The presence of thyroidal tissues in places in the body far removed from the usual pharyngeal embryonic source has often stimulated considerable speculation and discussion. For example, thyroidal follicles in the human ovary (struma ovarii) are believed by Means (5) to be local teratomatous tissues, and not metastatic growths, benign or other-

wise. In this report we propose to describe the occurrence of both normal and hyperplastic thyroidal tissue in fish organs as far anterior as the eye, and as far posterior as the kidney, spleen, and intestine. These findings, which appear to imply an extraordinary mobility of thyroid tissue in normal and goitrous fish, are presented for both their intrinsic interest and their possible value in the interpretation of atypically located thyroid tissue in man.

When first noted in a number of genetic strains of the platyfish (Xiphophorus maculatus), hyperplastic thyroidal tissue located in the kidney was described as a spontaneous kidney tumor (1). This atypical cell growth formed cysts and eventually caused the death of the affected fish. Further study disclosed that this hyperplastic tissue had the morphological and physiological properties of thyroid tissue in both the normal and hyperplastic state. Autoradiographs, with the use of radioactive iodine, showed that the tissue of the kidney tumors was capable of metabolizing iodine and binding it to a protein in the same way as the tissue of the thyroid gland itself. The distribution and properties of the thyroid-like tissue in kidney and other sites are the subjects of this report.

**MATERIALS AND METHODS**

Platyfish (Xiphophorus maculatus) of several genetic stocks were studied (Table 1). The “domesticated” strains were obtained from fish fanciers who had bred them previously for 15 or more years. The “wild” stocks were obtained by our expeditions to Central America in the years indicated in the table. Most stocks in our genetics laboratory have been maintained by brother-sister matings. All the stocks have been inbred closely, but not necessarily by brother-sister matings. All these strains have been maintained in this laboratory under similar conditions (4). The tumor incidence in the “Fury” stock was determined from records kept on living fish. The incidences of other stocks were estimated on counts of preserved animals which had been accumulated for several years in our “morgue.”

Tumorous animals to be studied histologically were fixed in Bouin’s fluid, decalcified with formic acid, sectioned serially at 8–10 μ, and stained with
hematoxylin and eosin. Forty tumorous fish of the various strains were studied in detail. Two of these were given injections intraperitoneally of 6–10 μc. of carrier-free I\(^{131}\) and sacrificed 24 hours later. For the purpose of obtaining radioautographs, the serial sections prepared from these radioactive animals were exposed to No-screen x-ray film for 48–60 hours. Four control animals of the nontumor-producing “30” strain were given injections of I\(^{131}\), fixed, sectioned, and the sections exposed to x-ray film in the same manner. In addition, four fish, caught in the Río Hondo, British Honduras, and maintained in our laboratory for only 1 week, were given injections of I\(^{131}\) and prepared in the same way to obtain comparable radioautographs.

**RESULTS**

**Morphological Observations**

**Normal kidney.**—The kidneys of normal platyfish are symmetrically paired organs lying in the dorsal region anterior to the air bladder, and just behind the head (Chart 1). They are bounded posteriorly by the anterior tip of the air bladder, which occupies an excavation of their caudal ends, and are separated posteriorly and ventrally from the peritoneal cavity by the pigmented peritoneal tumors in the kidneys have atypical thyroid follicles in all the areas shown and, in addition, in the spleen (not shown here). Some nontumorous fish have thyroid follicles in the kidneys and in the normal thyroid area, but nowhere else.
membrane. The kidneys are short antero-posteriorly, their total length being equalled by their depth. Together, the two platyfish kidneys resemble in their contours the form of a saddle: flat lateral flaps extend ventrally from the seat of the "saddle" on each side of the esophagus and terminate just above the heart (Fig. 3). The large, paired cardinal veins leave the central part of the kidneys and run in a ventral direction along the inner aspect of the lateral flaps. The veins separate from the kidney only a short distance above the sinus venosus.

**Tumorous kidneys.**—Fish with well advanced kidney tumors often became remarkably bloated (Fig. 2). On gross dissection the edematous condition was found to be caused by large, fluid-filled cysts that were contained in the kidneys. Frequently, one kidney was relatively normal in size and appearance, while the other was extremely cystic. The enlargement of the kidneys often dislodged the peritoneal membrane from the dorso-lateral body wall. (The membrane is pigmented and imparts a silvery coloring to the abdomen.) In tumorous fish, as the membrane was pushed down and away from the body wall, a dark area below the dorsal musculature became quite prominent (Fig. 2). This external feature is diagnostic of the presence of a tumor even before a fish becomes noticeably swollen.

Histological sections showed that the tumorous kidneys were always cystic to some extent. In many animals a few of the cysts were so large that the abnormal kidneys occupied most of the body cavity. The tumor tissue was entirely unencapsulated and was scattered throughout the kidney. Because of this, as the tumor enlarged, normal kidney tubules and lymphoid tissue became widely dispersed. The glomeruli were normal in size and appearance. The kidney tumors contained some areas of solid tumor which were composed of masses of solidly packed cells, exactly as in a follicular thyroid tumors, or of a mixture of follicular and follicular tissues. The follicles were of the type found and previously described in the thyroid tumors of the swordtail, *Xiphophorus montezumae*, a related species (3).

Fish with kidney tumors always had atypical thyroids; the latter will be described below. The growth pattern of the thyroid and the kidney tumors in the same individual were often closely similar (Figs. 4, 6). Many animals have an extensively papillary pattern of growth in both kidney and thyroid, similar to some kidney and thyroid papillary adenomas or adenocarcinomas in man (7). In the kidneys of most of the tumorous fish the cysts (swollen follicles) had some papillary extensions into their lumina. These enlarged follicles contained groups of loose tumor cells, macrophages, and a weakly eosinophilic granular material. The smaller follicles usually contained strongly eosinophilic colloid. The cells of the tumors were basophilic; those of the kidney tubules were acidophilic. The tumor cells were cuboidal to high columnar, and had characteristic, irregularly shaped, folded or grooved nuclei (Chart 2). The cells forming the largest cysts were frequently flattened. Mitotic figures were infrequent, although they were found much more often in the tumor cells than in the normal cells of kidney tubules and lymphoid tissue.

**Normal and tumorous thyroids.**—The thyroids of all tumorous fish which were sectioned serially were markedly hypertrophied. The thyroid in normal platyfish consists of unencapsulated, thin-walled follicles scattered among the connective tis-
 sue in the floor of the pharynx along the ventral aorta. Normal thyroid tissue was found in an area which extended antero-posteriorly from about the middle of the eye to about the level of the posterior of the pituitary (Chart 1). The hyperplastic thyroids, unlike the normal, extend posteriorly along the roof and sides of the pericardial cavity, often as far as the ventricle, and may make contact with the cardinal veins and spread around their walls. Many of the hyperplastic thyroids spread laterally into the gill arches and out along the course of the blood vessels of the gill filaments. In many fish, thyroid tissue which extends in a posterior direction makes contact with and is structurally continuous with the tumor tissue from the kidneys.

**Other sites of atypical thyroid growth.**—Search for other possible sites of tumor growth revealed that thyroid tumor tissue was present in numerous widely scattered locations within the body of afflicted fish. In tumors of abdominal fish, without exception, abnormal thyroid tissue was found among the ventricular muscle strands of the heart, in the spleen, and in the chorioid gland of the eye. In some fish the atypical growths in these locations were massive and were either solidly follicular or cystic (Figs. 8, 9). Most of the hearts contained only scattered clumps of small colloid-containing follicles (Fig. 10). These follicles were always adherent to the connective tissue sheathing the muscle bundles, never free in the circulating blood. They were only occasionally present in the auricle of the heart. The spleens were generally so filled with follicles that this organ was easily mistaken for an isolated solid tumor mass in the viscera. One spleen was so cystic that its outline was completely obliterated, and the walls of the cysts were squeezed out into loops weaving between and surrounding the coils of the intestine (Fig. 11). In the chorioid gland and surrounding it the tumor tissue had overgrown in three fish to such an extent as to force the eye out of its orbit almost completely (Fig. 8).

In one fish the tumor tissue was found in the wall of the intestine. In several it was found along the bony parts of the braincase in the ear region. None has been found in the liver, brain, or gonads, nor have recognizable thyroidal emboli been seen in any blood vessels.

Tumor tissue in the thyroid and in the kidney regions exhibited invasive and destructive properties. It frequently infiltrated muscle masses along their septa and surrounded muscle strands, which appeared to degenerate. Occasionally, tumor tissue from the kidney had completely perforated the muscular wall of the body and spread out under the skin (Fig. 12). Within the kidney itself the tumor tissue destroyed tubules, glomeruli, and intertubular hematopoietic elements. In some animals serial sections showed that scarcely any normal kidney tissues remained.

**Radioautographs**

Both tumorous fish studied had strong radioiodine autographs in the thyroid and kidney regions. Detailed examination revealed perfect correspondence between the positions of autographic spots and portions of the kidney tumor consisting of follicles containing colloid (Figs. 13, 14). There was also a correlation between the intensity of the autographic spots and the staining density of the contents of follicles and cysts in the kidney. Cysts containing little or no staining material retained little or no radioiodine. Both fish showed autographic spots which corresponded exactly in position to thyroidal follicles containing colloid in the heart and in the chorioid gland. No autograph was visible in areas of the kidney occupied only by tubules and lymphoid tissue.

The radioautographs prepared from two of four normal laboratory-reared fish of stock 30 showed no positive response in the kidney tissue. The other two showed a few small, distinct, positive areas in the kidney. These spots were found to correspond to a few isolated colloid-filled follicles (Fig. 15). These follicles had a very low epithelium like that of normal thyroid follicles. No autograph was obtained from any other parts of the normal fish except, of course, the normal thyroid area.

Four additional normal fish, caught in the Rio Hondo, British Honduras, and kept only 1 week in the laboratory, were given similar injections of $^{131}$I and tested by the radioautographic technic. Only their thyroids showed a positive response.

**DISCUSSION**

Like the thyroid tumors of *Xiphophorus montezumae*, reported by Gorbman and Gordon (3), the kidney tumors described here seem to appear relatively late in life. The youngest fish that have been found to have kidney tumors were 7 months old and sexually immature. In general, the disease appears in mature fish a year or more of age, and continues progressively until the death of the animal.

Of the xiphophorin fish species other than *X. maculatus* maintained in the laboratory, *X. montezumae, X. pygmaeus,* and *X. ziphidium* have also had thyroid tumors. However, as far as is known, none of these have involved the kidney, except for one exceptionally severe tumor in *X. montezumae. X. helleri* and *X. couchianus* have had neither thyroid nor kidney tumors.
Berg, Gordon, and Gorbman (2) have recently found that the aquarium water in which all our strains of fish have been reared is abnormally low in iodine. This may be attributed to the fact that the water has been re-used constantly for years, with the addition of new tap water only to replace evaporation and other minor losses. It is likely that the algae *Nitella* and other aquatic plants which are used as a shelter for the new-born fish remove some iodine from the water. Snails and the fish themselves also extract iodine (2).

The above authors (2) also reported that the thyroid tumors of *X. montezumae* may be prevented by the addition of iodide to their aquarium water and that established tumors regressed upon treatment with potassium iodide. We have some evidence that the kidney tumors also are responsive to treatment with iodide. This suggests that the kidney tumors develop in fish in response to their prolonged subject to an environment in which the concentration of iodine is abnormally low.

At present it is difficult to determine the point of origin of the kidney tumors. They may arise from cells or follicles of the unencapsulated thyroid gland by migration or metastasis, or they may arise in situ from unrecognized precursors. Migration is suggested by the presence of follicles that are attached to connective tissue on the outer surface of the kidney and heart, and around certain bones of the head. On the other hand, since isolated tumor foci have been most frequently found in blood-laden organs such as the heart, spleen, chorioid gland, and the kidney itself, their presence might be attributed to metastasis. In normal fish, however, where isolated thyroidal follicles are found within the kidney, there is no evidence for either migration or metastasis. In these animals consideration must be given to the possibility that the follicles arise de novo. Autographic studies on young fish of high tumor and nontumor strains now being carried on may yield some answers to this interesting problem.

The differences in incidence of kidney tumors between members of different genetic strains

Photographs for Figures 1 and 2 were taken by Sam C. Dunton, staff photographer, New York Zoological Society.

Fig. 1.—Normal male platyfish of the “Fury” stock. Life-size.

Fig. 2.—Male platyfish of the BH stock, with kidney tumor. The dark area caused by the detachment of the peritoneal membrane from the lateral body wall, a diagnostic character, is partially obscured by reflection. Life-size.

Fig. 3.—Cross-section through the kidneys of a normal platyfish. The cardinal veins (the large broad V-shaped structure) extend along the inner side of the two ventral extensions of the kidneys. The ventricle of the heart is below. Mag. ×18.

Fig. 4.—Section through a typical tumorous kidney. Some normal kidney tubules may be seen in the upper part of section. The remainder shows cystic and solid tumor tissue. The tumor tissue in the kidney and the tumor tissue in the thyroid (Fig. 6) are much alike. Mag. ×80.

Fig. 5.—Portion of an a follicular and microfollicular tumor mass in the kidney. One kidney tubule may be seen at the bottom center of the photomicrograph. Mag. ×125.

Fig. 6.—Thyroid tumor in a platyfish which also had a kidney tumor. The ventral aorta appears at the lower right. This thyroid tumor is unusually cystic and is distinctly papillary. Most kidney tumors (Fig. 4) closely resemble this example. Mag. ×160.

Fig. 7.—Thyroidal follicles within and on the surface of the chorioid gland of the eye in a fish with tumorous kidneys. The retina appears at the right, the choroid at the left of the photomicrograph. Mag. ×400.

Fig. 8.—A tremendous proliferation of thyroid tumor tissue in the chorioid gland (none of which is visible here) and around the optic nerve (left center) in a tumorous platyfish. This abnormal thyroid tissue growth had forced the eye out of its orbit. The retina may be seen in the upper left. The other eye was normal. Mag. ×80.
Fig. 9.—A prominent thyroidal outgrowth from the surface of the ventricle of the heart in a platyfish with tumorous kidneys. Discrete follicles may be seen among the muscle strands below the main mass of the abnormal growth. Masses of nucleated red blood cells are present among the muscle bundles within the ventricle at the bottom of the photograph. Mag. ×125.

Fig. 10.—Colloid-containing thyroidal follicles among the ventricular muscle bundles in the heart of a tumorous fish. Mag. ×540.

Fig. 11.—Section through the liver (left), spleen (center), and gut of a platyfish with tumorous kidneys. The entire central area consists of cystic thyroidal tissue in the spleen. The tumor tissue is so extremely cystic that the coils of the intestine are enveloped by its folds. Mag. ×18.

Fig. 12.—Cystic thyroidal tumor tissue in the kidney, which had invaded and perforated the lateral body musculature (left). Tumor tissue had expanded through the opening thus formed and spread under the skin. The muscular layer shows at the top and bottom of the section; the skin is the dark strip at the extreme left. Mag. ×80.

Fig. 13.—Section through the kidneys and liver of a fish with tumorous kidneys that was injected with I\(\text{131}\) for purposes of recording the radioautograph shown in Figure 14. Mag. ×18.

Fig. 14.—Radioautograph of Figure 13, at a slightly higher magnification. A strong autograph was obtained from the more solid left kidney where there is much follicular colloid-containing tumor tissue. The highly cystic right kidney did not autograph except along the edge, where there are small follicles containing colloid.

Fig. 15.—A portion of the kidney of a nontumorous (or pretumorous) fish of one of the high tumor strains. Several thyroidal follicles are present among the lymphoid tissue cells and along the surface of the kidney. These follicles have a normally low epithelium and are in all apparent respects identical with the thyroidal follicles found in the kidneys of the nontumor-producing strain of fish. Mag. ×125.
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...(Table 1), and the fact that some strains and some species apparently are not subject to such tumors at all, although living in a common environment, indicate that genetic differences are involved. Further support for a genetic basis lies in the observation that certain fish, particularly X. montezumae, develop thyroid tumors which proliferate extensively in situ and are not known to be found in the kidneys, whereas thyroidal tumors in the members of X. maculatus reported here proliferate extensively in the kidneys, but far less so in the thyroid area itself.

Differences in susceptibility to kidney tumors have been found in two closely related stocks. Platyfish from the Rio Jamapa that constitute our stock “30” and “168” were obtained in 1939. Subline number “30” was isolated early and has been inbred for twenty generations by brother-sister matings. No thyroid or kidney tumors have appeared in its members. Subline “168” was not inbred by brother-sister matings until six generations ago. Kidney tumors have often been found in the later generations of this stock.

As yet there is no evidence for linkage between any of the many known color genes and those genes which are probably responsible for susceptibility to kidney tumors. Experiments utilizing high tumor and nontumor strains are being carried on in the hope of analyzing the responsible genetic factors.

SUMMARY

Several laboratory strains of the platyfish, Xiphophorus maculatus, have been observed to have a high incidence of tumors involving the kidneys. By morphological and physiological criteria these tumors are thyroidal in nature. Radioautographs show that the follicles of the kidney tumors are similar to those of the thyroid gland in their ability to bind inorganic iodine to protein.

Thyroidal tumor tissue was found in the heart, spleen, chorioid gland, and other areas of the body, but there is no direct evidence for metastases. It is not known whether the tumors in the kidney and other sites are a result of migration from the thyroid itself, or whether they have differentiated in situ from unidentified precursor cells.

The tumors of the kidney are similar histologically to those of the thyroid, but are much more cystic. They, too, are able to invade and destroy muscle. The kidney tumor tissues may perforate the body wall, and, in addition, may cause almost total destruction of the normal kidney tissue.

The concentration of iodine in the laboratory aquarium water is abnormally low. Since some strains of platyfish develop thyroidal tumors in the kidney and other strains do not, while kept under similar conditions, it is inferred that there are genetic factors that mediate the differences in reaction.

As yet there is no evidence for genetic linkage between any of the many known color pattern genes and those which are probably responsible for various degrees of susceptibility to thyroid and kidney tumors.

REFERENCES


Functional Thyroid Tumors in the Kidneys of Platyfish


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