Utilization of Iodine by the Thyroid of the Platyfish, *Xiphophorus (Platypoecilus) maculatus*. (20481)

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The first experimental demonstration of the relation of iodine to thyroid function was made with teleost fish(1,2) in 1910, but since then teleost thyroidal physiology has received relatively little attention. A revival of interest in the comparative physiology of thyroid tissue has been made possible by availability of radioiodine and has resulted in recent work showing that marine teleost and elasmobranch thyroids can accumulate isotopic iodine and synthesize radiothyroxine (3-11). In regard to the function of the thyroid gland of adult freshwater teleosts, two important questions remain unanswered. 1) To what degree can the thyroid tissue accumulate iodide? 2) In what way is such iodide metabolized in the synthesis of thyroxine? In the experiments reported here, the freshwater killifish *Xiphophorus (Platypoecilus) maculatus* was used and quantitative data were sought to demonstrate the thyroidal accumulation rate and turnover of iodine given as iodide. By chromatographic analysis of thyroidal hydrolysates representing different time intervals after I^{131} administration, the varying proportions of iodide and iodinated amino acids during thyroxine synthesis was studied.

Materials and methods. Adult sibling platyfish, *Xiphophorus maculatus,* all of the same sex and 25 to 35 mm in standard length, were carefully chosen from inbred laboratory stocks* to give maximum animal unifomity. They were kept at all times in water taken from the general laboratory supply in which this stock is maintained. Twenty μc of I^{131} was given to each fish intraperitoneally. Groups of 4 fish were sacrificed at definite post-injection intervals and thyroid tissue (the entire lower jaws) was pooled for analysis for each group. The variation of the procedure in each of 5 separate experiments is summarized in Table I.

Thyroidal iodine uptake and loss, over a period of time, was followed by measuring radioactivity of aliquots of alkali-hydrolysed tissue samples. Radioactivity of the water in which animals were kept also was determined, and all results expressed in terms of per cent of the original dose given.

Extraction of the thyroidal hydrolysates and their analysis by filter paper chromatography was done by standard technics, and closely followed the procedure of Roche *et al.* (12) and Lissitzky(14). Two different developing solvents were used, n-butanol-acetic acid, and n-butanol-ammonia. As known reference substances, the following were employed: monoiodotyrosine, diiodotyrosine, diiodothyronine, d-l thyroxine, and potassium iodide. These also were used, in some instances, as "carriers". For the observation of the distribution of the radioactivity along the length of the filter paper chromatograms, a Geiger tube and shielding arrangement, resembling that of Lissitzky(14), was used.

Radioautography of paraffin sections of thyroid tissue of I^{131} injected *X. maculatus* was done by exposing such sections to squares of x-ray film.

Results. In the 4 experiments with fishes in normal stock water, relatively rapid, though somewhat variable, thyroidal uptake of radioiodine was found (Table II). The maximum uptake, 29%, occurred 24 to 48 hours after injection. The observed variation seemed to bear no relation to whether the water was changed frequently or whether the fishes were allowed to remain in contact with their excreted radioiodine. Even after 144 hours, and despite frequent water changes, the thyroids retained about 75% of their peak quantities of I^{131}, indicating that turnover of metabolized I^{131} was quite slow.

Since thyroidal uptake was so relatively high, and release of accumulated iodine so

* All fish were generously supplied by Dr. Myron Gordon, Genetics Laboratory, New York Aquarium, New York Zoological Society.

TABLE I. Summary of Experimental Procedure.

Strain	No., sex of animals	Culture conditions	Treatment	Time of sacrifice after exposure to radioiodine in hr 2	6	12	24	48	96	144	Type of hydrolysis
405	24 ♀♀	Groups of 4 fish in 400 cc water. Sacrificed together	Water not changed	*	*	*	*	*	*		2 N NaOH 18 hr 100°C
308	24 ♀♀	As in exp. 1	As in exp. 1	*	*	*	*	*	*		10 mg trypsin 48 hr pH 8.5 37°C
394	24 ♀♀	" " " "	Water changed once every 6 hr for 24 and once every 12 hr thereafter	*	*	*	*	*		*	1 N NaOH 16 hr 100°C
407	16 ♀♀	Groups of 8 fish in 500 cc water. Sacrificed 4 at a time	Water changed 3 times before the 12 hr and 24 hr sacrifice and 5 times before the 48 hr and 144 hr sacrifice			*	*	*		*	8% $Ba(OH)_2$ 13 hr 100°C
375	12 ♂♂	As in exp. 1	Treatment with 24 mg of KI/400 cc water initiated 1 wk prior to inj., continued until sacrifice. Water containing KI changed twice prior to inj., thereafter as in 394.			*	*		*		None

TABLE II. Uptake of Radioactive Iodine by Fish Thyroid Tissue, Expressed as % of Total Dose Injected.

	Water not changed		Water changed*		60 p.p.m. KI solution changed*
Strain	405	308	394	407	375
Time, hr					
2	5.8	13.1	6.5		
6	10.8	17.4	10.1		
12	11.3	19.6	19.6	15.0	2.9
24	14.1	24.4	16.4	29.5	3.1
48	14.7	29.9	16.8	19.5	
96	18.0	17.3			1.6
144			16.5	16.4	

* For details of intervals between changes of water, see Table I.

slow, it seemed possible that the fish were "iodine-starved". To test this an additional series of observations was made on animals kept in water which was 60 parts per million KI(4). They were placed in this solution one week prior to I^{131} injection and kept in it continuously until sacrificed. With this treatment the maximum thyroidal I^{131} uptake was only 3% of the injected dose. However, loss of this relatively small amount of radioiodine from the thyroid was slow as in the previous experiment.

As a correlate of the data on thyroidal iodine accumulation and loss, Fig. 1 shows the loss of I^{131} into the water by members of *X. maculatus* strains 407 and 375. Presumably, the differences between these sets of data and the data on thyroidal I^{131} indicate the amounts of radioiodine in the extra-thyroidal tissues of the animals. It may be

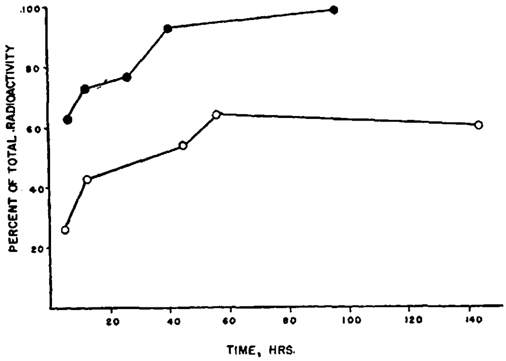

FIG. 1. The loss of radioactive iodine into water from fish injected with tracer dose of I^{131}. White circles: fish kept in small amount of water changed frequently (Table I). Black circles: fish treated with potassium iodide for one week before being given I^{131}. Water also changed frequently.

noted that the greatest rate of loss of I^{131} into the aquarium water from the injected fishes was during the first 12 hours. Whereas fish in iodide-enriched water had lost almost all of the injected I^{131} by 2 days, fish in normal (iodide-poor?) water still retained about 40% of the injected I^{131} in their thyroids and body tissues as long as 6 days after injection.

Radiochromatography. Different authors have recommended different hydrolytic technics for dissociation of the protein bound iodinated amino acids in thyroid(12-14). Because it is yet uncertain which method is most advantageous, 3 were used. As expected from the literature(13,14) NaOH-hydrolysis gave the highest iodide and lowest thyroxine values, presumably because it deiodinates thyroxine fairly readily. Trypsin gave relatively the lowest iodide and higher thyroxine figures, but it is not certain that in our hands it achieved complete hydrolysis. Barium hydroxide (8%) gave intermediate results. Despite these technical variables a unified picture can be constructed from the available data (Fig. 2) showing that thyroxine synthesis follows the same pattern in *Xiphophorus* as in mammals. Although the increase in radioiodide was not striking, radiothyroxine tended to increase, but on a small scale. Monoiodotyrosine and diiodotyrosine shifted in relative importance with time, MIT predominating earlier; DIT later. It is interesting that the largest proportion of radiothyroxine seen in any of the experiments was 15%.

Histology and radioautography. All the fish examined histologically had normal thyroid tissue. The follicles varied greatly in size. The epithelial cells were usually low cuboidal but in the largest follicles they were squamous. In most cases the colloid was dense, eosinophilic, and granular, but in some of the larger follicles it appeared to be nongranular. Especially strong radioautographs were made by the colloid. Unfortunately, with the autographic technic used it was difficult to determine whether the epithelium contained appreciable quantities of radioiodine.

Discussion. The four lower curves in Fig. 3 represent marine fishes; the 4 upper curves, freshwater species; the intermediate curve 5, the seaward migratory "smolt" form of the salmon. If such data, drawn from the work of several groups of investigators, can be taken to differentiate a freshwater from marine type of thyroid, it is possible that this variation is due to the differences in environmental iodine of such fishes. Both LaRoche (4) with immature salmon, and we, with adult platyfish, have demonstrated a decreased I^{131} uptake in freshwater fishes kept in iodine-enriched water. Such experimental curves, if plotted on Fig. 3, would fall within the family of curves representing the marine fish group. It may be noted that the Roussette shark(8), though it briefly accumulates a relatively high percentage of administered I^{131}, loses it promptly. A similar rapid loss of I^{131} was seen in our experiment with fish in iodine-enriched water (Fig. 1).

The data summarized in Fig. 2 show that synthesis of thyroxine in *Xiphophorus* follows the same pattern as it does in mammals(14), or in the shark(8). Monoiodotyrosine and diiodotyrosine are the precursors for thyroxine, with monoiodotyrosine being in excess in earlier phases and diiodotyrosine in excess in later intervals. It seems remarkable that despite the relatively high degree of thyroidal I^{131} accumulation, only a small proportion of it (maximum, 15%) was in the form of thyroxine as late as 6 days after injection. This could mean either that thyroxine synthesis is

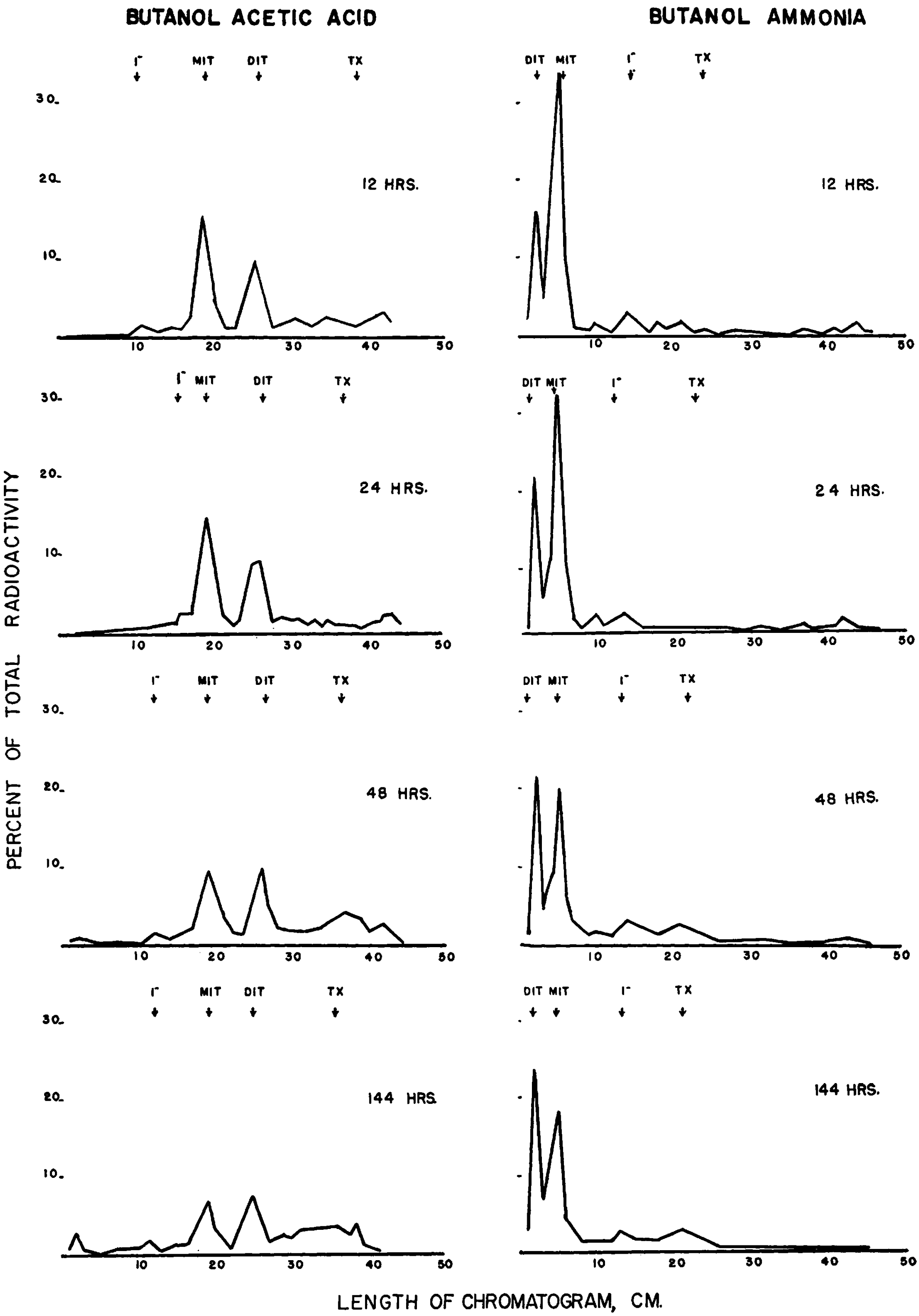
BUTANOL ACETIC ACID
BUTANOL AMMONIA
I⁻ MIT DIT TX
DIT MIT I⁻ TX
12 HRS.
24 HRS.
48 HRS.
144 HRS.
PERCENT OF TOTAL RADIOACTIVITY
10 20 30 40 50
LENGTH OF CHROMATOGRAM, CM.

FIG. 2. Radiochromatograms of barium hydroxide hydrolysates of thyroid tissue of platyfish sacrificed at intervals after injection of a tracer dose of radioactive iodine. Figures show distribution of radioactivity along length of chromatograms. Probable chemical form of I^{131} is indicated at top of each figure. Arrows indicate position of known iodinated compounds chromatogrammed as carriers or in parallel with thyroid hydrolysates. I, iodide; MIT, monoiodotyrosine; DIT, diiodotyrosine; Tx, thyroxine.

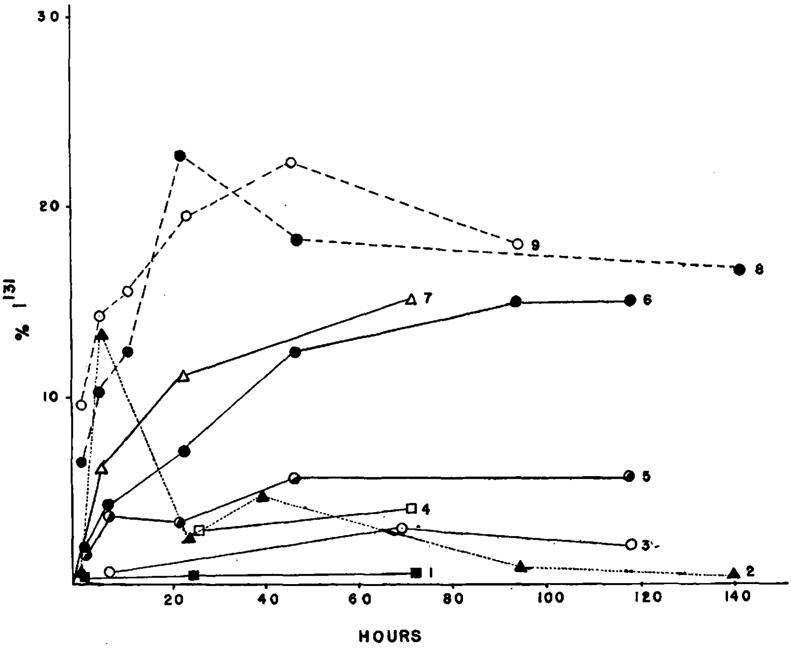

FIG. 3. Accumulation of radioiodine by thyroid glands of various fishes. Curves 3 and 4 are for marine teleosts whose thyroidal uptake of I^{131} is low or the turnover is rapid. Curves 1 and 2 for marine lamprey and shark, respectively, are similar. Curve 5 represents data from salmon smolt, caught in fresh water just before downstream migration. Note that I^{131} uptake resembles that of a marine fish. Curves 6 and 9 are for fresh water teleosts; I^{131} uptake is high and its loss from the thyroid is slow. 1. Marine lamprey, *Petromyzon marinus*, redrawn from Leloup(6). 2. Roussette shark, *Scyliorhinus canicula*, drawn from data of Gorbman *et al.*(8). 3. Marine mullet, *Mugil auratus*, drawn from data of Leloup(7). 4. Marine conger eel, *Conger conqer*, drawn from data of Leloup(7). 5. Salmon smolt, *Salmo salar*, drawn from data of Fontaine *et al.*(5). 6. Salmon parr, *Salmo salar*, drawn from data of Fontaine *et al.*(5). 7. Salmon parr, redrawn from LaRoche(4). 8. Platyfish, *Xiphophorus maculatus*, water changed frequently during experiment. 9. Platyfish, *Xiphophorus maculatus*, kept in same water for duration of experiment.

very slow, or that thyroxine is selectively released from the thyroid as it is formed. Our data do not favor either alternative explanation.

Summary. 1. One hundred platyfish, *Xiphophorus (Platypoecilus) maculatus*, 12 pretreated with potassium iodide, were injected with a tracer dose of carrier-free I^{131} and sacrificed at intervals up to 144 hours after injection. Groups of 4 fish were processed together. The lower jaws, containing the thyroidal tissue, were removed, hydrolysed, and measured for I^{131} uptake. Such hydrolysates then were analyzed by chromatographic technics and the relative rates of synthesis of monoiodotyrosine, diiodotyrosine, and thyroxine were determined. 2. The thyroid tissue accumulated about one-third of the injected isotope. Uptake was rapid but loss was slow so that 144 hours after injection 15% of the I^{131} was still held in the thyroid. Thyroid tissue of fish kept in 60 p.p.m. KI solution accumulated only 3% of the injected dose of I^{131}. 3. The loss of radioiodine into the water was much more rapid in fish treated with KI than in the others. 4. As in mammals, the thyroid first synthesized monoiodotyrosine, then diiodotyrosine, and finally, thyroxine.

The synthesis of thyroxine was relatively slow. A maximum value of 15% of the radioiodine in the thyroid was converted to thyroxine under the experimental conditions employed.

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Effect of Dihydrocholesterol (Cholestanol) Administration on Plasma Cholesterol and Atherosclerosis in the Rabbit.* (20482)

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We reported recently that the administration of dihydrocholesterol (DHC) to chickens fed a cholesterol-containing diet prevented the development of both hypercholesterolemia and the associated atherosclerosis(1). Since the feeding of this saturated steroid might offer a means of inhibiting the atherogenic effects of dietary cholesterol, it seemed desirable to extend our observations to mammals. The present report deals with the action of DHC in the rabbit.

Experimental. Rabbits weighing from 1850 to 2270 g were fed an adequate commercial pellet diet† for 6 weeks before being placed on the experimental diets recorded in Table I. During these 6 weeks, 2 blood samples were obtained from each animal by cardiac puncture, and the total cholesterol content of plasma was determined by the method of Sackett(2). At the end of this period, the rabbits were divided into 4 groups, and fed as shown in Table I. These experimental diets were fed *ad lib.* for a period of 2 weeks at the end of which time—because the supply of DHC was exhausted—all rabbits were placed on the normal pellet diet for the next 5 weeks. At week 13 the level of plasma cholesterol was again determined, and the feeding of the experimental diets resumed (Table I). The experiment was continued for 9 more weeks, and during this time 4 additional plasma cholesterol values were obtained. Each group consumed approximately the same amount of diet.

TABLE I. Composition of Diets. All diets consisted of commercial rabbit pellets to which was added one or more of the following constituents:

Constituent	Group I	Group II	Group III	Group IV
	%	%	%	%
Wesson oil	5	5	5	5
Cholesterol	0	1	1	0
DHC*	0	0	2	2

* The dihydrocholesterol used in this study was generously supplied by the Schering Corp.

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† This diet contained 60% alfalfa meal, 10% ground barley, 10% cottonseed meal, 10% rice polishings, 4% watergrass seed, 4% rice seed, 1% oyster shell flour, 0.5% cane molasses, and 0.5% salt.