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Received April 22, 1954. P.S.E.B.M., 1954, v86.

Normal and Altered Thyroidal Function in Domesticated Goldfish, *Carassius auratus.** (21037)

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The ability of thyroid follicles of fishes to accumulate radioactive iodine appears to be inversely proportional to the amount of iodine in their aqueous environment(1). Thus, it has been shown that marine fishes living in an iodine-rich environment seldom concentrate thyroidally more than 2 to 3% of a tracer dose of I^{131} while freshwater fishes living in a relatively iodine-poor medium accumulate up to 30% of an injected dose of I^{131}. The radioiodine turnover of such a freshwater fish can be reduced until it resembles that of a salt water fish by the addition of iodine to the water(1,8). It also seems to be true that euryhaline fish such as *Fundulus heteroclitus* that live in water which is salty at certain times, and almost fresh at others, can rapidly change their iodine metabolism while strictly marine fish such as *Fundulus majalis* are not so adaptable(5).

In a survey of the radioiodine turnover of freshwater fishes, we have found only one species, the common goldfish *Carassius auratus*, whose thyroid gland is not a relatively avid collector of radioiodine. The thyroidal epithelium of this species is normally squamous, denoting comparative inactivity, but it is extremely sensitive to thyrotropic hormone stimulation, so much so that it has been suggested as the basis for the bioassay of TSH (3,4). We were surprised to find that untreated goldfish make almost no thyroxine within the usual time required for such biosynthesis by other species, and undertook the following series of experiments with thyrotropic hormone to try to alter their exceptionally low level of iodine metabolism.

Material and methods. Eighty-four goldfish were injected intraperitoneally with 5 Junckmann-Schoeller units of thyrotropic hormone (TSH)† per day in .05 cc of 0.9% saline solution. Twenty-four animals received a total of 20 units of thyrotropin in 4 days; 60 others, 25 units in 5 days. The day after the last injection of TSH all were injected with about 20 μc of carrier-free I^{131} and sacrificed in groups of 6 at intervals thereafter up to 170 hours. Ninety-nine fish, used as controls received the tracer dose of I^{131} at the same time as the experimental animals and were sacrificed after a similar interval. Twenty-four additional fish were each injected with 2 frog pituitaries suspended in .05 cc of 0.9% saline solution. Forty-eight hours later they received tracer doses of I^{131}. Another group of 10 fish was kept for 2 months in conditioned aquarium water at the genetics laboratory in the American Museum of Natural History. This water is known to be extremely low in iodine (0.5

* These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and Columbia University (NR 163-208)

† The thyrotropic hormone was kindly supplied by Armour and Co. Their preparation number is P 589-80 and is stated to contain 10 J-S units per mg.

μg of iodine/liter as opposed to 2.3 μg/liter in New York City tap water), so low that xiphophorin fishes reared in it routinely develop thyroid tumors(2). Goldfish were kept in the museum aquarium water to see whether the need for more efficient use of available iodine in this extraordinarily iodine-poor environment might stimulate their normally quiescent thyroid glands. The fish were of the "Comet" variety, and were purchased from the Grassyforks Fisheries, Saddle River, N. J. They were kept in all-glass aquaria which were well-aerated by bubbling compressed air. The fish were fed a beef-liver-pablum diet during the period of the experiment(6). At the time of sacrifice all fish were killed rapidly in hot water, their lower jaws containing the thyroid follicles were removed and homogenized in 0.9% saline solution, and kept at 16°C overnight to permit the extraction of the iodinated compounds. Total thyroidal radioiodine content was then determined by measuring radioactivity of aliquots of the saline extract. The pH was adjusted to 8.5, and the homogenates were digested for 24 hours with trypsin and 24 more with papain (at pH 5.3), a modification of the technic of Roche *et al.*(11), and Lissitzky (9). The digested material was extracted first with chloroform to remove fats and then with butanol saturated with N/10 HCl. The acidified butanol, containing the iodinated amino acids, was dried under vacuum and the residue redissolved in 0.1 cc of butanol and analyzed by paper chromatography. N-butanol-acetic-acid and n-butanol-ammonia were used as developing solvents. Monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (TITn), thyroxine (Tx) and potassium iodide (KI) were used as known reference standards. For the observation of the distribution of radioactivity along the length of the filter paper chromatograms, a Geiger tube and shielding arrangement resembling that of Lissitzky(9) were used. Radioautographs were made by exposure of paraffin sections of the thyroid tissue of I^{131} injected goldfish to squares of x-ray film.

Results. In all 3 experiments with control goldfish, *i.e.* those not treated with TSH, thyroidal iodine uptake was consistently low

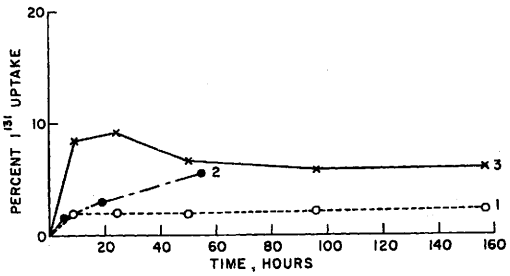

FIG. 1. Effect of thyrotropic hormone on peak accumulation and turnover of I^{131} by thyroid of the goldfish. Curve 1: Untreated goldfish. Peak value, 3% of injected dose of I^{131}, is reached 12 hr after injection. Curve 2: Goldfish pretreated for 48 hr with whole frog pituitaries. Curve 3: Goldfish pretreated 4 or 5 days with mammalian TSH. Note that TSH-treated fishes release about ⅓ of their peak accumulation of radioiodine after 48 hr, while the untreated fish hold 100% of their peak accumulation for the length of the experiment, 156 hr.

(Fig. 1, Curve 1), the maximum uptake was 3% of the injected dose and was reached 12 to 24 hours after injection. The thyroid evidently was not only sluggish in accumulating I^{131} but also was slow in releasing it because 100% of the peak accumulation was still held by the gland at the termination of the experiment 156 hours after it was started. TSH increased the ability of the gland to concentrate radioiodine over 3 times. The greatest uptake seen in any one fish, 17% of the injected dose, was after 5 days of TSH stimulation and 24 hours after I^{131} injection. However, an extra day of TSH treatment made no statistically significant difference in the I^{131} uptake by the thyroid. Therefore, the data on the effects of 4 and 5 days of stimulation by TSH are plotted together as a single curve in Fig. 1, Curve 3. The average uptake reached its peak value, 9% of the injected dose, 24 hours after I^{131} injection. After 156 hours the thyroid still held 66% of its peak accumulation.

Fresh whole frog pituitary preparations were also successful in stimulating the goldfish thyroid (Fig. 1, Curve 2). The response was not as great as with the purified mammalian product, possibly because the frog pituitary hormone was permitted to act for only 2 days.

Finally, the thyroids of the goldfish reared in the aquarium water at the American Mu-

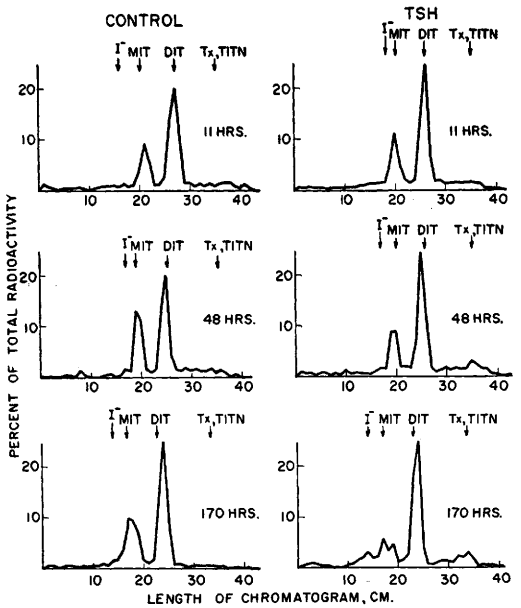

FIG. 2. Radiochromatograms of trypsin and papain hydrolysates of thyroid tissue of control and thyrotropic hormone (TSH) treated goldfish sacrificed at intervals after injection of a tracer dose of radioactive iodine. Figures show distribution of radioactivity along length of chromatograms in which butanol-acetic-acid was the developing solvent. Probable chemical formula of I^{131} is indicated at top of each figure. Arrows indicate position of known iodinated compounds chromatogrammed in parallel with thyroid hydrolysates. I, iodide; MIT, monoiodotyrosine; DIT, diiodotyrosine; TITn, triiodothyronine; Tx, thyroxine. Note absence of thyroxine in control series, and its presence after TSH treatment.

seum of Natural History accumulated 5% of the injected dose of I^{131} in 24 hours. This uptake is 167% of the 24-hour uptake by the fish reared in ordinary tap water.

Radiochromatography. The synthesis of iodinated amino acids followed the same pattern in the TSH-treated goldfish as in many other vertebrates. MIT and iodide predominated in the earlier hours, DIT in the later ones. Thyroxine first appeared in trace amounts between 11 and 24 hours, but was never present in large proportion. Even 170 hours after injection of the isotope it accounted for only 8.3% of the total radioactivity (Fig. 2).

Interestingly enough, in only one of the 3 control runs was there any thyroxine demonstrable. In that one case, 7% of the total radioactivity was radiothyroxine and the amount remained constant from 25 to 96 hours. In the other 2 experiments there was no thyroxine present. Triiodothyronine was absent in all cases.

Radioautography. The follicular epithelium of the control fish was squamous; that of the TSH-treated animals was high columnar. In both cases the thyroidal colloid autographed heavily.

Discussion. Although the function of the piscine thyroid gland is still essentially unknown, it has been assumed that the fish thyroid follicle produces a hormone of some functional importance to the animal(7,10). It is surprising, therefore, that these experiments indicate that for as long as 7 days after it enters the thyroid gland tracer iodine in the goldfish may not be incorporated into either thyroxine or triiodothyronine. It is possible, of course, that the hormone is released into the blood stream immediately upon production and is, therefore, not detectable on chromatograms of thyroid tissue. This, however, seems unlikely, because thyroxine was found in the thyroids of goldfish treated with TSH, an agent which is known to speed the release of thyroxine from the thyroid into the blood stream, at least in mammals.

In addition to being a poor thyroxine-producer the goldfish thyroid is sluggish in its response to iodine levels in the water. It does respond because the I^{131} uptake increases when the fish are placed in water known to be very poor in this element, but the response is not nearly as great as that of other species of fish(1,5). This low efficiency seems to be referable partially to a lack of pituitary thyrotropin because treatment with mammalian or amphibian TSH tends to alter the condition. Furthermore, there is no I^{131} turnover in the untreated animals, while the turnover in the fish pretreated with TSH is typical of that of any other fresh water teleost. Even with TSH stimulation, however, the goldfish thyroid is not as efficient as that known for the average freshwater fish. Possibly teleost TSH would be a more effective stimulant than the mammalian or amphibian material.

The goldfish has long been a domesticated

species living in quiet pools or aquaria. It is possible that the type of selection used by fanciers in developing the present strains of domesticated *Carassius* has in some way been selective also for a given state of thyroidal activity. Possibly it would be illuminating, therefore, to test the thyroidal activity of wild *Carassius*, or of the closely related carp.

Summary. 1. Normal goldfish, *Carassius auratus* accumulate a peak of 3% of an injected tracer dose of I^{131} intrathyroidally. This small amount of radioiodine remains in the thyroid at the same level for more than a week. Thyroids of thyrotropic hormone-injected goldfish accumulate 9% or more of the tracer I^{131}, but lose at least 40% of it within a week, indicating a greater turnover rate. 2. Radioiodine in normal goldfish thyroids is mostly in the form of monoiodotyrosine and diiodotyrosine, with little, if any, thyroxine formed even after one week. The thyrotropic hormone stimulated goldfish thyroid forms a small proportion of radiothyroxine as early at 24 hours after injected I^{131} and as much as 8% of the I^{131} is in the form of thyroxine by 7 days after injection of I^{131}. 3. Keeping the goldfish in water low in iodine had an effect on I^{131} metabolism similar to thyrotropic hormone treatment.

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Received April 23, 1954. P.S.E.B.M., 1954, v86.

Protective Effect of Hypoxia against Irradiation Injury of the Rat Bone Marrow and Spleen.* (21038)

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The reduction in radiation sensitivity by hypoxia has been demonstrated under a variety of experimental conditions(1). Dowdy, Bennett, and Chastain(2) have shown that the LD_{50} dose of x-irradiation is increased from 800 r to between 1200 and 1400 r when rats are irradiated in a 5% oxygen atmosphere.

In previous studies reported from this laboratory(3-5), the concentration of desoxyribonucleic acid has been used as a measure of the cellularity of the bone marrow and spleen and the rate of incorporation of radiophosphorus into this fraction as an index of mitosis rate. In the present investigation, these methods have been used in a study of the protective effect of hypoxia against irradiation damage to these organs.

Methods. Male rats of the Sprague-Dawley strain, 3 to 4 months old with an average weight of 290 g were used. Hypoxia was produced by submitting the animals to a stimulated altitude of 25,000 feet, previously described(5). Irradiated animals were given 800 r of total body irradiation at the rate of 21 r per minute from a 220 kv Maximar

* This study supported by funds under contracts between the USAF School of Aviation Medicine, Randolph Field, Texas, and between the U. S. Atomic Energy Commission and Northwestern University, and by the Armour Laboratories.

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