THE EFFECT OF TEMPERATURE ON RADIOIODINE METABOLISM AND THYROID HORMONE BIOSYNTHESIS IN BROOK TROUT, SALVELINUS FONTINALIS (MITCHILL)

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Carolyn Diane Dorey

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ABSTRACT

Radioiodine metabolism was investigated following radioiodine (^{125}I) injection in yearling brook trout acclimated to 10 and 16 C.

At 16 C thyroid accumulation of ^{125}I, ^{125}I excretion, intrathyroidal conversion of radiotyrosines to radiothyronines, and build up of radiohormones in the serum were more rapid than at 10 C. These data support previous preliminary data that the thyroid gland of the brook trout is more active at higher temperatures.

Maximum uptake of radioiodide by the thyroid was 6.5% at 16 C and 4.8% at 10 C. Release of radiohormone as PB^{125}I was detectable at 10 days at 16 C and at 25 days at 10 C. This slow radioiodide turnover both at 10 and 16 C may be due to the high serum iodide (^{127}I) pool where values over 500 µg/100 ml of serum were obtained.

A comparison of radioiodide metabolism at 10 C between two groups of trout of different ages, sizes and tested at different seasons revealed many similarities in ^{125}I metabolism.

Thin layer radiochromatography of butanol extracts of serum suggested that T_3, T_4 and iodotyrosines may be present.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE PAGE

LIST OF TABLES

INTRODUCTION

Although temperature has been found to influence thyroid function in some fish, conflicting evidence exists as to whether a rise or fall in temperature increases or decreases thyroid activity. The type of relationship seems to depend on the species and the method (histological or radiochemical) used for measuring the thyroid activity (see literature review).

In an attempt to resolve the discrepancy between histological and radiochemical methods for assessing thyroid function, Drury and Eales (1968) compared histological (thyroid epithelial cell height) and radiochemical methods (metabolism of injected ^{125}I) of measuring thyroid activity in brook trout. Histologically, thyroid activity was found to decrease with increasing temperature, but thyroid uptake (% dose in thyroid) and T/S (Thyroid:Serum) ratio increased with increasing temperature. The disappearance rate of administered radioactive thyroxine from the serum was more rapid at higher temperatures and therefore tended to support the thyroid uptake data.

However, the methods used by Drury and Eales to assess thyroid function by following the metabolism of inorganic radioiodide (^{125}I) by the thyroid were somewhat limited in scope.

Firstly, at all temperatures the metabolism of ^{125}I by the thyroid of the brook trout was found to be extremely slow. The conversion of injected ^{125}I to protein-bound ^{125}I in the serum (PB^{125}I) was so slow that the conversion ratio could not be applied as a measure of thyroid activity. For similar reasons the rate of release of radio-

iodide from the thyroid could not be followed. At present there is a complete lack of knowledge on the influence of temperature on the biosynthesis and release of hormones from the thyroid into the serum in this species.

Secondly, the methods used by Drury and Eales to assess the uptake of radioiodide by the thyroid are open to criticism since they did not completely take into account the modifying effects of altered iodide excretion via extrathyroidal routes. They did not calculate rate constants for the thyroid uptake of radioiodide.

Thirdly, Drury and Eales ignored the possibility that alterations in the blood iodide pool at different temperatures might influence the data.

The main objective of this study was to determine the influence of temperature on iodide uptake, hormone biosynthesis and hormone release of the thyroid gland of the brook trout. Where possible rate constants for the 3 phases of radioiodine metabolism have been calculated.

The influence of temperature was assessed by comparing radioiodine metabolism in fish acclimated to 10 C with that in fish acclimated to 16 C. No attempt was made to follow thyroid activity during the process of acclimation. These acclimation temperatures were chosen since (i) they provided an appreciable temperature difference, (ii) they were on either side of the preferred temperature for the species and (iii) could be maintained throughout the year by the limited facilities available.

A preliminary attempt was also made to determine the nature of

the iodoamino acids released into the serum. There are few data available on this in fish (see literature review).

LITERATURE REVIEW

This review has focussed on four aspects of thyroid function in fish directly related to this study. These are:

A. Radiochemical methods for measuring thyroid activity,

B. Influence of temperature on fish thyroid activity,

C. Influence of stable iodide levels on radioiodine metabolism,

D. Iodoamino acids in thyroid and serum.

Where possible information has been reduced to tabular form.

A. Radiochemical Methods for Measuring Thyroid Activity

Numerous investigations of thyroid function in fish have been made using radioiodide. From these previous studies it can be inferred that the biosynthetic pathway of the thyroid hormones in a fish thyroid is similar to that in a mammal. Injected radioiodide (^{125}I, ^{131}I) is taken up by the thyroid tissue ("iodide pump") from the inorganic serum pool and incorporated into the thyroglobulin molecule of the follicular colloid. Iodide is oxidized and carbon-iodine bonds are generated in the tyrosine molecules of thyroglobulin to form iodotyrosines. Oxidative coupling of iodotyrosines within the thyroglobulin molecule results in the formation of iodothyronines which are released by proteolysis to the serum. Thyroid hormones released into the serum become bound to serum proteins, the identity of which is not positively established in fish (a globulin and a prealbumin, Leloup, 1961; a prealbumin in brook trout on acrylimide gel, Falkner, unpublished data).

Different measures of thyroid activity using radioiodine have been developed.

(a) Thyroid uptake which is the % uptake by the thyroid of the injected dose of radioiodide (Pickford, 1957; Hoar, 1959; Berg, Gorbman and Kobayashi, 1959; Hoar and Eales, 1963; Eales, 1964). The greater the % uptake the greater the iodide requirements of the gland are considered to be and the more active the gland.

(b) Thyroid:Serum (T/S) Ratio which is the ratio of radioiodide in the thyroid to that in the serum. (Hoar and Eales, 1963b; Eales, 1964, 1965).

(c) Thyroid clearance rate which is the rate of clearance of radioiodide from the thyroid (Hickman, 1959; Baggerman, 1960).

$$\text{Thyroid clearance} = \frac{\text{rate of } {}^{131}\text{I uptake during t min}}{\text{mean blood conc of } {}^{131}\text{I during t min}}$$

(d) Thyroid release of radioactivity (Swift, 1955, 1959) is calculated by measuring the loss of radioiodine from the thyroid at various times following radioiodide injection. The regression coefficient for the slope of iodine loss from the thyroid by time is taken as an index of thyroid activity. The greater the slope, the greater the release of hormonal iodide from the gland.

(e) Conversion ratio which is the extent of conversion of the administered inorganic radioiodine into protein-bound

(hormonally incorporated) radioiodine of the serum after a given time following radioiodide injection (Hickman, 1959, 1961; Eales, 1963; Baggerman, 1963; Hoar and Eales, 1963a; Eales, 1964, 1965). The CR value gives more discriminatory power than the uptake of radioiodine by the thyroid gland, since the relative secretion rate is being measured, rather than merely the iodide-trapping activity of the gland (Hickman, 1961).

B. Influence of Temperature on Fish Thyroid Activity

Conflicting results have been determined for the influence of temperature on thyroid activity in fish (Table I).

A seasonal correlation exists between water temperature and thyroid epithelial cell height. Variation, however, occurs in the correlation of temperature and thyroid activity depending on the species of fish used and on the method used to determine the activity. Histological techniques usually show an inverse relationship whereas radiochemical methods show a direct relationship with temperature.

C. Influence of Stable Iodide Levels on Radioiodide Metabolism

Hickman (1962) states that the concentration of stable iodine in the body of teleost fish depends on environmental iodine availability. An interaction between stable iodine in the environment and temperature exists in determining the serum iodide level (Hickman, 1962). In the eel, it has been speculated that the content of stable iodine of the serum decreases significantly at higher temperatures due to increased loss of the inorganic fraction at higher temperatures (Leloup and

TABLE I. Influence of Temperature on Fish Thyroid Activity

Species	Temp. Range	Effect on Thyroid Activity (T.A.)	Method	Author
Phoxinus phoxinus	3, 14, 26C	Seasonal correlation between water temperature and thyroid epithelial cell height	histological	Barrington and Matty, 1954
Cyprinus carpio *Tinca tinca* *Anguilla anguilla* *Mugil auratus* *Scyllium canicula*	5-10C 20C	No difference detected after two months	histological	Olivereau 1955b, c
Salmo gairdneri	9-12C 20C	increased T.A. at lower temp after 9-10 days	histological, thyroid uptake (^{131}I)	Olivereau, 1955a
Fundulus diaphanus *Umbra limi*	7.7-15C 15-22C	direct correlation between T.A. and temperature Inverse correlation between T.A. and temperature	thyroid uptake (^{131}I)	Berg, Gorbman and Kobayashi, 1959
Anguilla anguilla (normal & hypophysectomized)	6.5 - 25C	direct correlation between T.A. and temperature	thyroid uptake	Leloup and Fontaine, 1960
Salmo gairdneri Yearling	6-18C	Inverse correlation between T.A. and temperature	histological	Eales, 1964,
Juvenile	4-13C	direct correlation between T.A. and temperature	CR, T/S	1965

TABLE I (continued)

Species	Temp. Range	Effect on Thyroid Activity (T.A.)	Method	Author
Salvelinus fontinalis	5, 12, 15C	Inverse correlation between T.A. and temperature direct correlation between T.A. and temperature	histological radiochemical (CR, thyroid uptake) T_4 clearance rate	Drury and Eales, 1968
Mugil auratus Cyprinus carpio	10-18C	direct correlation between T.A. and temperature	% thyroid, CR (^{125}I)	Leray and Febvre, 1968

Fontaine, 1960).

Stable iodine accumulation by the thyroid depends on the availability of environmental iodine (Robertson and Chaney, 1953; Gorbman and Berg, 1955; Srivastava, 1960). In general marine forms which live in saltwater rich in iodine, accumulate less stable iodine in the thyroid (Gorbman et al, 1952; Leloup, 1952) whereas stable iodine accumulation is high in freshwater fish (LaRoche, 1950; Fontaine, Leloup and Olivereau, 1953; Berg and Gorbman, 1953). Radioiodine excretion in fish also depends on the availability of stable iodine in the environment. In iodine-rich water the excretion of iodine is faster than that in iodine-poor water (Srivastava, 1960) since the rich outside supply reduces the affinity for what is in the body.

It must be emphasized that the internal environment (i.e. serum stable iodide) is critical to the level of thyroid activity and radioiodine metabolism. This internal level is dependent on the fishes ability to bind iodide in the serum (Leloup and Fontaine, 1960) as well as the environmental iodine availability.

D. Iodoamino acids in Thyroid and Serum

On the basis of evidence to date the biosynthesis of thyroid hormones in lower vertebrates seems to follow the same pathways as in mammals (Fontaine, Leloup and Olivereau, 1953; Leloup, 1958). Pitt-Rivers and Rall (1961) examined the proportion of thyroid compounds in the rat thyroid as well as that of the blood. They found, using a method of isotopic equilibrium, that the thyroid contained 13% iodide of the total iodine, 20% monoiodotyrosine (MIT), 46% diiodotyrosine (DIT),

18% T_4 and 3% T_3. The results for the blood confirmed older views that T_4 made up the majority (80%) of the total blood iodine, while T_3 made up 3.5% and the iodotyrosines were not present in significant amounts (0-4%). Inorganic iodide made up the remaining 16.5%. The data suggest that about 40% of the total metabolic effect of thyroid hormone is due to T_3, that is, that T_4 and T_3 contribute approximately half each of the total physiological activity of the thyroid hormones in mammals.

A considerable amount of data has been published on the identity and proportions of iodoamino acids in the thyroid gland of fish, but there are few data on the identity and proportions of the iodoamino acids in the serum. Available information is summarized in Table II. The first quantitative record of serum iodocompounds was made by Leloup (1955), in Lampetra planeri, who injected 10 μCi ^{131}I. Serum and endostyle tissue were extracted and chromatography of the extracts carried out on paper. They found that T_4 and T_3 represented 22% of the serum radioactivity. The same method was used by Leloup (1956, 1958) and Tong, Kerkof, and Chaikoff (1961).

Chavin and Bouwman (1965), Jacoby and Hickman (1966), and Osborn and Simpson (1969) used thin-layer chromatography to separate serum and thyroid iodocompounds. Osborn and Simpson carried the method one step further by forming derivatives of the thyroid hormones.

Traces of iodotyrosines were found in the serum by Leloup (1958) in Periophthalmus koelreuteri and by Jacoby and Hickman (1966) in Salmo gairdneri.

TABLE II. Summary of Radioiodocompounds of Thyroid and Serum of Fishes. Values are Maximum for Compound Over Experimental Time. Part of Table Taken from Berg, Gorbman and Kobayashi (1959) Table I.

	Thyroid Compounds (% of total radioactivity in the gland)					Serum Compounds (% of total radioactivity in the Serum)			
Species	MIT	DIT	T_4	T_3	?	T_4	T_3	Iodotyrosines	References
Salmo salar L. Atlantic salmon	+	+	+						LaRoche, 1950
Scyllium (Scyliorhinus) canicula-shark	+	+	53	?					Gorbman et al, 1952
Conger conger L conger -eel	61% together		29						Leloup, 1952
Mugil auratus L. mullet	64% together		33						Leloup, 1952
Anguilla anguilla hypophysectomized eel	+	+	17						Fontaine, Leloup; Olivereau, 1953
Anquilla anquilla normal eel	71% together		25						Fontaine, Leloup, Olivereau, 1953
Xiphophorus maculatus platyfish	40	45	15						Berg, Gorbman, 1953

TABLE II (Continued)

Species	Thyroid Compounds (% of total radioactivity in the gland) MIT	DIT	T_4	T_3	?	Serum Compounds (% of total radioactivity in the Serum) T_4	T_3	Iodotyrosines	References
Carassius auratus goldfish	+	+	8						Berg, Gorbman, 1954
Carassius auratus goldfish	40	60							Berg, Gorbman, 1954
Petromyzon marinus	24	34	18						Leloup, Berg, 1954
Lampetra planeri	21-33	55-58	10-22	?					Leloup, Berg, 1954
Fundulus heteroclitus	30% together		70						Gorbman, Clements, O'Brien, 1954
Fundulus diaphanus	50	10	20						Gorbman, Clements, O'Brien, 1954
Lampetra planeri lamprey	+	+	+	+		22% together			Leloup, 1955
Periophthalmus koelreuteri	35-50	35-51	2-4% of which 1/3 is T_3			26% (26-58% of total being T_3)		1.5(MIT)	Leloup, 1956
Protopterus annectens lungfish	80-90% together		10% together			27-61%			Leloup, 1958
Umbra limi mud minnow	+	+	+	+		+	+		Berg, Gorbman, Kobayashi, 1959

TABLE II (Continued)

Species	Thyroid Compounds (% of total radioactivity in the gland) MIT	DIT	T_4	T_3	?	Serum Compounds (% of total radioactivity in the Serum) T_4	T_3	Iodotyrosines	References
Eptatretus stoutii hagfish	22-47	13-35	0.7-2.2			<10			Tong, Kerkof, Chaikoff, 1961
Carassius auratus goldfish	+	23	56-62			+			Chavin, Bouwman, 1965
Salmo gairdneri rainbow trout	40-45% together		50-55% together		30-35	1.2	1.5	1-1.5	Jacoby, Hickman, 1966
Pleuronectes platessa L. plaice	+	+	+	+		3% together			Osborn, Simpson, 1969
Umbra limi	30	30	40						Berg, Gorbman, unpublished
Umbra limi	60	20		5					Berg, Gorbman, unpublished
Umbra pygmaeus	30	20							Berg, Gorbman, unpublished
Percina caprodes	15	50	10						Berg, Gorbman, unpublished
Notropis deliciosus	65	25		5					Berg, Gorbman, unpublished

+ represents presence, % unknown

The mud minnow (Umbra limi) showed some differences not observed in other fishes (Berg, Gorbman, and Kobayashi, 1959). Variations were noted in the thyroid hormones synthesized that depended on the area where the fish were caught. Michigan Umbra synthesized only T_3, Minnesota Umbra synthesized both T_3 and T_4, and Wisconsin Umbra only T_4 at low temperatures. The difference seems to be due to the amount of stable iodine in the water and the varying temperature (Leloup and Lachiver, 1955).

MATERIALS AND METHODS

A. Living Material

Brook trout from a common brood stock (Ontario Department of Lands and Forests, Dorion Hatchery brood stock) were held in a large outdoor tank at the Province of Manitoba Trout Hatchery, West Hawk Lake, Manitoba. Fish when needed, were taken from this stock and transported to the laboratory where they were held in a 560-liter fiberglass tank with continuously running dechlorinated tap water.

Seasonal changes in the water temperature of the holding tank are shown (Fig 1). Photoperiod was not controlled but approximated that in nature. At intervals fish were removed from this laboratory stock to 200-liter experimental tanks and acclimated to either 10 or 16 C. Acclimation took place over a period of at least 2 weeks. Brett (1946) states that up to 20 days or more are needed for acclimation in the region of 10 C. For the initial experiments (May, 1968) the fish ranged in size from 5.5 to 25.2 g and by the concluding experiments (March to April, 1969) ranged in size from 71.0 to 211.0 g.

Feeding of the stock fish was not rigorously controlled. It is estimated that each fish received approximately 2 g of

food/week, with total iodide content of 6.6 µg/100 g of food. The food consisted of a frozen homogenate of beef liver and ocean perch fillets (10:1 by weight) to which gelatin was added to hold the mixture together in the water.

Periodic outbreaks of fungus were cured by adding malachite green (1:15,000) each day to the tank and letting it slowly disperse with the turnover of water in the tank. Earlier attempts were made to eliminate fungus with methylene blue (1:1,000). Daily treatment checked, but did not eliminate, the fungus. Daily dipping of the fish for 5 minutes in 3% NaCl was also partially successful.

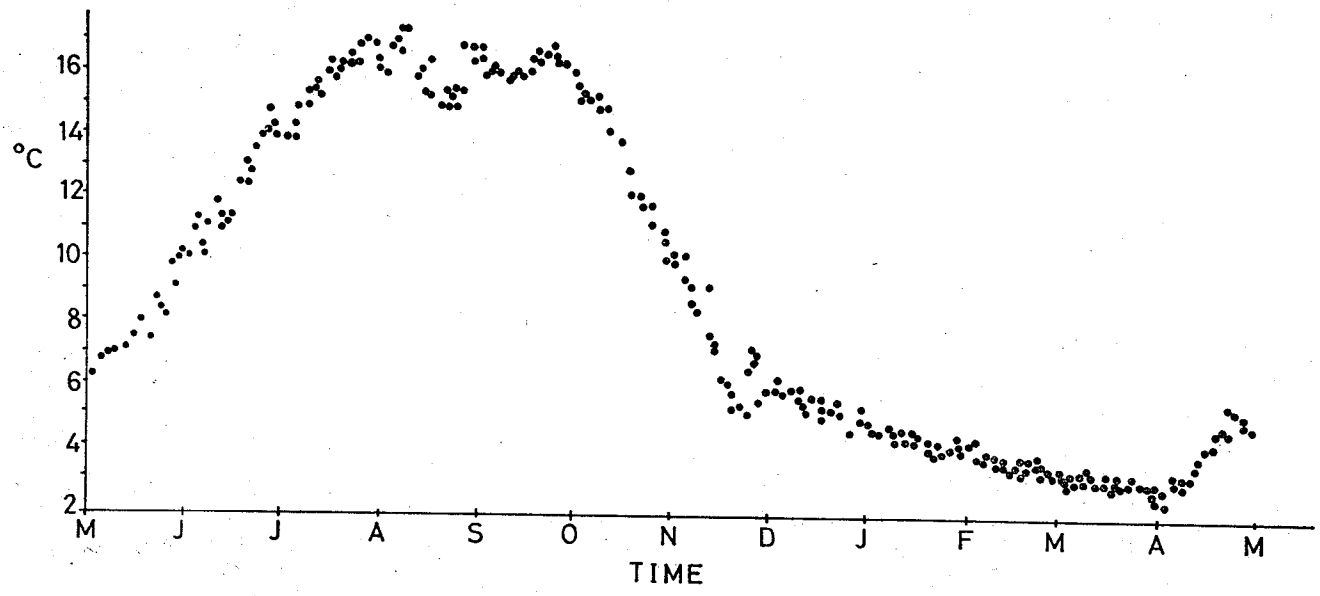
B. Injection of ^{125}I

Radioiodide, ^{125}I (Atomic Energy of Canada Limited, Commercial Products, Ottawa, Canada) was diluted with distilled water and 0.1 ml injected intraperitoneally. The fish were netted from the holding tank, injected through the fine-cloth mesh and released into the experimental tank.

Standards were prepared by injecting into a 100 ml flask a dose indentical to that injected into fish, and then diluting it to 100 ml with distilled water. Four ml of this represented 4% of the injected dose.

C. Collection of Blood and Thyroid Samples

Fish were anaesthetized with MS-222 (tricaine methane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sulphonate); weighed to the nearest 0.1 g, and measured to the nearest 0.1 cm. The caudal artery was severed by cutting off the tail. The small fish used in the early studies were bled into heparinized hematocrit capillary tubes and the blood centrifuged to separate the serum (International Microcapillary Centrifuge, Model MB) at 10,000 x g. The larger fish were bled into heparinized glass dishes, the blood pipetted into 1-ml plastic centrifuge tubes and centrifuged. The serum was used immediately or frozen for later use.

The thyroid region, between the first and third gill arches of the lower jaw, as mapped by Drury (1967), was cut out, counted and frozen at -20 C for analysis at a later time.

D. Separation of Protein-bound Radioiodide ($PB^{125}I$) and Inorganic Radioiodide ($I^{125}I$) of the Serum

Separation of $PB^{125}I$ from $I^{125}I$ in the serum was achieved by trichloroacetic acid (TCA) precipitation (Fig 2). In certain experiments listed below, variations of this method were used. They are not believed to have altered the estimates of $PB^{125}I$ and $I^{125}I$ significantly.

1. One instead of two washes with 4 ml 2.5% TCA was used (experiments from July to September, 1968 carried out on fish held at 10 and 16 C).
2. Serum was added to 0.5 ml 12.5% TCA in a 1-ml plastic centrifuge tube, centrifuged (10,000 x g, 10 min) and washed once with 0.5 ml of 2.5% TCA. The precipitate was dissolved in 1 ml of 4 N NaOH. $PB^{125}I$ and $I^{125}I$ fractions were diluted to 4 ml volume with 3 ml distilled water and counted