. Epon section. Azure II, x 175.

Fig. 11. Enlarged micrograph of a group of thyrotrophs (T) to show the vesiculated nature of the cytoplasm. "Vesiculated" gonadotrophs (V) are also evident, interspersed among the thyrotrophs. Epon section. Azure II, x 700.
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