Comparative Effects of Thyroidal Stimulants and Inhibitors of Normal and Tumorous Thyroids in Xiphophorin Fishes*

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Spontaneous thyroid tumors in salmonid fishes, which appear to originate as simple hyperplasias, according to Marine and Lenhart (10, 11) and Marine (9), ultimately become invasive and replace fibrous connective tissue, muscle, and bone. Upon treatment with iodine these tumors will regress even after they have become invasive. It is interesting to compare the tumor in fish with thyroidal growths in mice obtained after a year or more of constant treatment with thioureas. The induced thyroid tumors in mice often metastasize to the lungs; in some instances they may be successfully transplanted (6, 13). If the goitrogens are withheld in certain strains of treated mice, the thyroid tumors and their metastatic growths apparently regress. Thus the invasive cells of spontaneous thyroid tumors of fishes and experimentally induced thyroid tumors of mice may be controlled by chemotherapy.

This raises a question concerning the properties of thyroid tumors in fishes. To what degree do these atypical growths respond to the regulatory influences that affect normal thyroid cell growth and differentiation? This point is of great general interest because, in the past, the simultaneous possession of both invasive "carcinomatous" powers and normal responsiveness to physiological regulatory mechanisms has led to confusion in classification of these tumors and in comparing them with the thyroidal carcinomas in mammals. To pursue this question further we have studied the action of five agents upon the spontaneous thy-

roidal tumors of the swordtail: iodide, thyroxine, whole mammalian thyroid tablets, thiourea, and thyrotropic hormone. The atypical growth of thyroid cells of this Mexican viviparous freshwater fish is first manifested by a hyperemia and hyperplasia. Later the proliferating tissue cells become invasive and surround and replace muscle and bone (1, 7).

MATERIALS AND METHODS

Three species of xiphophorin fishes were used in two series of experiments. In the first series, adult fish treated with various drugs and their controls were maintained in conditioned aquarium water. In the second series, newborn treated and control fish were maintained in a medium made up of 2 parts conditioned water to 1 part aerated tap water.

Aquarium-conditioned water was originally obtained from New York City taps in 1941. It has been used since then for all fishes maintained in the Genetics Laboratory. When an aquarium needed cleaning, the water was drained, saved, and reused. About 5 per cent aerated tap water was added to make up for that lost in the cleaning process and by evaporation. Both the aquarium-conditioned and the tap water were analysed for iodine content.1

The adult fish were maintained in all-glass aquaria holding 8,000 cc. of water, which was changed twice weekly. In the aquaria holding the young fish, the water was changed once a week, at which time a fresh supply of drugs was added. All fish were fed a standard diet of pabulum-fiver food, dried ocean shrimp, living tubiflids and daphnids, according to the procedure described by Gordon (6).

Xiphophorin fishes of three species were used: Xiphophorus montezumae, the Montezuma swordtail; Xiphophorus variatus, the variatus platyfish; and Xiphophorus maculatus, the common platyfish. The natural habitat of the first two species is in the Rio Axtla, an upland tributary of the Rio Panuco, San Luis Potosi, Mexico; the habitat of the third species is in the Rio Costazacoalcos in the lowlands of southern Veracruz.

Thirty-five adult X. montezumae and seven X. maculatus with externally visible tumors, and 22 normal X. variatus were treated with thyroid hormones, goitrogenic agents, or thyroidal stimulants. Commercial whole thyroid tablets, KI, or powdered thiourea were dissolved in a small amount of conditioned water and were thoroughly mixed with the water in the experimental tanks before the fish were introduced. Thyroxine and thyrotropic hormone were injected intraperitoneally in the dosages indicated in Table 1.

1 Iodine analyses were done by the Chaney technic (5) in the Albert L. Chaney Chemical Laboratory, Glendale, Calif.
In the second series, ten newly born X. montezumae, of a strain in which thyroid tumors usually develop in 6 months, were maintained for 7 months in a 2:1 conditioned-water–tap-water mixture to which potassium iodide was added to produce an aqueous solution of a concentration of 0.1 mcg/cc (0.01 mg per cent). For purposes of controls, ten X. montezumae of the same strain were maintained in a similar water mixture, but with the omission of KI.

For routine histological examination the lower jaws of the fish were dissected, fixed in Bouin’s fluid, and decalcified with nitric acid and phloroglucin. Sections were cut at 7 μ and stained with either hematoxylin-eosin, Masson’s trichrome, Giemsa’s azur cosinate, or Bielschowsky’s connective tissue stain. The methods of counting and measuring the thyroid follicles were the same as previously described (1).

<table>
<thead>
<tr>
<th>No. ANIMALS</th>
<th>LENGTH OF TREATMENT (WEEK)</th>
<th>DRUGS</th>
<th>DOSE</th>
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<tbody>
<tr>
<td>montezumae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>maculatus (tumor)</td>
<td>15</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>variatus (normal)</td>
<td>15</td>
<td>Whole thyroid tablets</td>
<td>1 5-grain tablet, U.S.P., in 3,000 cc. of water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyroxine</td>
<td>50 μg in .05 cc. of .9 per cent saline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potassium iodide</td>
<td>10 μg/cc of water</td>
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<tr>
<td></td>
<td></td>
<td>Thiourea</td>
<td>500 μg/cc of water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thyrotropic hormone*</td>
<td>5 μg in .05 cc. of TSH .9 per cent saline</td>
</tr>
<tr>
<td>Totals 40</td>
<td>12</td>
<td></td>
<td>57</td>
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</tbody>
</table>

* The TSH was kindly supplied by Armour and Company through the courtesy of Dr. Iby Bunting. Preparation number 129–51; potency equal to approximately 50 per cent of TSH standard.

To obtain a rough indication of the effect of the drugs on the uptake of iodine by the tumors, radiographs of eight fish were made. Two of them were first treated with KI, two with thiourea, two with TSH, and two were untreated. All were immersed in tracer concentrations (50 μc in 200 cc. of water) of carrier-free 131I for 24 hours and then sacrificed. Radiographs were made by exposing the histological sections on glass slides, previously stained with hematoxylin-eosin, to No-Screen x-ray film.

RESULTS

The Structure of Normal Thyroids

Fifteen X. variatus, seven fixed at their site of capture in Mexico and eight from a laboratory-reared strain in which tumors were infrequent, were examined to establish the normal pattern of variation in thyroid structure. Of the laboratory-reared fish, four were relatively old (over a year and a half in age), and four were young adults (about 8 months old). The unencapsulated thyroidal follicles were distributed in the stroma along the ventral aorta and bases of the first, second, and third aortic arches. The smallest number of follicles counted in a wild fish was 65, the maximum 175. For the laboratory-reared fish the count varied between 50 and 185. Each follicle was ovoid in shape. It was composed usually of low cuboidal epithelial cells 2–3 μ in height, which enclosed a lumen filled with faintly acidophilic, evenly dispersed colloid. In the oldest laboratory-reared fish the follicles were frequently smaller than those in the young adults; they were scattered widely, being separated by much connective tissue stroma.

EFFECT OF DRUGS ON NONTUMOROUS FISHES

Effects of thyroxine and potassium iodide on normal fish.—Neither thyroxine nor potassium iodide, in the concentrations used, had any pronounced effects upon the normal thyroid tissue of the platyfish. The number of follicles, their distribution around the blood vessels, the height of the epithelial cells, and the condition of the colloid remained normal. Only the size of the follicles changed. In the thyroxine-treated animals the follicles were unusually small; in two of the five animals they were separated much more widely by connective tissue than in the oldest of the untreated fish.

Action of thiourea on normal fish.—Thiourea produced profound changes in the thyroids. The shapes of the follicles were so distorted that in most fish it was impossible to determine their total number. Follicles were found as far posterior as the base of the heart and as far laterally as the gills. The epithelial cells were low columnar (5–10 μ tall), and in all cases there was a great increase in vascularity around the follicles.

In two fish treated with thiourea the distribution of follicles was normal. One of these had 98, the other 250 active follicles. In the other similarly treated fish the thyroid tissue had spread along
the connective tissue septa around the muscle bundles to such an extent that individual muscle fibers were isolated and surrounded by thyroid cells.

In one specimen of this series a single area of the hyperplastic thyroid had enlarged follicles with low cuboidal epithelial cells and sparse colloid; these follicles appeared nearing cyst formation.

The histological picture of the thiourea-treated glands was comparable to that seen in early stages in the spontaneously developed tumors.

**Action of thyrotropic hormone on normal fish.**—

Two types of responses were found in the TSH-treated animals: (a) Three fish had between 58 and 70 normally distributed thyroid follicles with low cuboidal epithelium. The glands had no increase in vascularity and were apparently unaffected by the drug. (b) Two fish had glands with over 200 follicles. Epithelial cells varied in height from cuboidal to columnar and contained eosinophilic granules. The distribution of the follicles was normal, but they were closely spaced. In one animal, the interfollicular tissue was highly vascular. The glands were hyperplastic.

**The Structure of Spontaneously Developed Tumorous Thyroids**

Tumors were visible externally in the form of pink swellings at the ventral side of the head between the bones of the lower jaws (at the isthmus). Mid-sagittal sections of the tumors showed modified thyroid tissue which extended anteriorly to the mandible, posteriorly to the auricle of the heart, and dorsally to the roof of the mouth. Ventrally, the tumorous cells replaced the adductor mandibulae muscle. Laterally, the follicles spread into the gills. In some individuals much of the branchial epithelium was replaced by tumorous tissue. This probably caused a reduction in the available respiratory surface.

In *X. montezumae* the thyroid tumor was characterized histologically by the relative rarity of follicles (Fig. 1). The epithelial cells were greatly enlarged and arranged in closely packed cords that radiated from the center of the tumor to the periphery. Some cords were separated by a few connective tissue fibers or by blood sinuses and sinusoids filled with red blood cells. Connective tissue stroma and occasional macrophages were found between the few loosely packed peripheral follicles. The epithelial cells that form the cords were between 7 and 9 μ in height, oval or elongate in outline; they had faint cell borders and eccentric nuclei. Their cytoplasm stained weakly, and it was occasionally vacuolated or contained eosinophilic granules.

The radioautographs showed that only some of the few morphologically normal follicles accumulated radioiodine.

In *X. maculatus* the tumor was characterized by extremely large rounded follicles, some of which attained a diameter of 600 μ or more. Each follicle was composed of many epithelial cells and had a large lumen, usually completely filled with a dense, granular eosinophilic colloid. A few follicles, however, contained an almost chromophobic colloid. In rare instances one-half of the follicular lumen was filled with dense, the other half with stringy colloid. Some follicles were papillary and cystic. In the colloid of some follicles, epithelial cells and red and/or white blood cells were suspended. The follicles were in a loose, quite vascular connective tissue stroma, which was usually abundant but normal in appearance. The epithelial cells that formed the follicles were usually 2–5 μ in height; their cytoplasm was always faintly basophilic and granular. In a few cells eosinophilic granules were found.

**Effect of Drugs on Tumorous Fishes**

**Action of whole thyroid tablets, thyroxine, and potassium iodide on the tumors.**—These three substances had apparently similar effects on large and small externally visible tumors. After 1 week of treatment the pink color disappeared. After 2 weeks the small tumors were difficult to detect externally. After 4 weeks even the large tumors were no longer apparent.

One week after treatment the trabecular structure of the tumor of *X. montezumae* was no longer evident microscopically. Because of this it was not possible to distinguish *X. montezumae* tumors by histological criteria from those of *X. maculatus*. The tumors consisted of several hundred follicles. The follicular epithelium was usually composed of cuboidal columnar cells, but in some cases it was stratified squamous (Fig. 2). In some follicles the colloid was normal, in others it contained basophilic concretions. There was no increase in connective tissue; very few macrophages were present, and the blood vessels were not engorged as they were before treatment. Radioautographs revealed that some of the newly formed follicles contained organically bound radioiodine; others did not.

In animals treated for longer periods there was a decrease in the number of follicles and an increase in interfollicular connective tissue (Fig. 3). The follicular epithelial cells were usually cuboidal and sometimes had pyknotic nuclei (Fig. 4). Eosinophilic granules in the cytoplasm of epithelial cells were irregular in their distribu-
treated their distinguished swollen intracellular ranged. With the drugs and dosages used, and for the stated periods of treatment, none of the X. montezumae ever regained histologically typical thyroids. They all had an unusually large number of follicles in the thyroid region and some had follicles in the gill region. On the other hand, after 13 weeks of treatment, the once tumorous glands of X. maculatus were histologically indistinguishable from those of aged normal fish (Fig. 5).

One Montezuma swordtail with a large externally visible tumor was treated with whole thyroid tablets for 7 weeks, then left untreated for another 7 weeks. At the time of sacrifice the animal appeared to be tumor-free macroscopically. Histologically, however, the tumor was composed of both small and large follicles with hypertrophied columnar cells which contained eosinophilic granules. The follicular colloid was full of basophilic concretions. Connective tissue was more abundant than in untreated tumors in animals and extremely vascular; all blood vessels were engorged with red blood cells. Thyroid treatment, therefore, provided only a temporary correction of the conditions leading to tumorous growth.

Action of thiourea on the tumors.—Thiourea had relatively little effect on the tumors. Tumor cells did not appear to be more extensive than those of the tumorous untreated fishes, but less connective tissue was present. Follicular structure was almost completely absent even at the periphery of the growth; cells were either in cords or randomly arranged. Isolated groups of cells contained eosinophilic granules. Colloid was almost completely lacking; connective tissue was sparse; the state of vascularization was similar to that in an untreated tumor. Radioautographs were completely negative.

Action of thyrotropin on the tumors.—Tumors from animals treated with TSH were distinguished from those of untreated tumorous fish by the extraordinary hypertrophy of many of their follicle cells (over 20 μ tall), by their huge intracellular eosinophilic granules, and by their swollen and eccentric nuclei (Fig. 6). In the TSH-treated fish colloid was lacking, connective tissue was sparse, but the state of vascularization unchanged from that of untreated tumorous fish.

EXPERIMENT IN THE CONTROL OF TUMOR DEVELOPMENT WITH IODIDE

Ten Montezuma swordtails, from a genetic strain in which tumors usually develop in 6 months, were raised for 7 months in a 2:1 mixture of conditioned water and aerated tap water containing potassium iodide at a concentration of 0.01 mg. per cent. At the end of this period none of the fish had developed tumors. Histologically, the least number of follicles counted was 75, the maximum, 145. The follicular epithelium was 4-5 μ tall, the colloid acidophilic and nongranular. In two fish the thyroid region was highly vascular; in the others it was normal.

Ten control swordtails were reared under the same conditions, except that potassium iodide was omitted from the mixed tap and conditioned water. None of the control fish developed externally visible tumors. On histological examination small trabecular tumors were found in six animals. The other four had thyroids which were normal except for an increased number of follicles (between 200 and 300 follicles).

Almost half of the fifteen similar Montezuma swordtails reared in undiluted conditioned water for 7 months had small externally visible tumors.

IODINE CONTENT OF WATER

Iodine analyses revealed that aerated tap water from the American Museum of Natural History in New York City contains 2.3 μg of iodine/liter. The aquarium-conditioned water, on the other hand, contains only 0.5 μg/liter, even though it was originally obtained, approximately 13 years ago, from the New York City taps. Water from the Rio Axtla, the natural habitat of X. montezumae, has 3.3 μg. of iodine per liter.

AN EPITHELIAL TUMOR IN THE REGION OF A THYROID TUMOR

Histological examination of one young Montezuma swordtail raised in potassium iodide revealed a small epithelial cell tumor in the region of the thyroid. The tumor cells did not resemble thyroid tumor cells. A number of mitotic figures were noted, indicating active growth. Evidently the potassium iodide had no effect upon this tumor, although it completely inhibited the incipient thyroid tumor.

DISCUSSION

Thyroxine can maintain a euthyroid state in patients with carcinomas which are usually associated with a hypothyroid state (19), but ap-
parently it does not affect the carcinoma itself—at least insofar as its malignancy is concerned. The effect of iodine on such carcinomas is more difficult to assess, since a rapid review of the literature does not reveal any clinical attempt at control of thyroid carcinomas with iodine, although it is used for treatment of goiter (12). Apparently unlike human carcinomas, but like goiters, the thyroid tumors in xiphophorin fishes can be controlled by both iodine and thyroxine.

The responsiveness of the fish tumors to TSH, however, resembles that of certain human carcinomas. Sturgeon et al. (19) report that it is sometimes necessary to administer TSH to patients for as long as 6 months before their abnormal thyroid tissue can accumulate enough radioiodine to permit attempts to destroy the cancer by high doses of the isotope. A similar continued response to long periods of administration of TSH is characteristic of the fish tumor tissue and differs from the response of normal thyroid tissue in mammals, where repeated doses of TSH tend to induce a “refractory effect” (20).

Propyl thiouracil reduced hyperthyroid symptoms in certain patients with hyperactive cancers (16). In normal animals it is well known that goitrogens prevent the formation of thyroxine and thereby lead to pituitary-induced hyperplasia of thyroid tissue (4). The antithyroid compounds appear to act in normal and tumorous fish in a manner resembling their action in higher vertebrates.

The responsiveness of xiphophorin thyroid tumor tissue to iodine and thyroxine is, therefore, similar to that of human goitrous tissue, while its response to TSH resembles that of carcinomatous tissue.

Once again the importance of a lack of iodine in inducing thyroid tumors must be emphasized. On the basis of the water analysis figures supplied by the Chaney Laboratory, it is now possible to approximate the environmental iodine concentration needed to permit proper functioning of the Montezuma swordtail thyroid. Swordtails reared in aquarium water containing 0.5 μg of iodine/liter develop tumors. When aquarium water is diluted with 2 parts tap water, the resulting solution has a calculated iodine content of 1.7 μg/liter. We have found that this small amount of iodine is sufficient to delay, and probably in some animals to prevent, tumor formation. Finally, it can be assumed that the 2.3 μg/liter concentration of iodine in tap water is sufficient to prevent tumor formation. This assumption is based on the fact that tumor-free swordtails were reared for several generations when first established in the laboratory at the American Museum in aquarium tanks holding water recently taken from the city taps. City tap water has only slightly less iodine than the Río Axtla, original habitat of these fishes. The 100 μg. of iodine added to each liter of water in our experimental tanks is, therefore, more than sufficient to prevent tumor development, and, as expected, all the fish reared in such a medium are tumor-free.

Robertson and Chaney (17) have recently reported a marked hyperplasia of the thyroid in trout taken from some tributary streams of the Great Lakes. On chemical analysis they found such waters to contain 1–5 μg of iodine/liter. In trout from nearby streams, particularly those that have access to additional iodine in food discharged from a fish hatchery, no hyperplastic thyroids were detected. Thus, in order for the thyroid of the trout to function properly, the environmental water must have 5 μg of iodine/liter, while the swordtail can evidently manage on 2.3 μg/liter. This high sensitivity of the trout thyroid to a lack of iodine may help to explain the number of thyroid tumor epidemics in trout noted throughout the world.

The Great Lakes area is a famous goiter belt, and the water is notably low in iodine. In fact, until the advent of the catalytic technic for measuring iodine, Great Lakes water was assumed to be “iodine-free.” It was, therefore, surprising to us to find that our aquarium water has even less iodine (0.5 μg/liter) than the Great Lakes water (1–5 μg/liter). Iodine in the aquarium water is known to be extracted by algae (18), by snails, and by the fishes themselves. The slow extraction of iodine over an extended period evidently reduced the iodine concentration in the conditioned aquarium water to a critical point, despite the feeding of iodine-containing diets. Berg and Gorbman (3, 8) have shown that the thyroids of tumorous Montezuma swordtails make thyroxine more slowly than do thyroids of similar nontumorous fish. We regard this as an indication that fish of the tumorous strain require more iodine than the nonsusceptible ones, a suggestion made earlier by Gorbman and Gordon (7). Thus, the combined effects of a low iodine concentration in the water and a greater need for it by susceptible strains of Montezuma swordtails may result in a thyroxine deficiency. This, in turn, may lead to hypophyseal overstimulation of thyroid cell growth to an abnormal point. This chain of events is apparently reversible, at least in some measure, for treatment with either iodine or thyroxine produces a remission of the tumorous state.

* Gorbman, unpublished data.
The absolute level of iodine in the water may be just one of several factors involved in thyroid tumor development. From his study of thyroid tumors that develop in marine fishes, diagnosed in *Fundulus heteroclitus* and in *Cyprinodon variegatus* as interacinous adenomas and in *Angelichthys isabelita* as an adenocarcinoma, Nigrelli (14) suggests that factors other than iodine deficiency should be considered in their etiology, such as changes in oxygen tension, light intensity, salinity, calcium-magnesium ratios and enforced activity. The cyprinodont fish species were fed fresh clams, shrimp and salt-water fish, foods rich in iodine. They were maintained in aquaria containing sea water which had the following properties: pH varied from 7.1 to 7.4, the specific gravity from 1.017 to 1.018, and the iodine content was 17 to 20 μg/liter. Despite the seeming adequacy of iodine in salt water, Pickford (15) has reported that thyroidal hyperplasias in *Fundulus* reared in the laboratory in sea water can be prevented by the addition of iodine to their liver pabulum food. From this it would appear that even salt-water fishes may not be receiving enough iodine. Chaney (3) has analyzed two samples of sea water: one from the Pacific coast had 30 μg/l, and one from Woods Hole had 48.5 μg of iodine/liter. These values are considerably higher than the 20 μg reported by Nigrelli (14) and suggest that even sea water under certain circumstances may be relatively iodine-depleted in the laboratory. Although other factors must be considered, it is possible that the salt-water fishes respond to a prolonged decrease in the iodine in their water in the same manner as do fresh-water fishes.

**SUMMARY**

1. Adult *Xiphophorus montezuma* (a Mexican swordtail) and *Xiphophorus maculatus* (the common platyfish) with externally visible thyroid tumors and tumor-free *Xiphophorus variatus* (the variatus platyfish) were treated with thyroid hormones, goitrogenic agents, or thyroidal stimulators. The microscopic characteristics of the thyroid glands in these three fresh-water fishes were studied in serial sections.

a) Potassium iodide, thyroxine, and mammalian “thyroid tablets” induced regression of the tumors and replacement of tumor tissue by more normal and quiescent thyroid follicles. Thyroxine reduced the diameter of the follicles in the nontumorous thyroid tissue. Potassium iodide, in the concentration used, had no histologically visible effect on normal thyroid tissue.

b) Thiourea elicited growth of small thyroid tumors in ordinarily nontumorous fishes. It had little additional effect on the spontaneously developing tumors.

c) Long treatment with pituitary thyrotropic hormone had relatively little effect on the normal thyroid tissue but caused great hypertrophy in the tumor cells.

2. From these results it was concluded that thyroid tumor cells are particularly sensitive to iodide and thyrotropic hormone and are relatively insensitive to thiourea.

All tissues cross-sectioned at 7–10 μ and stained with hematoxylin-eosin.

**FIG. 1.**—Untreated thyroid tumor of *Xiphophorus montezuma* showing enlarged epithelial cells and virtual absence of follicular structure. Mag. X450.

**FIG. 2.**—Tumor of *X. montezuma* treated for 1 week with KI. The follicles shown are composed of stratified squamous epithelium, and the colloid contains basophilic concretions. Mag. X450.

**FIG. 3.**—Tumor of *X. montezuma* treated for 3 weeks with KI. Note the follicular constriction and large amount of connective tissue, in contrast to the condition of the untreated tumor shown in Figure 1. Mag. X110.

**FIG. 4.**—High power reproduction of section shown in Figure 3. Mag. X450.

**FIG. 5.**—Thyroid gland of a once tumorous *X. maculatus* treated for 13 weeks with KI. Gland is typical of aged normal animal. Mag. X110.

**FIG. 6.**—Thyroid tumor from an *X. montezuma* treated for 13 weeks with thyrotropic hormone. Note the enlarged cells filled with inclusions. Mag. X450.
3. Young fish of tumorous thyroid strains raised in water containing 100 μg of iodide/liter do not develop tumors. Young fish raised in a mixture of conditioned water and tap water containing 1.7 μg of iodine/liter develop tumors more slowly than those in undiluted conditioned water, which has 0.5 μg of iodine/liter. These results demonstrate the importance of environmental iodine in atypical thyroid growth in fishes.

REFERENCES