Spontaneous Thyroidal Tumors in the Swordtail *Xiphophorus montezumae**

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Early studies of the thyroid tumors of trout (3–5) have demonstrated the relationship between iodine supply and hyperplastic growth of thyroid cells. Experimental work with fishes provided much of the background for the present understanding of the etiology of human goiters. In contrast with encapsulated mammalian goiters, however, hyperplastic thyroids in fishes are remarkable for their invasive reaction to surrounding tissues. This apparently invasive property of unencapsulated fish thyroidal cells and follicles led earlier workers to consider the glandular tumors as "carcinomas" (1). However, later work showed that the regression of thyroidal tumors in trout could be induced by adding iodine to the water in which the fish were maintained (3).

Spontaneous thyroidal tumors have been reported in at least eighteen species of fresh-water and marine fishes. Like trout, all of them have been observed under cultivation in aquaria or in fish hatcheries (6). While the goiters in fresh-water forms are often caused by a low iodine supply in the water or food, this explanation hardly seems applicable for salt-water species which live in a relatively iodine-rich environment and which feed upon such iodine-rich foods as ocean shrimp and clams (6). The morphologies of thyroidal tumors in fishes are all quite similar.

In this report we propose to describe a spontaneous thyroidal tumor of high incidence in a species of Mexican fresh-water fish, the swordtail, *Xiphophorus montezumae*. Our knowledge of the environmental conditions under which these tumors develop makes it possible to suggest certain generalizations concerning their natural history and genetics. In addition, we have attempted to obtain an estimate of the degree of dysfunction of the tumorous thyroid tissue by means of the ability of these fish to utilize radioactive iodine. This was recorded autographically.

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METHODS

The original stock of *Xiphophorus montezumae* was obtained in 1939 from the headwaters of the Rio Axtla in the Mexican state of San Luis Potosi. Laboratory-bred stocks were propagated in 5-, 10-, or 20-gallon aquaria. These contained plants of the genera Nitella, Cryptochoryne, and Sagittaria, the rooted aquatics being planted in clean, coarse gravel. The overhead daylight illumination was filtered through the glass of the skylight. The water in the aquaria was originally obtained from the common New York City supply. Once utilized for aquarium purpose the water has been used over and over again for 10 years. From time to time aerated tap water is added to a common reservoir of the conditioned water. Other closely related fish species were kept in the laboratory under virtually identical conditions and management including the following: two species of Xiphophorus, *X. pygmaeus* and *X. hellerii;* and four species of Platypoecilus, *P. maculatus*, *P. xiphidium*, *P. variatus* and *P. couchianus*. A small colony of *Lebistes reticulatus* was maintained. All fishes were fed the same diet: a fresh-liver-Pablum-cereal-mixture (2), live tubificid worms, and dried, shredded ocean shrimp. In addition, particularly, during the first 2 months of life, the fishes were given live Daphnia and Aulophorus.

After eight generations of rather close inbreeding (but not by brother-sister mating) numerous montezuma swordtails (*X. montezumae*) developed pronounced pinkish swellings in the region of the isthmus (i.e., between the bones of the lower jaw) (Fig. 1).

A number of montezuma swordtails with externally visible thyroidal tumors were fixed. Others were isolated and kept under close observation. Fixation in Bouin's fluid made special decalcification procedures unnecessary. The lower jaws and the adjacent region of fourteen of these fish, some with and some without tumors, were serially sectioned for detailed microscopic study.

Eight tumor-bearing animals were placed in 1-liter aquaria containing 25–50 μc. of carrier-free radioiodine, I^{131}, for 24 hours. The serial sections of their entire lower jaws were exposed to squares of No-screen x-ray film, and radioautographs were prepared from them.

Morphology of thyroidal growths.—Gross examination of mid-sagittal sections revealed the anterior and posterior limits of the tumors which measured about 4×5 mm. Anteriorly, the thyroid was always only slightly forward of the gill-bearing visceral arches. Posteriorly, masses of thyroid tissue surrounded the base of the ventral aorta and the bulbar portion of the heart, extending almost as far back as the auricle. The tongue, which was much enlarged and consisted almost entirely of thyroid tissue (Fig. 3), extended dorsally nearly to the roof of the mouth. Ventrally, the thyroid tissue grew through the muscular mass of the lower jaw, between the elements of the visceral skeleton, and formed a nodular bulge clearly visible on the lower side of the head at the isthmus. Externally this characteristic swelling appeared pink and made identification of thyroid-tumorous fish an easy task. Laterally, thyroid tissue spread into the gills and to the bases of the opercula. The fairly loose connective tissue appeared to offer little resistance to the spread of the growth.

Penetration or invasion of the walls of the large aortic branches was not seen. However, the large masses of thyroidal tissue, apparently under some pressure due to expansive growth, enveloped the bulbar heart, the aorta, and aortic branches of the gills, probably impairing the efficiency of the vascular channels and thus interfering with the respiratory demands of the body.

In addition, tumor tissue penetrated the gills from below. The degree of gill involvement in various fish appeared roughly proportional to the size of the tumor growth. The tumor might, therefore, be regarded as a progressive type. In spreading into a gill the proliferating thyroid tumor cells caused the epithelium to pull away from its skeletal and vascular elements. The involved secondary gill filaments, enlarged by the tumor tissue, were found in various stages of pressure against one another and, finally, coalescence. Nests of gill epithelium were thus buried within thyroidal tumor masses. The epithelial cells in such nests were pyknotic. The available respiratory epithelium was radically reduced in this way, and it is probable that the expanding tumor growth imposed a real physiological burden upon the fish, destroying the efficiency of the gills as respiratory organs. This may contribute greatly, if not to a major extent, to the death of the tumor-bearing fish.

The tumors, though unencapsulated, appeared surprisingly regular at their peripheries, especially along the posterior border. Elsewhere, broad or narrow tongues of tumor tissue, arising from the main mass, invaded normal areas of the body. The penetration of muscle appeared never to be direct, but the invasion followed the endomysial connective tissue septa. In the process the muscles were divided into successively smaller units and finally isolated into individual muscle fibers. Such fibers appeared to undergo shrinkage and loss of cross striations. Seemingly, they finally degenerated completely, for only a few of the isolated muscle fibers were found imbedded in the main tumor mass.

Completely separated elements of the visceral skeleton also were found buried, and in a degenerative state, within the thyroid tumor (Figs. 2 and 3). Most cartilage and bone cells were pyknotic. Thyroid cells almost always were found within bone cavities. Eroded fenestrae in the bones (Fig. 2) were found, through which entry of the thyroidal tissue was apparently gained. Many tumor-imbedded fragments of bone were greatly reduced in size and abnormal in shape, giving further evidence of erosion. This relation between bone and the thyroid tumor implies that either (*a*) the bone undergoes some sort of autolytic change when isolated in this way, or (*b*) the thyroidal tissue itself has an erosive effect on bone.

The microscopic structure of the hypertrophied thyroid tissue was not uniform. Although the main mass of thyroid was microfollicular or afollicular, there was considerable variation in cell size and colloid content (Fig. 2). Afollicular areas of thyroid were usually located in the medullary portion of the tumor. However, in areas where penetration of neighboring tissues was in progress, especially in the gills, afollicular cells were found at the periphery. The individual cells were larger than in normal thyroidal tissue, and even though no follicular organization was obvious, the nuclei in most instances were eccentric in position. Frequently the cytoplasm was somewhat vacuolated and contained eosinophilic droplets of colloid-like substance.

Microfollicular areas in the tumor merged with the afollicular portions. The smallest follicles in such areas were less than 20 μ in diameter. Each consisted of a few cells grouped about a small droplet of colloid, or about a tiny empty lumen. The constituent cells of microfollicular portions of the tumor were quite similar to those of the afollicular parts and they appeared to be virtually as "invasive." They, too, were found within bones and in the gills.

In the tongue region the tumorous tissue frequently was less dense than in other parts. Here edematous spaces clearly separated the small follicles or individual cells. Some of the spaces were endothelium-lined, and, hence, seemed to be enlarged lymph channels. Seemingly in response to freedom from immediate pressure of neighboring cells, there was a rounding up of the cells within a small area. Under these circumstances the rounded cells of an afollicular thyroid region resembled young adipose tissue of the "brown fat" type.

There were relatively few follicles of approximately normal size, morphology, and colloid content. They occurred in groups either near the edges of the thyroidal growths, or especially near their posterior borders (Fig. 2).

Cystic follicles were found in the tumor of one animal only. The lumina of the cystic area, which was at the anteroventral end of the tumor, were irregular in shape, and some were in communication with one another through a series of gaps. The epithelium was cuboidal, and the lumen contained a scanty eosinophilic material, stringy in texture. The general histology of this cystic area resembled that of the mammalian seminal vesicle.

A few hemorrhages were found within the thyroidal tumors. They usually were associated with numerous pigment-laden macrophages.

Radioautographs.—The radioautographs of all afollicular, microfollicular, and cystic areas of the tumors were very weak, indicating that iodine accumulation in these areas was hardly, if at all, greater than in nonthyroidal areas (Fig. 3). This is in contrast to the behavior of thyroid tissue in unaffected fishes. However, evidence of approximately normal intensity of iodine storage was seen on most radioautographs in small spots at the periphery of the tumors. These dark autographs were found to have been made by the relatively few apparently normal follicles still present in the thyroidal growths.

DISCUSSION

In morphological features, at least, the thyroidal tumor of *Xiphophorus montezumae* is quite similar to the thyroidal "carcinomas" of trout and similar tumors of other fishes. The similarity extends, furthermore, to the fact that the tumors developed in a group of captive animals. We have no information concerning the incidence of such tumors under natural situations, except that among hundreds of specimens collected in the field from various tributaries of the Rio Axtla, no externally visible thyroidal tumors were seen.

One seemingly significant difference between the tumors of *X. montezumae* swordtails and those of trout is in respect to the age at which they develop. The thyroidal tumors of trout were relatively large in young animals. They diminished or even regressed completely (3) in older trout, if the animal survived beyond a critical point. In *X. montezumae* the growths did not appear until the animals had reached almost adult size, and they increased in size until the death of the diseased fish.

Some of the circumstances attending the development of the tumors seem worthy of consideration, since they further define the character of the growths. While the thyroidal tumors of trout appear to form in goitrogenic response to a low concentration of iodine in the environment (3, 6), this does not appear to apply to *X. montezumae.* Dried ocean shrimp, which composed a large part of the diet of our aquarium-reared fishes, have a high content of iodine and actually had a curative effect upon the thyroidal tumors of trout studied by Marine (3). It is conceivable that our laboratory stock of *X. montezumae* has an extremely high iodine requirement—one which exceeds its dietary supply.

In our laboratory the following species kept under the identical dietary and environmental conditions develop thyroidal tumors very rarely: *Xiphophorus pygmaeus*, *Platypoecilus maculatus*, and *P. xiphidium*. The tumors have never been found in *Xiphophorus hellerii*, *Platypoecilus variatus*, or *Platypoecilus couchianus*. In nature, *X. montezumae*, *X. pygmaeus*, and *P. variatus* are found living together occasionally in the Rio Axtla at Axtla, San Luis Potosi, Mexico. *X. montezumae*'s greater susceptibility, whether or not it is due to a higher exogenous iodine requirement, may, therefore, be mediated by a specific genetic difference.

SUMMARY

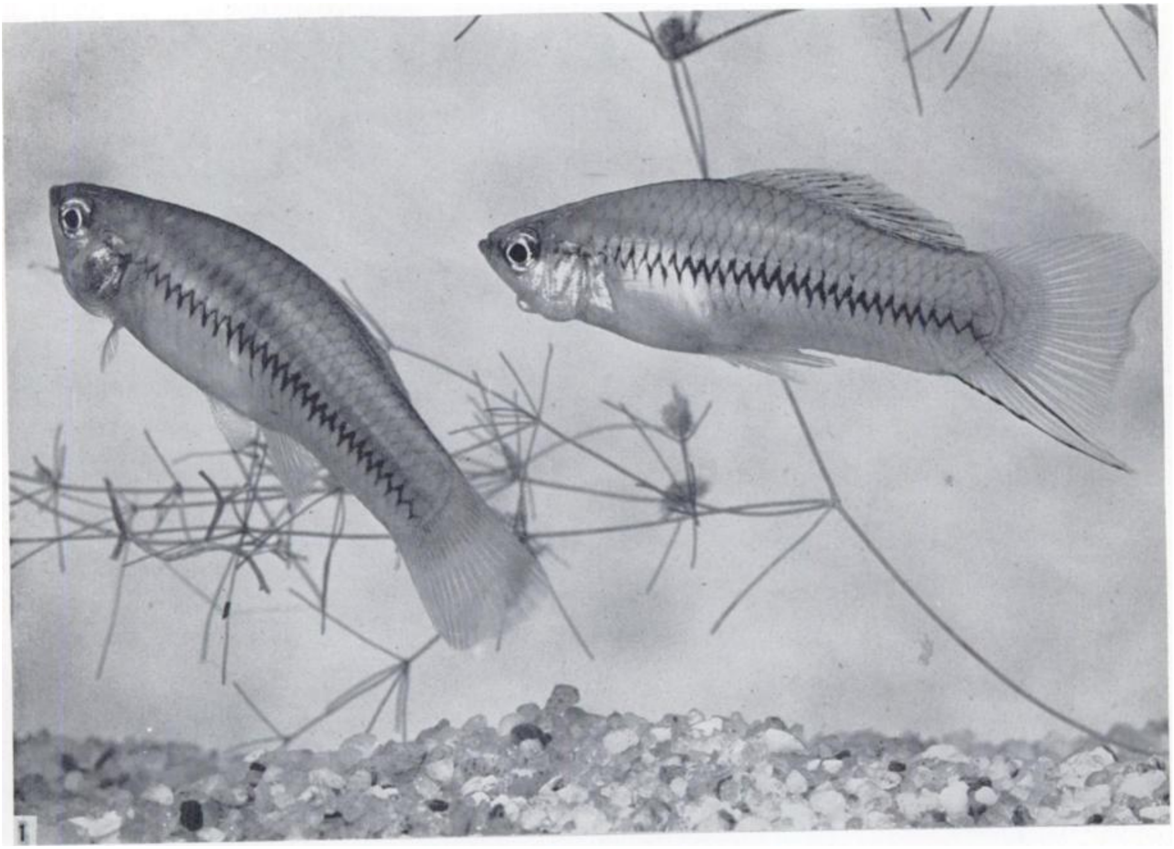
In a laboratory population of swordtails (*Xiphophorus montezumae*) obtained originally in 1939 from the Rio Axtla in Mexico, a high incidence of spontaneous thyroidal tumors has been observed. In two other species of Xiphophorus, four species of Platypoecilus, and in *Lebistes reticulatus*, kept under identical conditions, thyroidal tumors are very rare or are never seen.

The thyroidal tumor is not uniform in microscopic structure, consisting of areas of normal follicles, very small follicles, nonfollicular cell masses, and a few cystic follicles. It has the property of

FIG. 1.—Two adult *Xiphophorus montezumae*, a female on the left, a male on the right. Both have thyroidal tumors. The tumor of the male is visible as an outgrowth below the jaw. In both the opercula are lifted away from the head due to the infiltration and thickening of their bases.

FIG. 2.—Section of thyroidal tumor. All normal tissue beneath the mucosal epithelium (top) has been supplanted by thyroidal tissue. Two bones are visible in this section (middle and left). Both have been perforated, and contain thyroid. The only follicles which metabolize radioiodine (revealed by autograph in Fig. 3) are the large ones at the top edge of the growth, just beneath the mucosal epithelium (near point *A* in Fig. 3). A few degenerate muscle fibers are visible as very dark spots about the diameter of a small thyroid follicle. The large blood vessels are branchial vessels. The remainder of the growth is mostly microfollicular, with several afollicular areas (near center).

FIG. 3.—Section of the entire lower jaw (left) of the same animal as in Figure 2, and the radioautograph (right) made by this section. In the photomicrographs of both the section and the autograph, corresponding points *A*, *B*, *C*, and *D* have been placed for reference. The area *A-C-D* is the much enlarged tongue. The area to the right of the line *B-C* is the portion of the tumor which projects visibly externally as a dimple on the lower side of the jaw (see Fig. 1). Most of the growth does not metabolize iodine normally. A few follicles at or near the edge of the growth produce a normal autograph. Parallel and posterior to (below) the line *A-C* are several bones and bases of gill arches.



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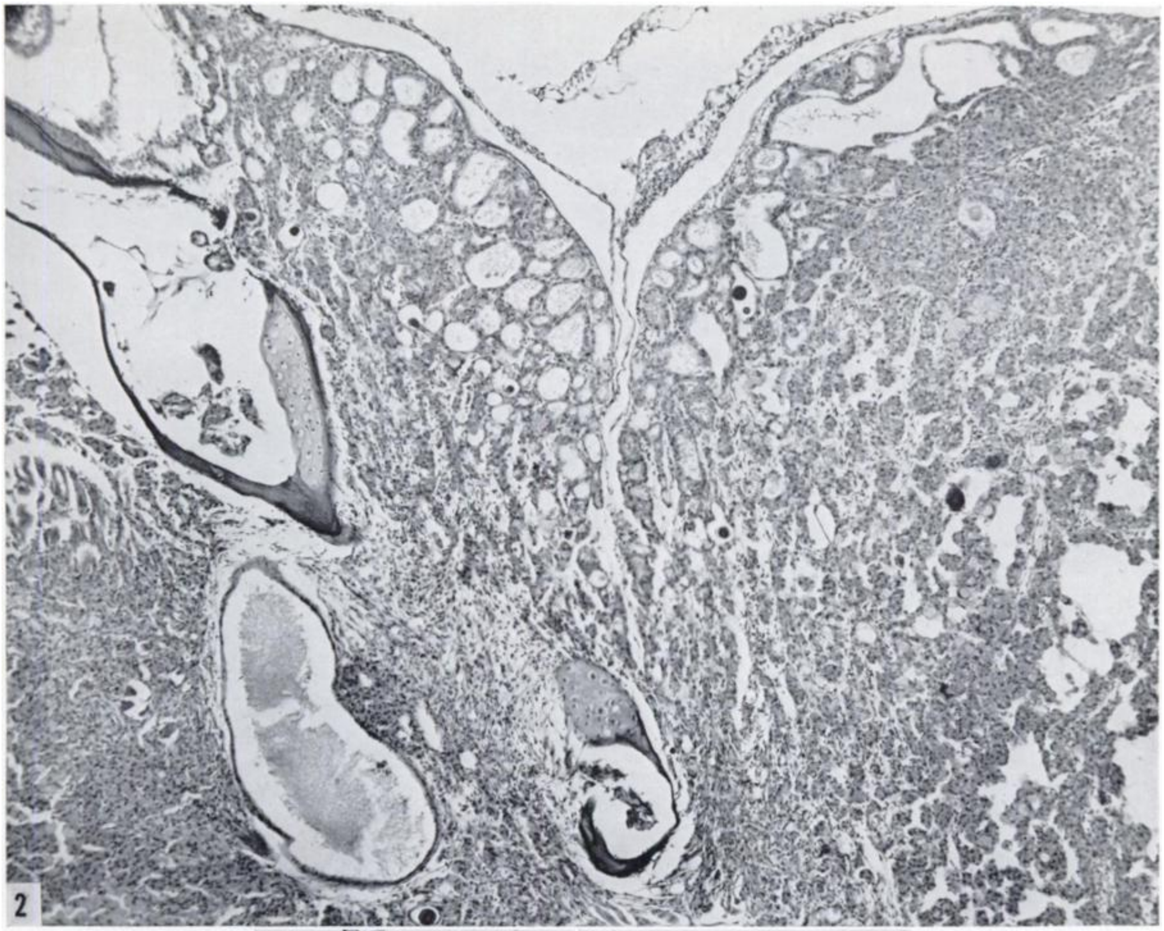
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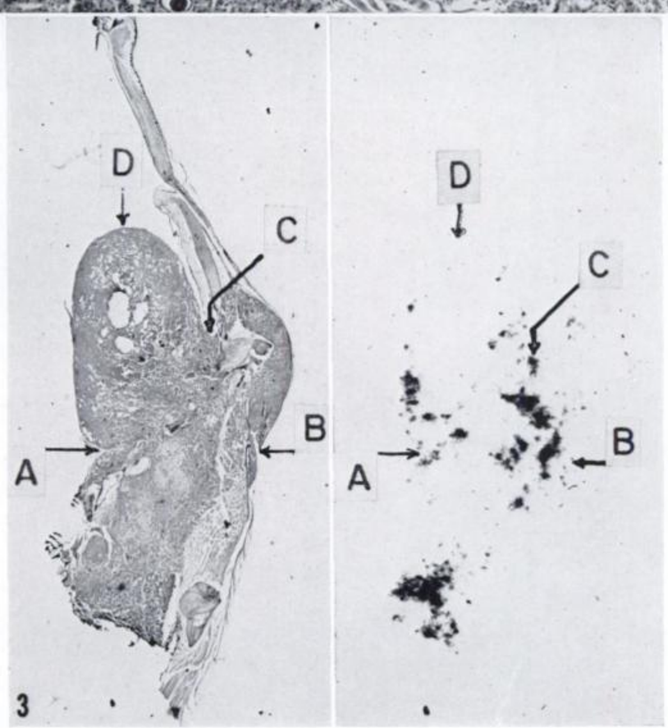
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invading bone. By gradually filling the visceral arches it apparently interferes with respiration, contributing finally to the death of the fish.

In radioautographs of thyroidal tumors of swordtails given radioactive iodine it appears that the few normal follicles are normally functional in respect to iodine metabolism. The remaining areas of the tumor, on the other hand, seem to lack this ability to a large extent.

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