

Iodine Utilization by Tumorous Thyroid Tissue of the Swordtail* *Xiphophorus montezumae*

OLGA BERG† AND AUBREY GORBMAN

(Departments of Zoology, Barnard College and Columbia University, New York, N.Y.)

Evidence from radioautographic work indicates that metastasizing thyroidal carcinomas are not normal in their metabolism of iodine (4, 7); but surprisingly little detailed study has been made of the physiological properties of such cancers. Seidlin *et al.* (11) found thyroxine in a human thyroid metastasis. Wollman *et al.* (12) were able to demonstrate that some thyroxine is produced by a transplantable thyroid carcinoma, obtained originally by chronic goitrogenic treatment of mice. There is, therefore, a dearth of information concerning the pattern of function in the tumorous thyroid which would enable a direct comparison with the normal thyroid.

Xiphophorin fish form a ready source for tumorous thyroidal tissue, since such tumors develop spontaneously in high incidence in certain laboratory-bred strains. The tumors are large enough to be visible externally in their more advanced stages. Their morphology, formation, and invasive properties have been described (3, 5), and their growth can be prevented and controlled by the administration of iodine, thyroxine, or mammalian whole thyroid tablets (2).

This report deals with iodine uptake and loss and the rate of synthesis of thyroxine in tumorous thyroid tissue of *Xiphophorus montezumae*. The tumorous tissue is compared to normal thyroid tissue of the closely related platyfish, *Xiphophorus maculatus*, which has been previously described by Berg and Gorbman (1).

MATERIALS AND METHODS

Thirty sexually mature *Xiphophorus montezumae*, between 30 and 40 mm. in standard length, were selected for analysis.¹ Of these,

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seventeen had large, externally visible thyroid tumors; the remaining thirteen were assumed to have small tumors, not yet sufficiently large to be seen externally but invariably found on histological examination of their siblings. The iodine accumulation and chromatography data confirmed the diagnoses. The fish were kept at all times in water taken from the same tanks in which they were bred and were fed a standard laboratory diet which included dried shrimp (6). Twenty $\mu\text{c.}$ of carrier-free I^{131} were injected intraperitoneally into each fish. Two to ten animals were sacrificed at definite post-injection intervals. Thyroidal iodine uptake and loss, over a period of time, was traced by measuring the radioactivity of aliquots of saline extracts of individual tissue samples. Radioactivity of the water in which the animals were kept also was measured. All results were expressed as per cent of the original dose given. The fraction of iodine in the body was calculated by subtracting the fraction of iodine in the water from 100 per cent.

The varying proportions of iodide and iodinated amino acids produced during thyroxine synthesis were determined by chromatographic analysis. In one series of experiments the saline extracts of two to four thyroids were pooled for each time interval, in order to minimize individual variation; in another series the thyroids were analyzed individually. Homogenates were hydrolysed to dissociate the protein-bound amino acids by one of three methods: (a) with trypsin for 48 hours at 37° C., (b) with 2 N NaOH at 100° C. for 12 hours, or (c) with 8 per cent Ba(OH)₂ at 100° C. for 18 hours. Extraction of the thyroidal hydrolysates and their analysis by filter paper chromatography was done according to the procedures of Roche *et al.* (10) and Lissitzky (8). *n*-Butanol-acetic acid and *n*-butanol-ammonia were used as developing solvents. Monoiodotyrosine, diiodotyrosine, triiodothyronine, DL-thyroxine, and potassium iodide were used both as reference standards and as "carriers." The distribution of radioactivity along the length of the filter paper chromatograms was determined with an end-window Geiger tube and

shielding arrangement resembling that described by Lissitzky (8).

Radioautographs were made from paraffin sections of thyroid tumors by exposure to squares of no-screen x-ray film.

RESULTS

The thyroidal uptake of radioiodine was relatively rapid and surprisingly high, although individual variation was great. Maximum uptake occurred 12–24 hours after injection of the isotope

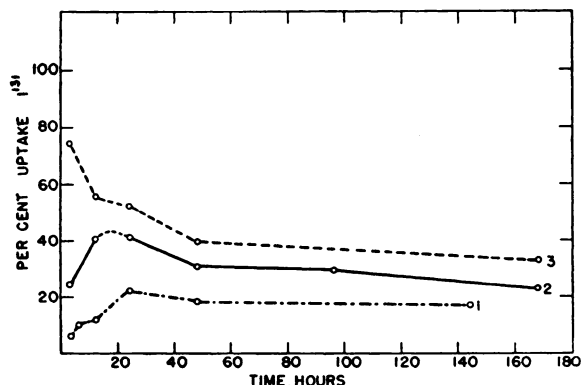


CHART 1.—Curves 1 and 2 show the radioiodine uptake and loss over a period of time in normal (1) and tumorous (2) xiphophorin fish. Curve 3 diagrams the total amount of radioiodine retained in the body of the tumorous swordtails over the course of 168 hours.

and varied from 63.5 per cent in one fish with an externally visible tumor to 11.3 per cent in another. The mean 24-hour uptake based on ten animals was 40.8 per cent \pm 17.9. Although the thyroid accumulated radioiodine rapidly, it released it slowly, and after 168 hours 50 per cent of the peak quantities of I^{131} were still held by the gland, as shown in Chart 1, curve 2. This slow turnover of metabolized I^{131} was also characteristic of fish with normal thyroid tissue (Chart 1, curve 1).

Excretion of radioiodine into the water was rapid for the first 24 hours, after which period it tapered off. The excretion data have been plotted in Chart 1, curve 3, as the total amount of radioiodine retained by the fish over the course of 168 hours. When so plotted the space between curves 2 and 3 represents the per cent of radioiodine held extrathyroidally and shows that an equilibrium is rapidly reached between the amount of radioiodine in the thyroid and that in all other body tissues.

Different hydrolytic technics for dissociating iodinated amino acids from thyroglobulin gave somewhat different proportions of thyroxine and iodide. Since it is not yet known which method is

the most desirable, three were used. The results were fairly consistent with all three methods, except that the NaOH hydrolysates had the most iodide, presumably because strong hydrolysis deiodinates the thyronines (9). Ba(OH)₂ and trypsin, in our preparations, may not always have accomplished complete hydrolysis. The tumorous tissue, like normal tissue, first made monoiodotyrosine (MIT), then diiodotyrosine (DIT), and finally thyroxine, but the conversion of radioiodide to radiothyroxine was extraordinarily slow. Three hours following injection only 8 per cent of the I^{131} was organically bound as MIT; by 12 hours after injection up to 60 per cent of the iodine was in the form of MIT, but only about 5 per cent was DIT. Ninety-six hours after injection the fish still had not made an appreciable amount of thyroxine; in fact, it was not until 168 hours after injection that about 10 per cent of the radioiodine was incorporated into thyroxine. Triiodothyronine was not detectable on any of the chromatograms.

The slow metabolism of radioiodine in the tumors is contrasted with that of the normal platyfish thyroid tissue in Chart 2. Note that in the normal thyroid tissue 48 hours after injection of I^{131} there were approximately equal proportions of radioactive DIT and MIT, and at least 10 per cent of the I^{131} had been incorporated into newly formed thyroxine.

The histological description of the tumors by Gorbman and Gordon was found to characterize the growths used in this study. The tumors were not uniform in microscopic structure, but consisted primarily of nonfollicular epithelial cell masses interspersed with very small follicles and cystic follicles. Although the tumors were not encapsulated, they were quite regular in boundary, and it was in the peripheral area that apparently normal follicles were seen most often. The radioautographs indicated that the histologically normal follicles accumulated organically bound radioiodine, but the remaining areas of the tumor seemed to lack this ability—or possessed it to a much smaller degree.

DISCUSSION

The very high degree of accumulation of radioiodine by thyroidal tumors of *X. montezumae* appears, at first glance, to indicate that the tumorous tissue has a greater than normal avidity for iodine. It must be remembered, however, that the mass of the thyroidal tumors is many times greater than that of normal thyroidal tissue. The thyroid of a normal Montezuma swordtail is composed of relatively few follicles and occupies only a small area in the connective tissue of the lower

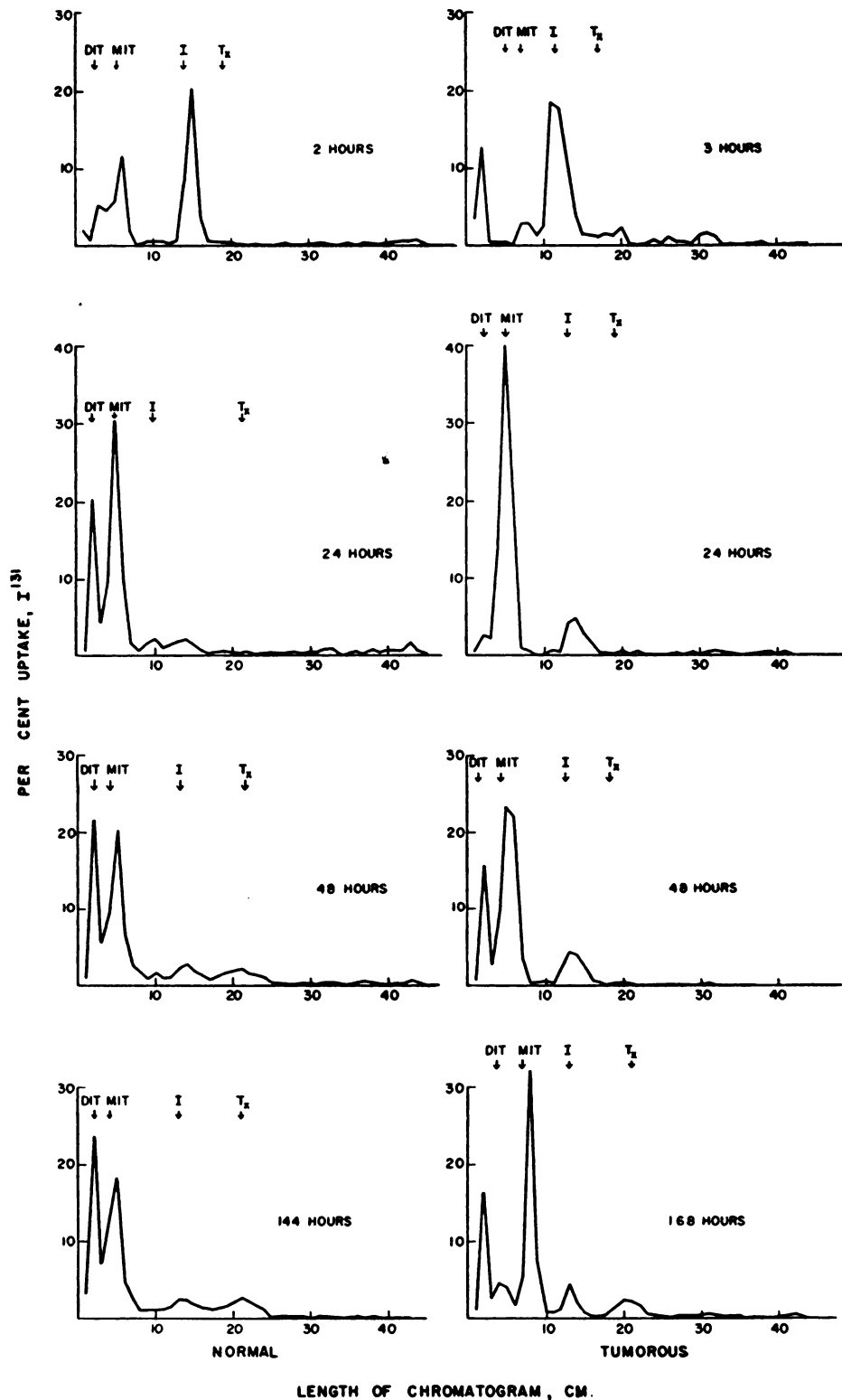


CHART 2.—Radiochromatograms of hydrolysates of thyroid tissue of normal (left) and tumorous (right) xiphophorus fishes sacrificed at intervals after injection of tracer doses of radioactive iodine. Graphs show distribution of radioactivity along the length of chromatograms developed in *n*-butanol-ammonia.

Probable chemical form of I¹³¹ is indicated at the top of each figure. Arrows indicate position of known iodinated compounds chromatogrammed as carriers and in parallel with thyroid hydrolysates. I, iodide; MIT, moniodotyrosine; DIT, diiodotyrosine; TX, thyroxine.

jaw between the first and third gill arches. The tumor, on the other hand, extends from just behind the protrusible jaws to the ventral aorta and replaces much if not all the adductor mandibulae muscle. This large growth actually contains many more histologically normal follicles than the non-tumorous thyroid. The high uptake of I^{131} by the tumor is, therefore, probably referable to an increase in mass rather than an increase in the uptake of individual follicles. Wollman *et al.* (13) have shown in mice with transplantable thyroid tumors that the I^{131} uptake/mg of tissue was always less for the implanted tumor than for the host's own thyroid gland.

The synthesis of thyroxine in the tumorous fish follows the same pattern as in normal fish and in mammals, but its rate is extremely slow. Thus, diiodotyrosine which is normally present within 6 hours after injection does not appear in appreciable quantities until 24 hours post injection. Since diiodotyrosine is not absorbed into the blood stream, its late appearance must be related to its slow synthesis. Thyroxine is not present on the chromatograms until 1 week after injection. The small proportion of thyroxine could, of course, be due to its unusually rapid and selective loss into the blood stream, and this may account for its complete absence on the early chromatograms. However, in view of the slow production of diiodotyrosine by the tumor tissue, it seems likely that the production of thyroxine is also retarded.

Gorbman and Gordon (5) have suggested that the initial development of the tumors may be related to an exceptionally high iodine requirement of the fish, and this suggestion seems to have confirmation in the remedial effect of iodide (2). The lack of efficiency of the tumors in synthesizing thyroxine provides an additional basis for the genesis of the tumor. If thyroxine production is slow in the pretumorous fishes that have few thyroid follicles, the level of thyroxine in the blood will be correspondingly low, which could lead to overproduction of hypophyseal thyrotropic hormone. An increase in the number of follicles, as seen in the tumor, would, of course, tend to raise the thyroxine blood level to par in animals in which the rate of thyroxine synthesis is slow. The efficacy of thyroxine in preventing formation of tumors in thyroid-susceptible *Xiphophorus* and its ability to reduce existing tumors are in agreement with this hypothesis.

The radioautographs located most of the radioiodine in the apparently normal colloid-containing peripheral follicles and indicated the virtual absence of organic I^{131} in the more anaplastic parts of the growths. For this reason, the abnormal bio-

chemical characteristics of I^{131} metabolism seen in our chromatographic data would seem to be referable to these apparently normal follicles. A further conclusion, therefore, seems to be justifiable—that these morphologically normal structures are physiologically different from the normals, or that the presence of tumorous tissue disturbs or distorts the normal pattern of iodine metabolism.

SUMMARY

1. Thirty swordtails, *Xiphophorus montezumae*, with thyroid tumors were given injections of a tracer dose of carrier-free I^{131} and were sacrificed at intervals up to 168 hours after injection. The tumors were removed from the fish, extracted in saline solution, and measured for radioactive iodine uptake. The extracts were hydrolysed and then chromatographed to reveal the relative rates of synthesis of monoiodotyrosine, diiodotyrosine, triiodothyronine, and thyroxine.

2. The thyroid tumors accumulated up to 60 per cent of the injected isotope. Uptake was rapid, but turnover was slow; 168 hours after injection the thyroid still held 50 per cent of its peak quantity of radioiodine.

3. The thyroid tumor tissue transformed I^{131} in the following sequence: first, monoiodotyrosine, then diiodotyrosine, and then thyroxine. The rate of synthesis was unusually slow. The tumorous thyroid required 3 times as long as the normal glands to convert 10 per cent of the radioiodine to radiothyroxine.

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