THE RELATIONSHIP BETWEEN THYROID FUNCTION AND SKIN PURINE LEVELS IN THE BROOK TROUT, SALVELINUS FONTINALIS (MITCHILL)

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by

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ABSTRACT

The thyroid function and skin purine (guanine and hypoxanthine) levels of brook trout, Salvelinus fontinalis were investigated under four treatments: (i) thiourea, (ii) thyroxine, (iii) thyroid powder, and (iv) thyrotropic hormone (TSH). The methods used were chromatographic, spectrophotometric and radiochemical. The main objective of the study was to find out the relationship between thyroid function and skin purine levels of brook trout.

The skin purine was extracted and then analysed qualitatively and quantitatively using descending paper chromatography and ultraviolet spectrophotometry. No alteration in guanine and hypoxanthine levels was observed after thiourea and thyroxine treatments at 10° C for 21 days. But the purine levels were increased after thyroid powder and TSH treatments at 15° C for 35 days. Thyrotropic hormone was found to be more effective than thyroid powder in increasing purine deposition.

When radioiodide ($Na^{125}I$) was injected intraperitoneally, it was taken up into the blood and then absorbed by the thyroid tissue. The radioactivity in the thyroid relative to the inorganic radioactivity in the serum (thyroid : serum = T/S) and the % of radioiodide uptake (% thyroid) were used as indices of thyroid activity. The conversion ratio was also tested in the thyroid powder-treated group and the TSH-treated group. On the basis of these methods, thyroid activity was found to be suppressed after thiourea, thyroxine and thyroid powder treatments but stimulated after TSH treatment.

An ambient concentration of thiourea solution as low as 0.0001 %

was found to inhibit the thyroid as effectively as a 0.01 % thiourea solution.

TABLE OF CONTENTS

CONCLUSIONS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The presence of guanine in the silvery layers of fish skin was accepted by Peschen (1939), Sumner (1944), Hitchings and Falco (1944), and Robertson (1948), but due to the lack of discriminating qualitative analytical methods, the identification of guanine and the presence of other purines in the fish skin were by no means certain. However, Ziegler-Gunder (1956 a, b), using chromatographic and spectrophotometric methods found, besides guanine, small amounts of hypoxanthine present in the skin of European chub, Squalius cephalus and the bleak, Alburnus lucidus. Guanine and significant levels of hypoxanthine have since been demonstrated in the skin layers of several fish including the brook trout, Salvelinus fontinalis (Eales, 1969).

During the parr-smolt transformation prior to marine life in juvenile salmon, there is, in addition to increased silvering due to dermal purine deposition, an increase in activity of the thyroid gland as observed by Hoar (1939), Robertson (1948), and Baggerman (1960). Silveriness in certain salmonids can also be induced by the administration of thyroid or pituitary extracts to the fish (Landgrebe, 1941; Robertson, 1949; Hoar et al., 1951; Smith, 1956; Massey and Smith, 1968). Since silveriness and increased thyroid activity were frequently found accompanying each other in the salmonid species (Robertson, 1948) it is possible that the thyroid hormones are in part responsible for this colour transformation. However, earlier workers usually judged the degree of silveriness subjectively by the optical appearance of the fish or by histological methods using a microscope. Discriminating qualitative and

quantitative methods of measuring the purines in the skin objectively have usually not been used in these studies.

The influence of thyroid hormones on the deposition of purines in the skin of brook trout, Salvelinus fontinalis has not been mentioned in the literature.

The main objectives of the present study were to attempt to establish the relationship between thyroid function and skin purine levels (guanine and hypoxanthine) in the brook trout using objective methods to measure both thyroid activity and skin purine levels. The experimental approaches were to (i) inhibit thyroid activity with thiourea, (ii) stimulate thyroid activity with thyroid stimulating hormone (TSH) and (iii) to increase circulating thyroid hormone levels by administration of exogenous hormones. Following such prolonged treatments dermal purine levels were compared to those of controls using qualitative and quantitative methods adopted from Johnston and Eales (1967). Conclusions were drawn concerning the influence of thyroid function on skin purine levels.

LITERATURE REVIEW

A. Historical background of silvery coloration in fish

Silveriness in fish skin has usually been attributed to deposition of guanine crystals. Peschen (1939) and Sumner (1944) are some of the early works who isolated silvery substance, which they claimed to be the purine guanine, from the skin extract of fish. Peschen (1939) also reported that the percentages of purine for the scales of different species of fish are different and that there is more purine in the skin than in the scales. Hitchings and Falco (1944) used guanase to identify guanine. Robertson (1948) prepared purine crystals which he claimed to be guanine from the skin strips of rainbow trout under a dissecting microscope. However, Ziegler-Gunder (1956 a, b), using more discriminating chromatographic and spectrophotometric methods, managed to identify not only guanine but also hypoxanthine in the skin of European chub, Squalius cephalus and the bleak, Alburnus lucidus. Markert and Vanstone (1966), in addition to guanine and hypoxanthine, found guanylic acid in the belly skin of parr and smolt salmon (Oncorhynchus kisutch). Johnston and Eales (1967) found guanine and appreciable amounts of hypoxanthine in the skin and scales of parr and smolt of Atlantic salmon (Salmo salar). Massey and Smith (1968) estimated the guanine content of the skin of trout (S. trutta fario L.) from the level of Folin-Ciocalteau positive material which on the basis of chromatographic separation they believed to be largely guanine. Hayashi and Saito (1968), using anion-exchange chromatography, found acid-soluble nucleotides and related compounds, hypoxanthine and small amounts of uridylic acid, cytidylic acid and inosinic acid, in the

skin of carp, rainbow trout and chum salmon. Eales (1969) from a study on channel catfish (Ictalurus punctatus), burbot (Lota lota), brook stickleback (Culaea inconstans), pike (Esox lucius), walleye (Stizostedion vitreum), sauger (S. canadense), goldfish (Carassius auratus), trout perch (Percopsis omiscomayus) and brook trout (Salvelinus fontinalis) concluded that both guanine and hypoxanthine probably contributed to the silveriness of the skin of fish generally.

B. Evidence for thyroid influence on fish silvering

(i) Observations during smoltification

Some species of teleost fish undergo a striking external transformation at a certain stage in their life cycle. This transformation, smoltification, comprises a distinct loss of dark skin pigments and replacement with a brillant silveriness.

Landgrebe (1941), Robertson (1949), and LaRoche and Leblond (1952) report that the phenomenon of silveriness in the skin of salmonid fish is due to the increased deposition of guanine crystals in the guanophores (iridophores or leucophores). This heavy layer of guanine obscures the melanophores in the skin (Robertson, 1948) thus making the fish look more silvery than in the parr stage.

Besides the changes in the skin colour, another physiological change in the fish that frequently accompanies the parr-smolt transformation is the increase in the activity of the thyroid gland. Some workers like Hoar (1939) made significant investigations in this field and found that at the time of transformation from the parr to the smolt stage in the Atlantic salmon, there is a great increase in the thyroid activity

judged histologically. Baggerman (1960) found that the increase of thyroid activity actually occurs before the loss of the parr marks and the other characteristics of smolt phase in the Pacific salmon. Robertson (1948) found that the changes occurring in the transformation of rainbow trout parr to smolt are closely related to the increased thyroid activity. Pickford and Atz (1957) reported that during the period of smoltification, increased thyroid activity was correlated with degranulation of the basophil cells of the adenohypophysis, indicating hypersecretion of thyrotropin.

(ii) Thyroid and guanine deposition

Numerous authors have observed the silvering in the skin, especially in salmonids, that results from thyroxine treatment. Landgrebe (1941) induced silvering in parr of Salmo salar either with thyroid or pituitary extracts of mammals. Robertson (1948) also induced silvering in parr of S. gairdnerii by the administration of thyroid or pituitary extracts. LaRoche, Leblond and Prefontaine (1950), LaRoche and Leblond (1952), and LaRoche (1953) have shown that treatment with thyroid hormone elicits changes resembling those of smoltification, such as pallor due to a moderate decrease in number of pigment cells, increased opacity of skin and pronounced thickening of the epidermis. Smith has been successful in inducing silvering in S. trutta with purified TSH though Landgrebe (1941) failed to do so but the failure of the pituitary preparation can probably be attributed to an insufficient dose.

Ball et al. (1963) while studying pituitary transplants in the teleost, Mollienesia formosa found that in the hypophysectomized group with reduced thyroid activity due to lack of TSH, the skin was dull and

poorly reflecting, due presumably to loss of guanine, and that the dull skin was restored to the normal silvery state by treatment with thyroxine. It seems clear that thyroxine may therefore affect integumentary guanine metabolism, and some evidence to this effect was furnished recently by Matty and Sheltawy (1967) who report that prolonged thyroxine treatment increases the guanine content of teleost skin and also increases incorporation of ^{14}C-labelled glycine into newly synthesized guanine. However in most cases the estimations of guanine were done subjectively by the optical appearance of the fish skin or by histological methods using a microscope.

The effect of thyroxine on skin silvering is a good indication of a direct relationship between this hormone and guanine deposition in the skin of some salmonids and in eels (Landgrebe, 1941; Robertson, 1948). Although observations show that thyroid hormone may stimulate guanine production, it does not specifically control this phase of fish metabolism (Hoar and Bell, 1950; Hoar et al., 1951) because Landgrebe (1941) was unable to produce a premature silvering in the yellow eel (Anguilla vulgaris), which under natural conditions becomes silvery before returning to sea, and chum salmon (Oncorhynchus keta) which becomes silvery before migration without the evidence of thyroid hyperactivity (Hoar and Bell, 1950).

(iii) Thyroid and nitrogen metabolism

The origin of the intracellular guanine crystals in the fish is not yet clearly known (Premdas, 1967, unpublished) but guanine is liberated as one of the products in the natural degradation of

nucleic acid (Keilin, 1959). Therefore, there is the possibility that the fish thyroid controls some aspect of nitrogen metabolism probably involving nucleic acid turnover, The metabolism of the purines is shown in Figure 1.

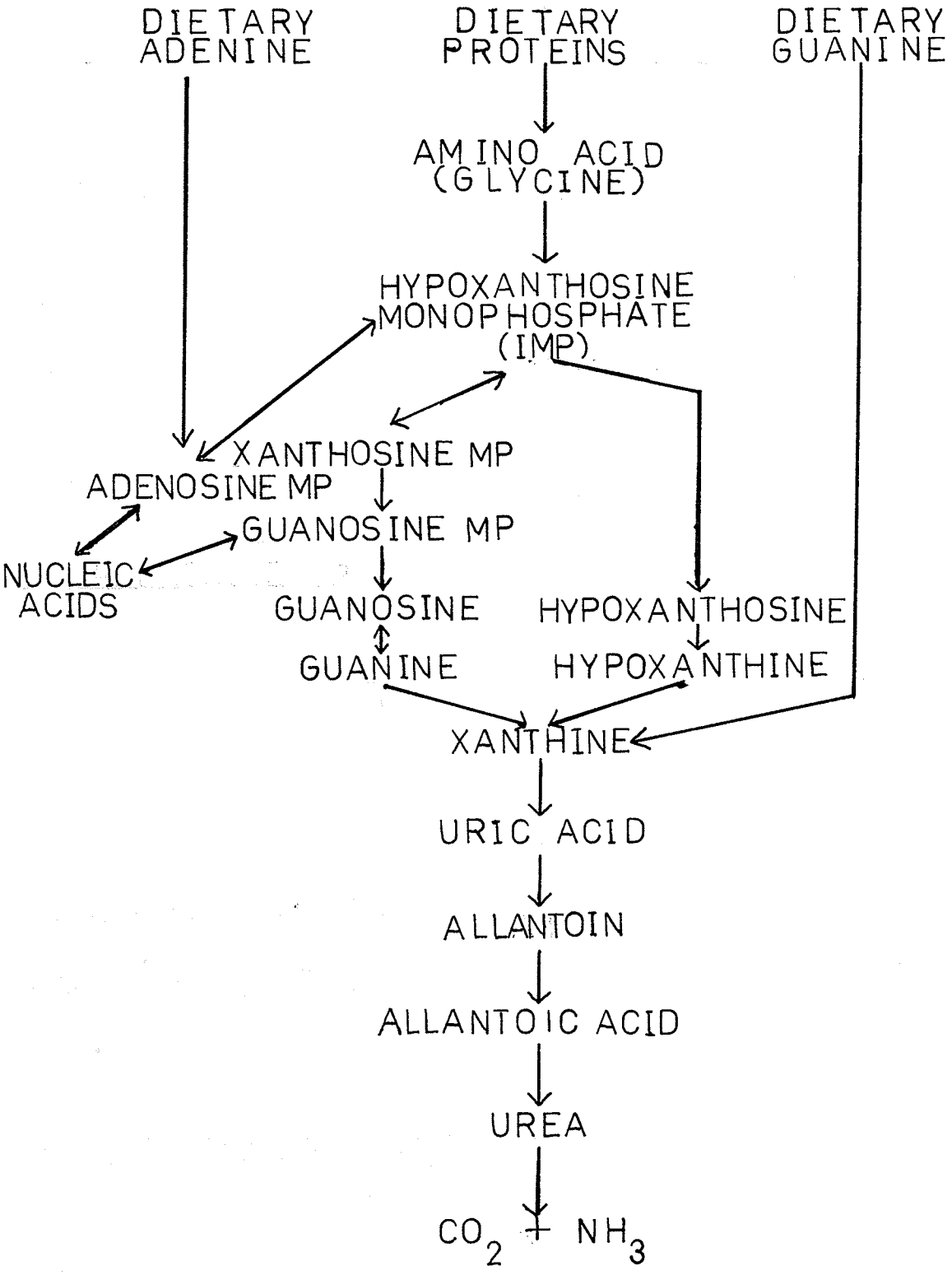
Hoar (1958) claimed that increased ammonia excretion by goldfish is another metabolic function of thyroxine, and he observed a 100% increase in ammonia excretion in goldfish within one week of immersion in thyroxine. Thornburn and Matty (1963) have shown that thyroxine affects nitrogen metabolism in the brown trout as well as in goldfish and it stimulates incorporation of ^{14}C-labelled leucine into protein. They also found an increase in amino acid nitrogen in the muscle and liver of these fish.

There is ample evidence to show that the growth and development of the teleosts are closely related to the activity of the thyroid gland (Hoar et al., 1951). The effects of thyroidal treatment on growth (Pickford and Atz, 1957) are some of the observations to indicate a direct relationship of hormone and nitrogen metabolism. Hoar (1958) concluded that the thyroid hormone stimulates some aspect of protein metabolism from the amino acid level upwards and the action of the hormone is paralleled in mammals and the goldfish.

C. Influence of thiourea on fish thyroid activity

In most teleosts, the thyroid gland consists of numerous isolated units, the follicles which are scattered diffusely over a comparatively wide region (Hoar, 1939). Therefore surgical thyroidectomy is impossible and 'chemical thyroidectomy' is often used to study the

FIGURE 1. Metabolism of purines


DIETARY ADENINE
DIETARY PROTEINS
DIETARY GUANINE
AMINO ACID (GLYCINE)
HYPOXANTHOSINE MONOPHOSPHATE (IMP)
XANTHOSINE MP
ADENOSINE MP
GUANOSINE MP
NUCLEIC ACIDS
GUANOSINE
HYPOXANTHOSINE
GUANINE
HYPOXANTHINE
XANTHINE
URIC ACID
ALLANTOIN
ALLANTOIC ACID
UREA
CO_2 + NH_3

effects on thyroid insufficiency. A commonly used drug is thiourea. Administration of thiourea to higher vertebrates induces some metabolic changes and also external symptoms similar to those attending the removal of the thyroid gland (Charipper and Gordon, 1947). Most of the thiourea compounds are more active when administered orally than when injected in the mammals (Astwood, 1944-5) and their effectiveness also depends on the individuals. Charipper and Gordon (1947) found that older rats had better tolerance to the toxic effects of thiourea than younger ones and also that compounds differing even slightly in their chemical composition will operate in different ways to produce their antithyroid action. Scott (1953), in a study using zebra fish, found that thiourea has a more pronounced effect on the younger fish which were six weeks old than on the older ones.

Astwood (1955) suggested that the action of thiourea on thyroid is the prevention of iodination of tyrosine possibly due to interference with the enzymatic oxidation of iodide to iodine and the enzyme responsible for this oxidation is the peroxidase. Chambers(1953) found that treatment of normal males of Fundulus heteroclitus with thiourea injections at a subtoxic dose had a profound effect on the liver besides hypertrophy and hyperplasia of thyroid. Charipper and Gordon (1947). Pickford and Atz (1957), and Goodman and Gilman (1956) also state that thiourea can affect tissues other than the thyroid gland itself. Massey and Smith (1968) in a study of mitochondrial respiration in the trout reported that thiourea or other antithyroid drugs are known to produce side-effects which might affect metabolism. The histology

of the thiourea-treated group indicates that the pituitary-thyroid axis was being altered and that the concentration (0.10%) used by Massey and Smith (1968) was very high. Therefore the extrathyroidal effect must be taken into consideration in experiments using high doses of thiourea. Concentrations from 0.01% to 0.02% of thiourea in solution are enough to cause hypertrophy and hyperplasia in the thyroid of Lebistes reticulatus (Lever et al., 1949) and Gambusia affinis (Buser-Lahaye, 1953).

The thiourea concentration Baggerman (1960 and 1963) used for her experiments on salmon was 0.03 % and after five days of treatment at this concentration she was able to observe suppression of thyroid activity. The widely used effective dose of thiourea by research workers is 0.03 % or 0.0033%. Kinnear (1960) performed experiments to find out the minimum dose of thiourea affecting starry flounder thyroxine synthesis and he found that the range between 0.0025 % and 0.03% effectively inhibits thyroid hormone synthesis though there was some production of monoiodotyrosine and diiodotyrosine. He also found that concentrations from 0.001 % to 0.0001 % did not inhibit thyroid hormone biosynthesis in flounder. This is the only case where a lower limit has been established.

Higher doses of thiourea in solution such as 0.10 % (Lever et al., 1949; Olivereau, 1952a and 1954; Barrington and Matty, 1952; Fortune, 1956; Massey and Smith, 1968), 0.33 % (Scott, 1953), 0.36 % (Hoar and Bell, 1950; Hoar et al., 1951) and 0.60 % (Lever et al., 1949) have also been used, but Lever et al. (1949) reported that 0.60 % of thiourea in solution is toxic to Lebistes reticulatus. Fortune (1953, 1955, and 1956) in her studies on Phoxinus phoximus and Lebistes

reticulatus found that using 0.05 % solution the histological responses of thyroid gland takes place more rapidly at higher temperature (25° C to 30° C) than at lower temperature (15° C to 20° C).

Table 1 gives a summary of some of the work done on fish using thiourea as the antithyroid agent.

D. Influence of thyroid hormones on fish thyroid activity

Treatment with thyroid hormone results in an inhibition of autogenous thyroid function (Pickford and Atz, 1957). This effect is mediated primarily through an inhibition of the release of pituitary thyrotropin, the well-known homeostatic mechanism of Aron et al. (1931). However, in the absence of the pituitary there appears to be a residual regulation depending upon an inhibition of the iodide-concentrating mechanism of the thyroid gland itself (Vander Laan, 1955). In mammals the relationship between thyroid hormone production and thyrotropin activity has been extensively investigated, a normal or high level of thyrotrophic activity seems to depend on stimulation from the hypothalamus and requires an intact hypothalamo-pituitary link (Szentagothai et al., 1962). A reduction of thyrotropic activity can largely be attributed to the inhibitory action of thyroxine acting both on the hypothalamus and also directly at the pituitary level (Von Euler and Holmgren, 1956 a, b; Yamada and Greer, 1959; Bogdanove and Crabill, 1961). Normal regulation of thyroid function is controlled by the pituitary which responds to changing levels of circulating thyroxine in some mechanism which is yet imperfectly understood (Pickford and Atz, 1957).

TABLE I. Influence of thiourea on silvering and thyroid activity in fish.

Authors & dates	Species	Treatments	Results
1) Goldsmith *et al.* (1944)	*Xiphophorus maculatus*	0.033 % solution for 73 days room temperature (?)	after 49 days: hypertrophy, hyperplasia, increase # of follicles with scanty colloid. growth retardation.
2) Nigrelli *et al.* (1946)	*Lebistes reticulatus*	0.03 % solution for 3 months	thyroid: hyperplastic.
3) Evropeitzeva (1949)	*Coregonus lavaretus ludoga* 6 days old fry	0.033 % solution for 17 days	increase follicular cell height.
4) Lever *et al.* (1949)	L. *reticulatus*	thiourea in: 0.01 % 0.10 % 0.60 % for 7 to 35 days at 24° C	0.60 % is toxic. increase follicle cell height after 1 week; decrease in follicle size; thyroid hypertrophy and hyperplasia.
5) Rasquin (1949)	*Astyanax mexicanus*	0.033 % solution for 4 to 12 weeks at room temperature	increase follicle cell height.
6) Hoar & Bell (1950)	*Oncorhynchus keta*	0.36 % solution for 21 days at 10° C	silvering is not pronounced, no parr marks.
7) Hoar *et al.* (1951)	O. *keta*	0.36 % solution for 21 days at 10° C	thyroid: hyperplastic.

TABLE I (continued)

8)	Barrington and Matty (1952)	Phoxinus phoxinus	0.10 % solution for several months at 10° C	1st month--little change in thyroid 2nd month--increase thyroid cell height.
9)	Gaiser (1952)	L. reticulatus	0.03 % solution at 22° C	thyroid:hyperplastic.
10)	Lelpup (1952)	Mugil auratus	1 g/l for 8, 11, 14, & 21 days	uptake of ^{131}I reduced after 24 hr. synthesis of organic radioiodide was severly impaired and no thyroxine was formed.
11)	Olivereau (1952a & 1954)	M. auratus	0.10 % solution for 12 hr to 31 days. work done in 1949, 1950, & 1951 at 14-15° C or 19-21° C	1949 & 1950 : increase cell height and activity of thyroid after 5-7 days. 1951 : hypertrophy onset after 17-28 days.
	Olivereau (1952b & 1954)	Conger conger	1 g/l for 10-33 days at 19-21° C	histo-radioautographs showed total inhibition of fixation of radioiodide
12)	Vivien & Gaiser (1952)	L. reticulatus	0.03 % solution for 48-600 hr.	histo-radioautographs showed suppression of radioiodide uptake completely.
13)	Atz (1953)	A. mexicanus	0.033 % solution for 4 weeks at room temperature	increase height of follicle cell in thyroid.
14)	Buser-Lahaye (1953)	Gambusia affinis	0.20 g/l or 0.02 % solution	sections of thyroid gland showed false appearance of activity, hypertrophy and hyperplasia.

TABLE I (continued)

15)	Chambers (1953)	Fundulas heterochitus	intraperitoneal injection 0.5 g/g wt. twice/wk fish died after 4 wks. 0.25 g/g wt. for 2 wks at 15-18.5° C, fish survived	thyroid: hypertrophy hyperplasia hyperemia
16)	Fortune (1953 & 1955)	P. phoxinus	0.05 % solution at: 3° C, 15° C, 20° C, 25° C.	increase cell height and decrease colloid changes take place more rapidly at high temperature. max. effect: 25° C 7-10 days 20° C 10-14 days 15° C 28 days 3° C 5 weeks
17)	Scott (1953)	Brachydanio rerio	0.33 % solution for 14 or 16 weeks at 76-78° F.	thyroid: hyperlpasia hypertrophy hyperemia.
18)	Berg et al. (1954)	Xiphophorus variatus	300µg/cc solution for 9-14 days	induced growth of small thyroid tumors.
19)	Honma & Murakawa (1955)	O. keta	1/3,000 solution for 6 wks.	hypertrophy and decrease size of follicles which are lack of colloid.
20)	Fortune (1956)	L. reticulatus	0.05 % solution at 20° C, 25° C, 30° C.	increase cell height and decrease colloid. max. effect: 30° C 7-10days 25° C 10-14days 20° C 17-21days
		Carassius auratus	0.10 % solution for 5 weeks at 30° C.	no effect.

TABLE I. (continued)

21)	Kinnear (1960)	*P. stellatus*	0.0025 % to 0.03 %	hormone synthesis inhibited
			0.001 % to 0.0001 %	not effective.
22)	Baggerman (1960)	*O. kisutch*	0.036 % at 10-12° C	less radioiodine accumulation less radioactive hormone secretion.
23)	Hickman (1961)	*P. stellatus*	0.04 % solution	decrease thyroid activity & conversion ratio
24)	Baggerman (1963)	*O. gorbuscha*	0.10 % solution	decrease thyroid activity.
25)	Massey & Smith (1968)	*S. trutta fario L.*	0.10 % solution	silvery substance in skin was increased slightly and mitochondrial enzyme activity decreased but phosphorylation increased.

In the teleost the circulating levels of thyroid hormones undoubtedly influence thyrotropin release from the hypothalamus by a feedback mechanism as in the higher vertebrates (Ball et al., 1963; Fontaine and Leloup, 1964). Treatment with thyroxine or thyroid preparation causes a decrease in thyroid function, as reflected in the histological picture (Rasquin, 1949; Robertson, 1949; Hoar et al., 1951; Fontaine and Wurtz-Arlet, 1952; Gaiser, 1952; Hopper, 1952; Honma and Murakawa, 1955; Massey and Smith, 1968) and in the diminished ability to fix radioactive iodine (LaRoche, 1950; Vivien and Gaiser, 1952; Berg and Gorbman, 1953; M. Fontaine, Baraduc and Y. A. Fontaine, 1955; Baggerman, 1960). Studies by Chavin (1956)on the goldfish and studies by Baggerman (1960) on O. kisutch suggest that thyroxine has a direct inhibitory action on thyroidal iodine metabolism.

Baker (1965 and 1969) studied S. gairdnerii and Anguilla anguilla, and Sage (1967) studied Poecilia reticulata; both concluded that thyroxine acts directly on the pituitary, inhibiting both TSH release and TSH synthesis rather than an increased storage of TSH in the pituitary. Gorbman et al. (1964) reported that artificial administration of thyroid hormone to goldfish modifies cerebral electrogenesis acting not only on the hypothalamus but also on higher structural levels of the central nervous system.

Table II gives a summary of some of the work done on fish using thyroid hormones.

E. Influence of TSH on thyroid activity in fish

In all classes of vertebrates the thyrotropic hormone causes

TABLE II. Influence of thyroid hormones on silvering and thyroid activity in fish.

	Authors & dates	Species	Treatments	Results
1)	Landgrebe (1941)	*S. salar*	fed ox thyroid extract≡ to 0.5 g fresh tissue twice/wk.	1 month--silvering shown 2 months-more silvering and no parr marks.
2)	Robertson (1949)	*S. gairdnerii*	thyroxine injection 0.1 mg to 0.5 mg for 6 weeks	no increase in silvering deposition thyroid abnormally quiescent, B.M.R. normal, no local pallor, and no exophthalmos.
			thyroid powder injection 2 % in solution	3 weeks--silvery tint shown 7 weeks--look like silvery trout with dense mat of silver, thyroid showed hyperactivity, contraction of melanophore.
3)	Hoar & Bell (1950)	*O. keta*	0.36 % thyroxine	increase growth rate.
4)	Hoar *et al.* (1951)	*S. salar*	fed compressed thyroid	silvering increased but not pronounced; thyroid activity increased.
		S. fontinalis	fed compressed thyroid	no silvering but thyroid activity increased.
		O. kisutch fingerlings	fed desiccated thyroid gland	silvering increased but not pronounced; thyroid activity increased.
			immersion in thyroxine (1:1,125,000)	decrease in pigmentation.

TABLE II (continued)

		O. kisutch fry	immersion in thyroxine (1:2,000,000)	silvering more pronounced no parr marks.
		O. kisutch alevius	immersion in thyroxine (1:1,000,000)	silvering not pronounced no parr marks.
5)	LaRoche & Leblond (1952)	S. salar	sodium thyroxinate 200 µg in 0.05 cc injection thyroid extract fed with 50 % beef liver beef thyroid fed with 25 % beef liver	integumentry pallor, reduced radioiodine fixation, increase in skin thickness and retardtion on growth.
6)	Atz (1953)	A. mexicanus	fed ½ grain of desiccated thyroid (Burroughs Wellcome tablets)	depresses height of epithelium of thyroid follicle.
7)	Baggerman (1960)	O. kisutch	thyroxine solution ($1:10^6$)	reduced radioiodine uptake.
8)	Barrington et al. (1961)	S. gairdnerii	thyroxine 2 mg/l	growth rate increased in 2 weeks.
9)	Sage (1967)	P. reticulata	1/2,000,000 in water for 4 weeks	no changes occur in TSH producing cells and thyroid follicle cells judged histologically, increase in growth.
10)	Massey & Smith (1968)	S. trutta fario L.	thyroxine 3.75 µM	increase in growth, increase in skin silvery substance.

growth and hyperplasia of thyroid tissue. Thyroid functions such as iodide accumulation, hormonal synthesis and release are stimulated by TSH (Gorbman and Bern, 1962). For the teleost fish, there is an abundance of evidence which indicates that the pars distalis contains a thyroid stimulating hormone and that surgical removal of this gland leads to involutional changes in the thyroid and reduction of its functions (Fontaine et al., 1953; Pickford, 1959).

In mammals the rate at which the thyroid clears iodide from the blood is under some circumstances dependent on TSH and, under the influence of the latter there is a rise in protein-bound-iodide in blood following discharge of iodine from the thyroid (Condliffe and Robbins, 1967). Hickman (1961) in a study of the flounder concluded that in the blood, the most sensitive indicator of TSH action appears to be the ratio of protein-bound-iodide to iodine, or the ' conversion ratio' which can easily indicate the state of the thyroid in fish.

Injection of mammalian anterior lobe pituitary extract to Conger conger (Leloup, 1952), Carassius auratus (Berg and Gorbman, 1954; Chavin, 1956; Hoar and Eales, 1963), Salmo gairdnerii (Olivereau, 1955), Platichthys stellatus (Hickman, 1961), and O. kisutch (Baggerman, 1963) increases the radioiodine uptake and secretion of radioactive hormones. The increase in activity of the thyroid gland is also shown by the alterations in the histological picture of the gland after pituitary extract injection in Carassius auratus (Gorbman, 1940 and 1946; Olivereau, 1954), Fundulus heteroclitus (Albert, 1945; Pickford, 1954), Salmo gairdnerii (Robertson, 1949), Salmo Salar (Hoar et al., 1951), Xiphophorus variatus (Berg et al., 1954), and Salmo trutta (Smith, 1956).

As yet, no action of TSH peripheral to the thyroid gland has been demonstrated (Hoar and Eales, 1963).

Crude extracts of teleost pituitary capable of stimulating intensely the thyroid of teleosts have no action on the thyroid of mammals in the range of doses which was studied, but the teleost thyroid is responsive to mammalian TSH, (Gorbman, 1940; Ortman and Billig, 1966). One reason for the apparently greater responsiveness to various pituitary preparations by the fish thyroid than by the mammalian thyroid is that fish thyroid may not discriminate closely among pituitary hormones (Y.A. Fontaine, 1967). This zoological specificity suggests the existance of physico-chemical differences between TSH of various origins (Y.A. Fontaine and Condliffe, 1963).

Experiments of Olivereau (1955) and M. Fontaine, Baraduc and Y.A. Fontaine (1955) have shown that cold temperatures profoundly decrease the histological response of the teleost thyroid to exogenous thyrotropin and actually reverse the normal, stimulating action of this hormone on the synthesis of organically-bound thyroidal iodine. It was suggested that at low temperatures thyrotropin promotes a prompt release of thyroid hormone but that the subsequent synthesis of newly iodinated amino acid was retarded. Delsol and Flatin (1956) in a study of goldfish thyroid response to TSH at different temperatures confirmed the observation of Olivereau and concluded that more TSH must be liberated by the poikilotherm pituitary to maintain the same level of thyroid activity at low than at high temperature.

Table III gives a summary of some of the work done on fish using TSH as thyroid stimulator.

TABLE III. Influence of TSH on silvering and thyroid activity in fish.

Authors & dates	Species	Treatments	Results
1) Gorbman (1940 & 1946)	C. auratus	purified sheep & pig TSH intraperitoneal injection daily for five days 0.25-5 mg	min. dose required to induce 50 % increase in cell height was 0.25 mg
2) Landgrebe (1941)	S. salar	injection=0.5 g/week of ox ant. lob. pit. for 2 months	silvering increased
	S. trutta	injection= 1 g, bi-weekly of ox ant. lob. pit.	silvering increased
	A. anguilla	injection= 1 g fresh tissue weekly or bi-weekly for 2-3 months	no induction of silvering.
3) Albert (1945)	F. heteroclitus	sheep TSH intraperitoneal injection of 200 mg≡ of fresh gland at 20-25° C	thyroid hypertrophy and hyperplasia after 3 days.
4) Robertson (1949)	S. gairdnerii	beef TSH intra. injection 3/week with gradually increasing in dose (1, 1.5, & 2 mg) and a total of 24 mg.	skin was bright silvery, thyroid increase in activity.
5) Hoar et al. (1951)	S. salar	injection of mammalian ant. pit. extract	silvering increased slightly, thyroid increase in activity with hypertrophy and prompt release of colloid.
6) Leloup (1952)	C. conger	TSH 15 JSU injection/day for 6 days	increase ^{131}I fixation in thyroid and increase T_4 synthesis.

TABLE III (continued)

7)	Berg & Gorbman (1954)	C. auratus	TSH 5 JSU injection/day for 4-5 days	increase radioiodide uptake and synthesis of thyroxine.
8)	Berg et al. (1954)	X. variatus	5 µg/injection (? daily) for 13 weeks	very little effect on thyroid 3 normal and 2 hyperplastic thyroid glands.
9)	Olivereau (1954)	C. auratus	TSH injection/day for 3 days total dose=150 guinea pig units 6° C and 23.5° C	at 6° C_{o} no effect at 23.5° C increase cell height and formation of intracellular colloid droplets.
	Olivereau (1955)	S. gairdnerii	daily injection of 5 JSU to 10 JSU for 1, 3, 5, days at 10° C	slight increase in cell height no increase in radioiodide uptake
			3 daily injections of 10 JSU each at 20° C	strong increase in cell height and increase in radioiodide fixation.
10)	Pickford (1954)	F. heteroclitus	TSH injections 3/week for 1 month at 20° C 0.01, 0.10 and 1 µg/g wt.	cell height increased in thyroid.
11)	Chavin (1956)	C. auratus	TSH injection 0.3 USP/fish for 3 days	increase thyroid uptake of radioiodide from 9.6 % to 20-21 %.
12)	Smith (1956)	S. trutta	TSH injection 2 or 3 days	
			0.04 USP units/fish/day	41 % increase in cell height
			0.11 USP units/fish/day	72 % increase in cell height
			0.2 USP units/10 g/week for 24 days	induced silvering.

TABLE III (continued)

13)	Hickman (1961)	P. stellatus	2 injections of 0.01 IU at 14° C	increase % I uptake and CR (1.72-40.5)
14)	Baggerman (1963)	O. kisutch	injection 0.05 IU for 3 days at 10-12° C	increase % I uptake and CR increase secretion of radioactive hormone.
15)	Hoar & Eales (1963)	C. auratus	0.05 USP/injection 0.10 USP/injection	increase radioiodide uptake and release of hormone
16)	Ortman & Billig (1966)	C. auratus	21 ± 0.6° C 1) 0.024 IU 2) 0.080IU 3) 0.240 IU 4) 0.800 IU 4 daily injections	increase in cell height 1) 4.6 μ 2) 7.3 μ 3) 12.5 μ 4) 18.1 μ

MATERIALS AND METHODS

A. Living material

One-year-old or two-year-old brook trout, Salvelinus fontinalis (Mitchill) from the Province of Manitoba Trout Hatchery, West Hawk Lake, were held in the laboratory in running freshwater and fed ground ox liver on alternate days. Fish were acclimated for at least two weeks to the experimental temperature before use.

B. Killing of fish

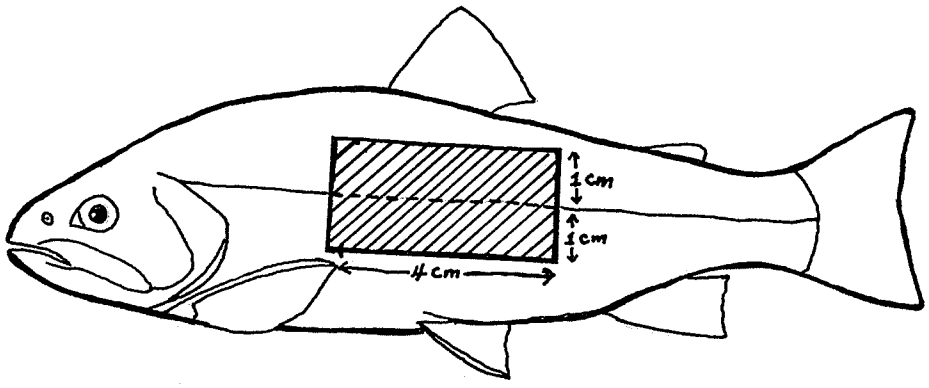
The fish were sacrificed by means of a blow to the head region or immersion in a lethal dose of tricaine methanesulfonate (MS-222), 1:6,000 in the water. The chemical, MS-222 acts on the nervous system of the fish and causes their death. The fish were then weighed and their fork lengths recorded. The tails were cut off using a sharp scalpel blade and individual blood samples were collected in heparinized capillary tubes from the dorsal aorta at the caudal region.

C. Extraction of purines from the skin

The qualtitative and quantitative methods were similar to those used by Johnston and Eales (1967). The fish were placed in the freezer for several hours until they were frozen and then the skin was removed. A measured area of skin directly below the dorsal fin of each fish, 1 cm above and 1 cm below the lateral line and 4 cm in length, was removed from both sides of the fish making a total of 16 cm^2 of skin for purine analysis. (See Figure 2.)

The skin was cut into small pieces and placed in a Stendor

FIGURE 2. Drawing of a brook trout with designated area of skin removed for purine analysis.


1 cm
1 cm
4 cm

dish. Four ml of 1N HCl were added to each sample as extraction solution, and the pair of scissors was rinsed with a portion of this volume. The solution was agitated for approximately 30 min with a magnetic stirrer to extract the purines. Usually the skin suspensions were left overnight for effective extraction. Preliminary experiments were done to test the completeness of extraction of purines from the skin with this volume (4 ml) of extraction solution. The results showed that 4 ml of 1N HCl were sufficient for extracting all the purines (guanine and hypoxanthine) in this amount of skin.

The skin and extract were poured into a 12-ml plastic centrifuge tube and the Stemdor dish was washed out twice with 1 ml of 1N HCl each time, making a total of approximately 6 ml of solution. The skin and extract were centrifuged at 3,500 x g for 15 min and the supernatant was decanted into a 10-ml graduated cylinder. The skin was resuspended twice in 1ml 1N HCl each time. After centrifuging, the two washings were added to the volume in the cylinder. The final volume of the extract was recorded for calculations later on.

The skin was placed in a pre-weighed drying dish and left in the oven at 70-80° C overnight to dry. The dish was uncovered when it was in the oven and covered while weighing again the next day in order to avoid any moisture from coming into contact with the dry skin.

D. Chromatographic method of separation

The concentration of purines in the skin of fish is usually

extremely low. It is therefore practically impossible to obtain tissue extracts which, in the few microlitres used for spotting in thin-layer chromatography, would contain a sufficient amount of the substances necessary for quantitative determination of microgram amounts of purines using the spectrophotometer. On the other hand, paper chromatography allows application of much larger volumes of solutions and hence, also larger amounts of examined substance (Niemierko and Krzyzanowska, 1967).

The chromatographic development was performed in a rectangular glass jar (50 x 20 x 54 cm high) with polished surfaces covered with a well-fitting ground-glass cover (50 x 20 cm). Since the solvent system used (methanol-conc.HCl-water, 70:20:10 v/v, Kirby, 1955) was very corrosive and caused metal instruments in the laboratory to tarnish, the glass tank was placed in the fumehood throughout the experiment.

Experiments showed that equilibration did not improve the resolution of the purines and it was therefore omitted (Niemierko and Krzyzanowska, 1967). In order to keep the atmosphere inside the jar as constant as possible, a beaker of solvent was placed at the bottom of the jar throughout the chromatographic development.

The solution of the samples to be separated was spotted as a band, 5 cm in length, on the paper at a distance 9.5 cm from the edge and 4 cm from each other by gently expelling a few microlitres of the liquid from a 100-lambda disposible micropipette. Whatman No. 1 filter paper was used for all the separations in descending chromatography. Duplicate samples were made and the bands were dried by a blast of warm air from a lady's ordinary hair drier.

After development the purines were viewed under a short-wave ultraviolet lamp (Mineralamp UVS.11, 253.57 mμ). The dark blue spots of guanine and the almost black spots of hypoxanthine were outlined under U.V. with a soft pencil and afterwards they were cut out as rectangles, 5 cm and 1-3 cm wide. Each of them was cut into smaller pieces which were placed in a 12-ml plastic centrifuge tube for elution. The samples with a reference blank (an area equal in size and corresponding to the same Rf value as the samples on the same chromatogram) were cut from the chromatograms and separately eluted in plastic centrifuge tubes for 12 hr in 4 ml 1N HCl.

E. Spectrophotometric method of quantitative analysis

At the start and end of the elution the tubes were agitated with a Vortex shaker and following agitation the pieces of filter paper were removed. The remaining solution was centrifuged at 3,000 x g for 10 min to remove paper fibers in the solution. The supernatant was poured carefully into a clean tube and the aliquots placed in the cuvette were analysed against their blanks on a SP 800 Spectrophotometer (UNICAM Instruments Limited) between 320 and 220 mμ.

The concentration of guanine or hypoxanthine in each extract was determined by substituting the absorbance value at 248 mμ in the following equation:

$$\text{Concentration (moles/litre)} = \frac{\text{Xg x sample vol}}{\text{M. wt. x Extraction vol (ml) x Elution vol(ml)}} \times 10^3$$

$$= \frac{\text{Absorbance}}{\text{Molar Extinction Coefficient}}$$

$$Xg = \text{Absorbance} \times \frac{\text{M. wt.}}{\text{sample vol} \times E \times 10^3} \times \frac{\text{Extraction vol(ml)}}{\text{Elution vol(ml)}}$$

Where Xg = weight of purine in an extract

E = Molar Extinction Coefficient

The extinction coefficient at 248 mμ and pH 1 was 11,400 for guanine and 10,800 for hypoxanthine (Beaven et al., 1955). The purine bases extracted were expressed as % of the dry weight of the tissue and purine:

$$\% \text{ purine extracted} = \frac{\text{purine (mg)} \times 100\ \%}{\text{dry tissue + guanine + hypoxanthine (mg)}}$$

The purines were also expressed in mg/cm^2 skin which is a more useful indication of the quantities of purines present in the skin, because this value is not affected by any increase in the skin thickness which might be the result of the treatment using thiourea, thyroxine, thyroid power and TSH.

F. Estimation of thyroid activity

After intraperitoneal injection of Na^{125} I the thyroid activity was measured in three ways: (i) % dose in thyroid, (ii) thyroid/serum (T/S) ratio, and (iii) conversion ratio (CR). The serum radioiodide level was also measured. The conversion ratio and serum radioiodide level were only measured in Experiment (v), after the fish were treated with TSH.

(i) % dose in thyroid

The thyroid of the fish is usually not an organized gland. The follicles are scattered singly or in small clusters in the loose connective tissue under the pharynx. They usually follow the course of

the ventral aorta and the roots of its branches into the gills (Gorbman and Bern, 1962, pp 100). In order to remove the entire thyroid gland, part of the gill arches and gill filaments were unavoidably included. The radioactivity in the thyroid was counted at the same time as the standards which were prepared by putting 0.1 ml of ^{125}I from the vial used for injection into the fish, in a clean counting tube. Four standards were usually prepared and the average counts were taken.

$$\% \text{ dose in thyroid} = \frac{\text{total counts in thyroid} \times 100\ \%}{\text{average counts in the standards}}$$

(See Figure 3 for drawing of the thyroid gland of the brook trout, from Drury, 1967).

(ii) T/S ratio

After centrifugation of the whole blood at 13,500 x g for 3 min to separate the serum and corpuscles in the capillary tube, the serum was weighed and put into a counting tube with 4 ml 4N NaOH. The radioactivity of the measured portion of serum was counted.

The uptake of radioiodine by the thyroid can be approximately assessed by the following ratio:

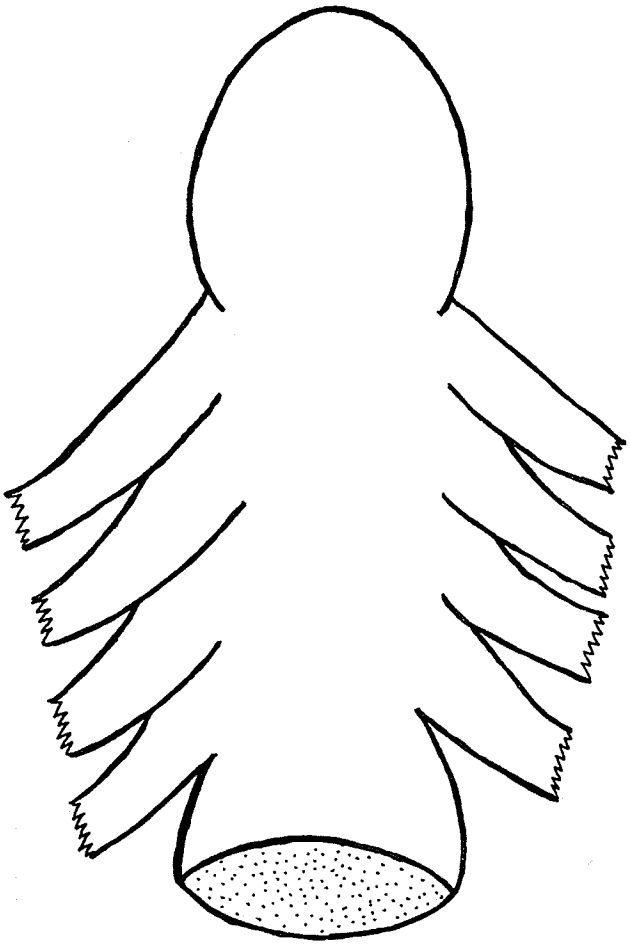
$$T/S = \frac{\text{cpm/g thyroid}}{\text{cpm/g serum}}$$

(iii) Conversion ratio

To a 12-ml centrifuge tube with 2 ml 12.5% trichloroacetic acid (TCA), 0.1 ml serum was added, a white precipitate of protein was formed immediately and another 2 ml 12.5 % TCA was added to wash down all the precipitate stuck on the edge of the tube. The precipitate was

FIGURE 3. Drawing of the thyroid gland of brook trout (Drury, 1967)

ANTERIOR



GILL

ARCHES

POSTERIOR

broken up with a glass rod and centrifuged down for 20 min at 3,500 x g.

The supernatant which contained inorganic iodide ($I^{125}I$) was poured into a clean test tube. The precipitate was washed twice with 2 ml 2.5 % TCA each time and recentrifuged for 10 min. The washings were added to the supernatant to make up a total of 8 ml and 4 ml of this TCA wash were used for counting the radioactive inorganic iodide present. The total inorganic iodide count was obtained by multiplying this value by 2.

The precipitate was broken up by using 1.9 ml distilled water and then dissolved in 2 ml 4N NaOH which gave a 4 ml-solution containing the protein-bound-iodide ($PB^{125}I$).

The total counts in the serum = $PB^{125}I + I^{125}I$

The conversion ratio is one of the best methods to determine thyroid activity due to the fact that it expresses the extent of conversion of administered radioiodine into protein-bound-iodide (Baggerman, 1963) and the changes in the output of hormones is detected radioactively.

$$\text{Conversion ratio} = \frac{PB^{125}I \times 100}{PB^{125}I + I^{125}I}$$

Serum radioiodide levels

The level of serum radioiodide was expressed as a percentage of the injected dose per unit mass of serum. This expression was corrected for each fish to a standard weight of 100 g. This was done to allow for alterations in radioiodide distribution space resulting from a uniform dose of ^{125}I being injected into fish of different sizes.

The correction was made as follows:

$$\text{Serum radioiodide} = \frac{\%\text{ of injected } {}^{125}\text{I in serum x body wt of fish(g)}}{\text{wt of serum (g) x 100}}$$

where $\%\text{ of injected } {}^{125}\text{I} = \frac{\text{serum sample radioactivity (cpm) x 100}}{\text{injected rad. when sample was counted}}$

G. Thin-layer chromatography (TLC)

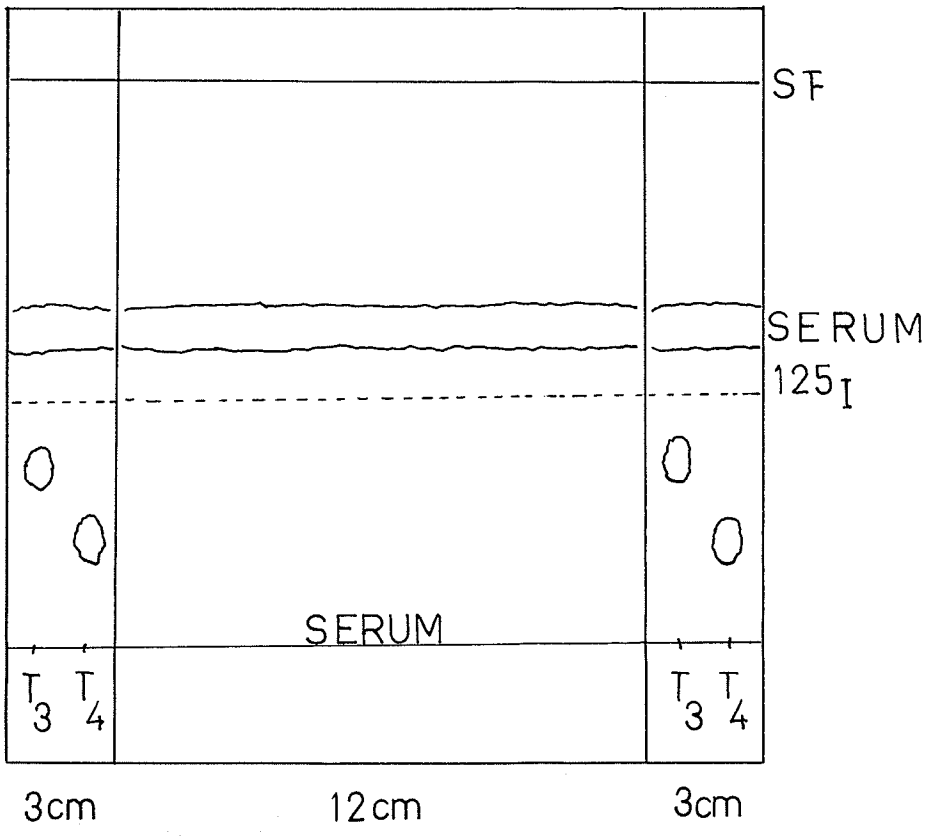
After TSH treatment, an attempt was made using TLC to detect the radiohormones synthesized from injected ^{125}I and secreted into the blood of the fish with the highest CR. The solvent system used was butanol-ethanol-6N ammonia (50:40:10, v/v). About 25 lambda of the serum sample were applied in a line 12 cm long on 0.1 mm, MN Silica gel S-HR/UV 254 (Starch as binder) on plastic sheets (20 x 20 cm).

Standards of triiodothyronine (T_3), thyroxine (T_4), and radioactive iodide (^{125}I), were applied on the same sheet as the serum but on either side of the serum band (see Figure 4). Methyl mercapto imidazole was added to the serum to prevent the oxidation of iodide to iodine. After development in a glass jar for $2\frac{1}{2}$ hr the positions of the hormone standards were located under U.V. and the spots outlined by a soft pencil. A protective coating was sprayed on the TLC before cutting it into strips of 4 mm in width for counting. The strips were placed in clean counting tubes and counted for 30 min for each strip, since the radioactivity on each strip was very low.

H. Experiments

Five experiments were performed between May 1969 and May 1970

FIGURE 4. Thin-layer Chromatography.


SF
SERUM
125I
SERUM
T3 T4
T3 T4
3cm
12cm
3cm

to determine the influence of thiourea, thyroxine, thyroid powder and TSH (thyrotropic hormone) on skin purine (guanine and Hypoxanthine) deposition and thyroid activity.

(i) Influence of different concentrations of thiourea

Forty fish previously acclimated at 10^{o} C were divided into four equal groups. Each group (av. wt.* 21.67 - 24.06 g; av. length** 12.90-13.95 cm) was held at 10 ± 0.5^{o} C in identical white plastic containers holding 17.4 l of aerated water or thiourea solution. The four conditions were:

(a) Control (dechlorinated Winnipeg City Water)

(b) 0.01 % thiourea (in dechlorinated Winnipeg City Water)

(c) 0.05 % thiourea (in dechlorinated Winnipeg City Water)

(d) 0.10 % thiourea (in dechlorinated Winnipeg City Water)

These four conditions were maintained from May 20 to June 9, 1969 (21 days). The fish in each tank were fed 8 g of chopped ox liver on alternate days. Four to five hours following feeding, the water or thiourea solutions were changed. Seventy-two hours before the fish were sacrificed, 0.25 μCi of $Na^{125}I$ in 0.1 ml of distilled water was injected intraperitoneally into each fish. The activity of the thyroid was expressed as the thyroid to serum (T/S) ratio and % uptake of an injected dose of radioiodide by the thyroid.

(ii) Influence of different concentrations of thyroxine powder

* av. wt. of fish in each group.
** av. length of fish in each group.

Forty fish previously acclimated at 10° C were divided into four equal groups. Each group (av. wt. 17.12-25.29 g; av. length 11.92 -13.31 cm) was held at 10 ± 0.5° C in identical white plastic containers holding 17.4 l of aerated running water. The fish were injected on alternate days with a total of 10 injections for each group. The four conditions were:

(a) Control (0.1 ml 50 % aqueous propylene glycol/injection)

(b) 1 µg L-thyroxine powder (in 0.1 ml dissolved in 50 % propylene glycol).

(c) 10 µg L-thyroxine powder (in 0.1 ml dissolved in 50 % propylene glycol).

(d) 100 µg L-thyroxine powder (in 0.1 ml dissolved in 50 % propylene glycol).

The L-thyroxine powder used was the sodium salt Grade II supplied by Sigma Company. These four conditions were maintained from June 22 to July 12, 1969 (21 days). The fish in each tank were fed 8 g of chopped ox liver on alternate days. The containers were thoroughly cleaned out every four days. The L-thyroxine solution was injected intraperitoneally on alternate days prior to the feeding. Seventy-two hours before the fish were sacrificed, 0.25 µCi of $Na^{125}I$ in 0.1 ml of distilled water was injected intraperitoneally into each fish. The activity of the thyroid was determined as in Experiment (i).

(iii) Influence of low concentrations of thiourea

This experiment consisted of 4 groups of fish, each treated with different concentrations of thiourea which were lower than those used in the first experiment. The objective of this experiment was to

test the lowest dose of thiourea which would cause thyroid inhibition and to determine also if there were any influence on purine levels.

Sixty fish previously acclimated for two weeks at 10°C were divided into four equal groups . Each group (av. wt. 64.30-73.20g; av. length 19.23 - 19.83cm) was held at 10 ± 0.5°C in identical white plastic containers holding 17.4 l of aerated water or thiourea solutions. The four conditions were:

(a) Control (dechlorinated Winnipeg City Water)

(b) 0.0001 % thiourea (in dechlorinated Winnipeg City Water)

(c) 0.001 % thiourea (in dechlorinated Winnipeg City Water)

(d) 0.01 % thiourea (in dechlorinated Winnipeg City Water)

These four conditions were maintained from Oct 18 to Nov 7, 1969 (21 days). The fish in each tank were fed 8 g of chopped ox liver on alternate days. Four to five hours following feeding the water or thiourea solutions were changed. Seventy-two hours before the fish were sacrificed, 0.25 μCi of $Na^{125}I$ in 0.1 ml of distilled water was injected intraperitoneally into each fish. The activity of the thyroid was expressed as in Experiment (i).

(iv) Influence of thiourea and thyroxine

The objective of this experiment was to confirm the findings of Experiments (i) and (ii). The procedures of treatment were essentially the same as in those two previous experiments.

Sixty fish previously acclimated for two weeks at 10°C were divided into four equal groups. Each group (av. wt. 117.91-148.55 g; av. length 24.03-25.15cm) was held at 10 ± 0.5° C in identical white

plastic containers holding 17.4 l of aerated running water or thiourea solution. The four conditions were:

(a) L-thyroxine powder control (0.1 ml 50 % aqueous propylene glycol/injection)

(b) 3.5 µg L-thyroxine powder (in 0.1 ml dissolved in 50 % propylene glycol)

(c) Thiourea control (dechlorinated Winnipeg City Water)

(d) 0.05 % thiourea (in dechlorinated Winnipeg City Water)

These four conditions were maintained from Nov 15 to Dec 5, 1969 (21 days). The fish in each tank were fed 8 g of chopped ox liver on alternate days. The L-thyroxine powder solution was injected on alternate days and the 0.05 % thiourea solution was changed on alternate days. Due to the large size of these fish, each time before thyroxine injection, the fish were anaesthetized by immersion in a dilute solution of MS-222 (0.3 g/6 l) for a few minutes.

Seventy-two hours before the fish were sacrificed, 0.25 µCi of $Na^{125}I$ in 0.1 ml of distilled water was injected intraperitoneally into each fish. The activity of the thyroid was expressed as in Experiment (i).

(v) Influence of thyroid powder and TSH

Fifty-eight fish, acclimated for two weeks at 10° C and another two weeks at 15° C, were divided into four groups. Each group (av. wt. 98.78 - 127.58 g; av. length 21.64 - 22.78 cm) was held at 15 ± 0.5° C in identical white Styroform coolers holding 30.5 l of aerated running water. The fish were injected on alternate days with a total of 17 injections for each group. The four conditions were:

(a) 1 mg thyroid powder suspended in 0.1 ml 50 % aqueous propylene glycol

(b) Thyroid powder control (0.1 ml 50 % aqueous propylene glycol)

(c) 5 JSU (0.5 IU) TSH suspended in 0.1 ml solvent (10 ml solution containing 0.03 g phenol and 0.05 g dextrose)

(d) TSH control (0.1 ml solvent/injection)

These four conditions were maintained from April 14 to May 18, 1970 (35 days). The fish in each tank were fed 25 g of chopped ox liver every day to ensure an ample source of dietary nitrogen. The fish were injected on alternate days, after being anaesthetized by immersion in solution of MS-222 (0.3 g/6 l) for a few minutes. The activity of the thyroid was expressed as in Experiment (i) and the conversion ratio also determined. In order to obtain a higher conversion ratio (CR), index of radiothyroxine production, 0.25 μCi of $Na^{125}I$ in 0.1 ml of distilled water was injected intraperitoneally into each fish 6 days before the fish was sacrificed.

The TSH (Bovine Pituitary Sterile Powder) and the thyroid powder (Grade I) were supplied by the Sigma Chemical Company.

RESULTS

A. Verification of guanine and hypoxanthine

The ultraviolet absorption spectra for the extracts were compared with that of the standard solutions of guanine and hypoxanthine. The results (Figure 5) showed that the purines extracted from the skin consisted mainly of guanine and hypoxanthine.

B. Experimental results

(i) Thiourea treatment (Table IV)

(a) Thyroid activity

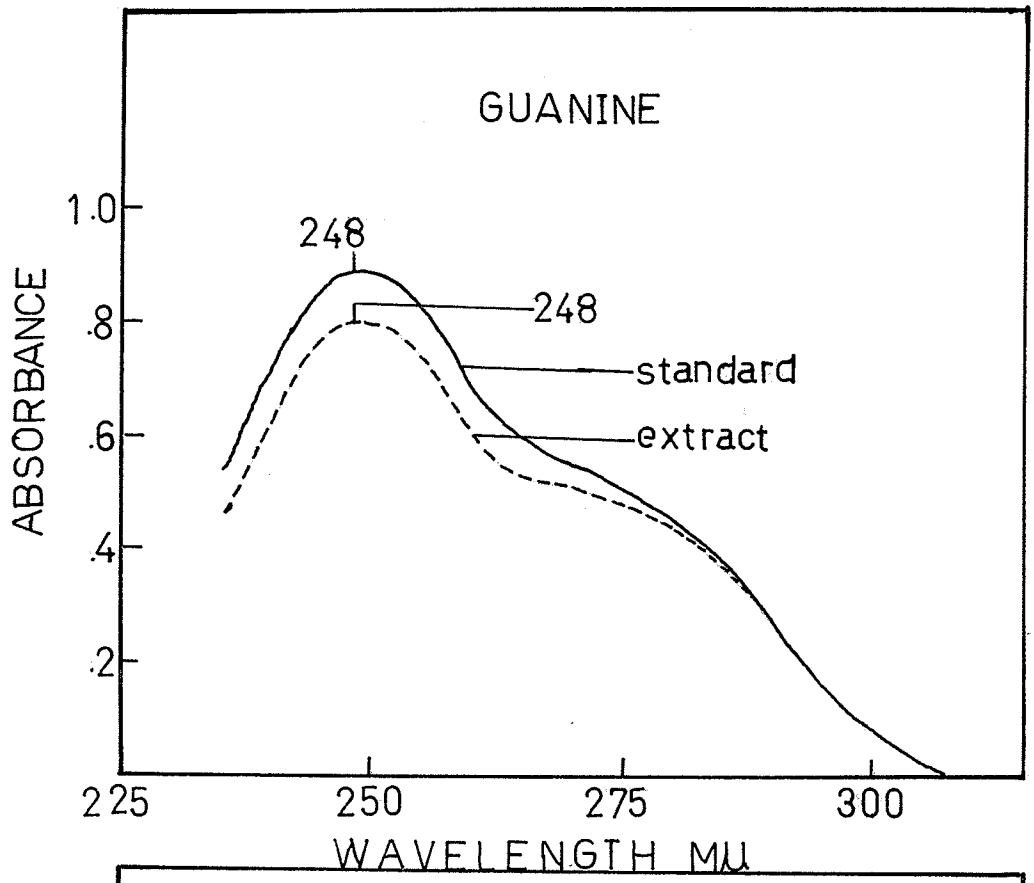
Thyroid activity was greatly suppressed by treatment with thiourea at a concentration as low as 0.01 % in the water. In the control group the T/S ratio was 6.38 $\pm$ 0.71. This was ten times the T/S for the group treated with 0.01 % thiourea (T/S = 0.61 $\pm$ 0.03). Higher levels of thiourea (o.o5 and 0.10 %) did not substantially decrease thyroid activity any further.

(b) Skin purine deposition

The effects of thiourea on skin purine deposition were less obvious than those on the thyroid though there was a slight decrease in guanine or hypoxanthine % in skin. The weight of purine (mg)/area of skin (cm^2) decreased too. However, there was no difference between the treatments at 0.05 % level, by analysis of variance.

The weight of skin/cm^2 area (an index of skin thickness) and G/H were not significantly changed, and these had been tested at 0.05 % levels, by analysis of variance.

FIGURE 5. The ultraviolet absorption spectra for the extract and standard solutions of guanine and hypoxanthine in 0.1N HCl.


GUANINE
ABSORBANCE
1.0
.8
.6
.4
.2
248
248
standard
extract
225
250
275
300
WAVELENGTH MU

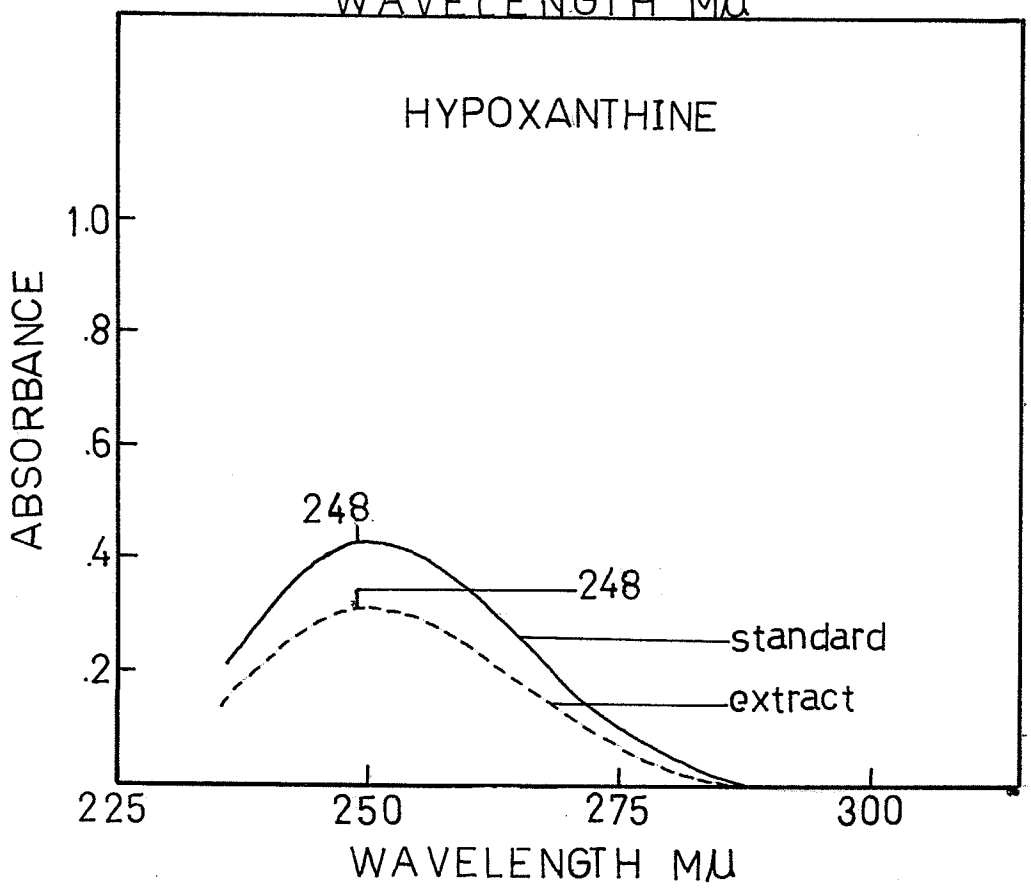

HYPOXANTHINE
ABSORBANCE
1.0
.8
.6
.4
.2
248
248
standard
extract
225
250
275
300
WAVELENGTH MU

TABLE IV. Effect of thiourea on thyroid activity and purine deposition.

Treatment	number of fish	average length (cm)	average weight (g)	thyroid activity T/S	thyroid activity % thyroid	purines: guanine % in skin	guanine mg/cm^2	hypoxanthine % in skin	hypoxanthine mg/cm^2	G/H	skin* mg/cm^2
Control	10	13.70	24.06	6.38	20.95	5.91	0.167	2.10	0.059	2.94	2.87
S.E.		±0.19	±1.18	±0.71	±2.65	±0.28	±0.010	±0.16	±0.004	±0.20	±0.20
0.01 %	10	12.90	21.67	0.61	2.80	5.00	0.151	1.70	0.053	2.89	3.10
S.E.		±0.28	±1.56	±0.03	±0.27	±0.38	±0.009	±0.14	±0.003	±0.13	±0.08
0.05 %	10	13.95	22.67	0.53	1.73	5.40	0.137	1.60	0.042	3.33	2.53
S.E.		±0.15	±0.76	±0.02	±0.14	±0.34	±0.010	±0.08	±0.003	±0.24	±0.08
0.10 %	10	12.95	21.93	0.43	1.45	4.42	0.140	1.57	0.050	2.88	3.27
S.E.		±0.25	±1.12	±0.02	±0.11	±0.35	±0.010	±0.13	±0.003	±0.21	±0.27

* Wt. of skin / unit area of skin.

(ii) Thyroxine treatment (Table V)

(a) Thyroid activity

Due to the fact that the fish in this experiment were kept in running water and the excreted ^{125}I had less chance to be reabsorbed into the blood system than for those fish of the first experiment, the T/S and % thyroid in the control group of this experiment (T/S = 1.68 ± 0.14; % thyroid = 6.67 ± 1.11) were three times lower than those of the control group of the first experiment (T/S = 6.38 ± 0.71; % thyroid = 20.95 ± 2.65).

The thyroid activity was suppressed by the presence of excess thyroxine in the blood system which presumably acted on the hypothalamo-hypophyseal axis and inhibited the secretion of TSH from the pituitary gland. Therefore the activity of the thyroid gland was reduced.

Doses as low as 1 μg thyroxine/fish/injection every two days reduced the thyroid activity to more than half of the control group. The T/S ratio was reduced from 1.68 ± 0.14 to 0.77 ± 0.08 and % thyroid was reduced from 6.67 ± 1.11 to 1.98 ± 0.34. It seemed that further increase in thyroxine doses to 10 times (10 μg) and 100 times (100 μg) did not reduce these two values to any considerable extent, in fact they were slightly higher.

(b) Skin purine deposition

The purine levels in the skin were negligibly increased and statistically insignificant. The skin thickness and G/H were not significantly changed, these had been tested at 0.05 % level, by analysis of variance.

TABLE V. Effect of thyroxine on thyroid activity and purine deposition.

Treatment	number of fish	average length (cm)	average weight (g)	thyroid activity T/S	thyroid activity % thyroid	purines: guanine % in skin	purines: guanine mg/cm^2	purines: hypoxanthine % in skin	purines: hypoxanthine mg/cm^2	purines G/H	skin mg/cm^2
Control	10	13.31	25.29	1.68	6.67	5.41	0.165	1.76	0.054	3.07	3.08
S.E.		±0.26	±1.85	±0.14	±1.11	±0.36	±0.011	±0.10	±0.003	±0.09	±0.14
1 µg	10	11.92	17.12	0.77	1.98	5.03	0.163	1.79	0.058	2.78	3.37
S.E.		±0.36	±1.66	±0.08	±0.34	±0.49	±0.014	±0.11	±0.003	±0.13	±0.24
10 µg	10	13.05	21.94	0.68	2.50	5.69	0.197	1.73	0.060	3.07	3.50
S.E.		±0.31	±1.32	±0.06	±0.33	±0.32	±0.009	±0.07	±0.002	±0.20	±0.08
100 µg	10	13.04	22.31	0.87	2.14	5.76	0.191	1.80	0.060	3.20	3.30
S.E.		±0.48	±2.45	±0.11	±0.23	±0.27	±0.012	±0.08	±0.004	±0.10	±0.14

(iii) Thiourea treatment at low concentrations (Table VI)

(a) Thyroid activity

Further investigations on the effects of thiourea on thyroid gland were performed in Experiment (iii). The sample size was increased from 10 to 15 and fish in this experiment were three times as big as those in Experiment (i). The doses were reduced to 0.0001 %, 0.001 %, and 0.01 %. The purpose of this experiment was to find out the lowest level of thiourea which would affect the activity of the thyroid gland in this species (Salvelinus fontinalis), and also to determine if there were any effect on skin purine levels.

The T/S ratio and % thyroid were reduced in the treated groups. Comparing the results of the control group in this experiment with those of the control group obtained in Experiment (i), it was found that the T/S and % thyroid in this group were about 40 % lower than those in the first experiment, this could be due to the increase in body size of these fish. However the results for 0.01 % thiourea treatment in both groups came out quite close to each other (T/S: 0.61 and 0.57; % thyroid: 2.80 and 2.56).

At a level as low as 0.0001 % of thiourea in the ambient water, the T/S and % thyroid were reduced to about 40 % of the control group, and 0.0001 % thiourea was almost as effective as 0.01 % thiourea.

(b) Skin purine deposition

Only the skin purines of the control group and the 0.01 % thiourea group were tested and the results showed that both the guanine and hypoxanthine levels were not changed. The G/H ratio was slightly but not significantly decreased, also the skin thickness did not seem to

TABLE VI. Effect of thiourea on thyroid activity and purine deposition.

Treatment	number of fish	average length (cm)	average weight (g)	thyroid activity T/S	thyroid activity % thyroid	purines guanine % in skin	purines guanine mg/cm^2	purines hypoxanthine % in skin	purines hypoxanthine mg/cm^2	purines G/H	skin mg/cm^2
Control	14	19.23	65.25	2.73	8,48	2.97	0.146	1.18	0.059	2.47	5.24
S.E.		±0.39	±4.88	±0.76	±2.37	±0.28	±0.012	±0.07	±0.004	±0.08	±0.45
0.0001 %	15	19.52	66.00	0.94	3.03						
S.E.		±0.50	±4.59	±0.15	±0.60						
0.001 %	13	19.53	64.30	1.34	3.24						
S.E.		±0.68	±6.88	±0.37	±0.74						
0.01 %	15	19.83	73.20	0.57	2.56	2.63	0.138	1.19	0.062	2.22	5.38
S.E.		±0.64	±6.92	±0.06	±0.32	±0.16	±0.009	±0.07	±0.003	±0.08	±0.36

be altered.

(iv) Thiourea and thyroxine treatments(Table VII)

(a) Thyroid activity

In order to confirm the results obtained so far, another experiment was performed with thiourea and thyroxine at concentrations known from previous experiments to effectively suppress the activity of the thyroid gland. Since the fish used in this experiment were 3-4 times larger than before, the dose of thyroxine was also increased to compensate for the larger distribution volume.

The T/S and % dose in the two control groups were much lower than the values of the control groups obtained in the previous experiments, this might be due to the increase in the size of these fish. The T/S value in the thyroxine-treated group decreased from 0.74 $\pm$ 0.06 to 0.54 $\pm$ 0.06, and the % thyroid decreased from 2.47 $\pm$ 0.32 to 1.79 $\pm$ 0.17. The T/S value in the thiourea-treated group decreased from 1.03 $\pm$ 0.13 to 0.41 $\pm$ 0.03, and % thyroid decreased from 2.78 $\pm$ 0.34 to 0.82 $\pm$ 0.08. The magnitude of decrease in the thiourea-treated group was greater than in the thyroxine-treated group. The thyroid did not seem to be completely inhibited by the thyroxine.

(b) Skin purine deposition

The purine levels were not significantly altered as found in the previous experiments. The skin thickness in the thyroxine-treated group increased slightly and in the thiourea-treated group it was increased too. The G/H value increased significantly ($t = 3.59$, $df = 28$, $p<0.01$) in the thyroxine-treated group but decreased slightly in the thiourea-treated group.

TABLE VII. Effect of thiourea and thyroxine on thyroid activity and purine deposition.

Treatment	number of fish	average length (cm)	average weight (g)	thyroid activity		purines					skin
				T/S	% thyroid	guanine		hypoxanthine		G/H	mg/cm^2
						% in skin	mg/cm^2	% in skin	mg/cm^2		
Control (thyroxine)	15	25.15	148.55	0.74	2.47	2.64	0.160	1.27	0.078	2.12	6.28
S.E.		±0.37	±8.64	±0.06	±0.32	±0.16	±0.009	±0.08	±0.005	±0.12	±0.45
thyroxine 3.5 µg	15	24.79	135.41	0.54	1.79	2.70	0.190	1.04	0.072	2.68	7.39
S.E.		±0.39	±5.90	±0.06	±0.17	±0.19	±0.014	±0.10	±0.006	±0.10	±0.63
Control (thiourea)	15	24.03	117.91	1.03	2.78	3.20	0.180	1.45	0.082	2.27	5.67
S.E.		±0.23	±5.88	±0.13	±0.34	±0.16	±0.009	±0.08	±0.005	±0.12	±0.26
thiourea 0.05 %	13	24.48	128.72	0.41	0.82	3.06	0.185	1.57	0.960	2.00	6.20
S.E.		±0.58	±8.27	±0.03	±0.08	±0.19	±0.011	±0.12	±0.008	±0.11	±0.39

(v) Thyroid powder and TSH (Table VIII)

The fish in this experiment were kept at a higher temperature, 15 ± 0.5° C and for a longer period, 35 days. Instead of plastic containers the fish were kept in white Styrofoam coolers holding 30.5 l.

(1) Thyroid powder

(a) Thyroid activity

The % thyroid was decreased (from 17.54 ± 2.75 to 4.89 ± 1.12) by thyroid powder treatment as compared to the propylene glycol-treated controls. The T/S ratio decreased (from 5.42 ± 1.10 to 1.46 ± 0.25) almost to 25 % of the control group.

Blood serum was collected for protein and inorganic radioiodide analysis. The conversion ratio was decreased slightly (from 1.26 ± 0.07 to 1.02 ± 0.06) which could be an indication of the suppression effect on thyroid activity.

Protein-bound-iodide and inorganic iodide corrected for body weight were not significantly altered when tested statistically.

(b) Skin purine deposition

The weight of guanine (mg)/area of skin (cm^2), was significantly increased from 0.153 to 0.202 mg/cm^2 ($t = 4.36$, $df = 27$, $p<0.01$). The weight of hypoxanthine/cm^2 of skin was also found to be significantly increased from 0.070 to 0.086 mg/cm^2 ($t = 3.34$, $df = 27$, $p<0.01$).

However the G/H ratio, the skin thickness, % hypoxanthine and % guanine were not significantly altered though slightly increased.

(2) Thyrotropic hormone

(a) Thyroid activity

TABLE VIII. Effect of thyroid powder and TSH on thyroid activity and purine deposition.

Treatment	number of fish	average length (cm)	average weight (g)	thyroid activity T/S	thyroid activity % thyroid	serum $I^{125}I$	serum CR	purines guanine % in skin	purines guanine mg/cm^2	purines hypoxanthine % in skin	purines hypoxanthine mg/cm^2	purines G/H	skin mg/cm^2
Control (thy.p.)*	14	21.64	106.76	5.42	17.54	5.21	1.26	3.46	0.153	1.59	0.070	2.30	4.58
S.E.		±0.55	±7.36	±1.10	±2.75	±0.43	±0.07	±0.25	±0.008	±0.16	±0.004	±0.16	±0.23
1 mg thy.p.*	15	22.78	127.58	1.46	4.89	5.71	1.02	4.05	0.202	1.17	0.086	2.42	5.32
S.E.		±0.57	±10.80	±0.25	±1.12	±0.52	±0.06	±0.32	±0.010	±0.16	±0.005	±0.12	±0.39
Control (TSH)	14	21.68	98.78	4.24	14.90	5.08	1.37	3.93	0.164	2.04	0.086	1.95	4.29
S.E.		±0.38	±5.55	±1.17	±3.68	±0.49	±0.18	±0.23	±0.004	±0.12	±0.004	±0.09	±0.18
5 JSU TSH	15	21.87	100.94	29.57	29.13	2.65	9.94	5.10	0.232	2.51	0.113	2.05	4.68
S.E.		±0.59	±8.38	±8.45	±4.56	±0.52	±3.02	±0.35	±0.012	±0.18	±0.004	±0.08	±0.12

* thy.p. = thyroid powder.

After TSH treatment the thyroid gland was greatly stimulated, the T/S ratio increased almost seven times (from 4.42 ± 1.17 to 29.57 ± 8.45) and % thyroid almost doubled that of the control group (from 14.90 ± 3.68 to 29.13 ± 4.56).

Blood serum samples were collected for protein and inorganic iodide analysis and the conversion ratio was greatly increased from 1.37 ± 0.18 to 9.94 ± 3.02 ($t = 2.74$, $df = 27$, $p<0.01$). Protein-bound-iodide corrected for body weight was doubled (from 6.71 ± 1.12 to 12.61 ± 1.36) and the inorganic iodide corrected for body weight (from 5.08 ± 0.49 to 2.65 ± 0.52) decreased by 50 %. These changes were tested at 0.01 % level using the t test, and found to be statistically significant.

(b) Skin purine deposition

The increase in purine content of the skin was even more noticeable after TSH treatment than thyroid powder treatment. Guanine increased from 0.164 to 0.232 mg/cm^2 of skin, ($t = 6.16$, $df = 27$, $p<0.01$). Hypoxanthine increased from 0.086 mg to 0.113 mg/cm^2 of skin, ($t = 7.35$, $df = 27$, $p<0.01$). Percentage guanine also increased significantly at 0.05 % level, but % hypoxanthine, G/H and skin thickness were not altered significantly though increased slightly.

The weight of guanine/cm^2 skin and H/cm^2 skin in the control group for thyroid powder (with propylene glycol injected) were lower than those of the control group for TSH. In fact these values for the thyroid powder-treated group (G/cm^2 = 0.202; H/cm^2 = 0.086) were quite similar to the values of the control group of TSH (G/cm^2 = 0.164; H/cm^2 = 0.086). This could be due to the effect of the 50 % propylene glycol treatment. The H % in skin (2.04) of the TSH control was in fact higher than in the

thyroid powder-treated group (1.71).

(c) Hormone production by the thyroid gland

The serum of the fish with the highest conversion ratio (fish No. 14) was used for the preliminary identification of radiohormones produced by the fish after TSH treatment. The technique used was thin-layer chromatography.

Results from the graph showed that both T_3 and T_4 were probably produced by the fish after TSH treatment. (Figure 6.)

(d) Total iodide in serum

Total iodide in serum samples of ten fish were analysed through the help of Miss Linda A. Welsh, to whom the author is extremely grateful.

The results showed that, with the exception of one fish, the conversion ratio decreased as the μg % of iodide in the blood serum samples increased. The great variation in the conversion ratio may be explained by the variation in the amount of stable iodide present in the iodide pool in the fish before the radioactive iodide was injected. (Figure 7.)

FIGURE 6. Thin-layer chromatographic separation of radioiodinated amino acids and inorganic radioiodide on the serum of a brook trout injected with ^{125}I.

O = origin
SF = solvent front
BGC = background count
CPM = counts per minute
T_3 = triiodothyronine
T_4 = thyroxine
I^- = inorganic iodide

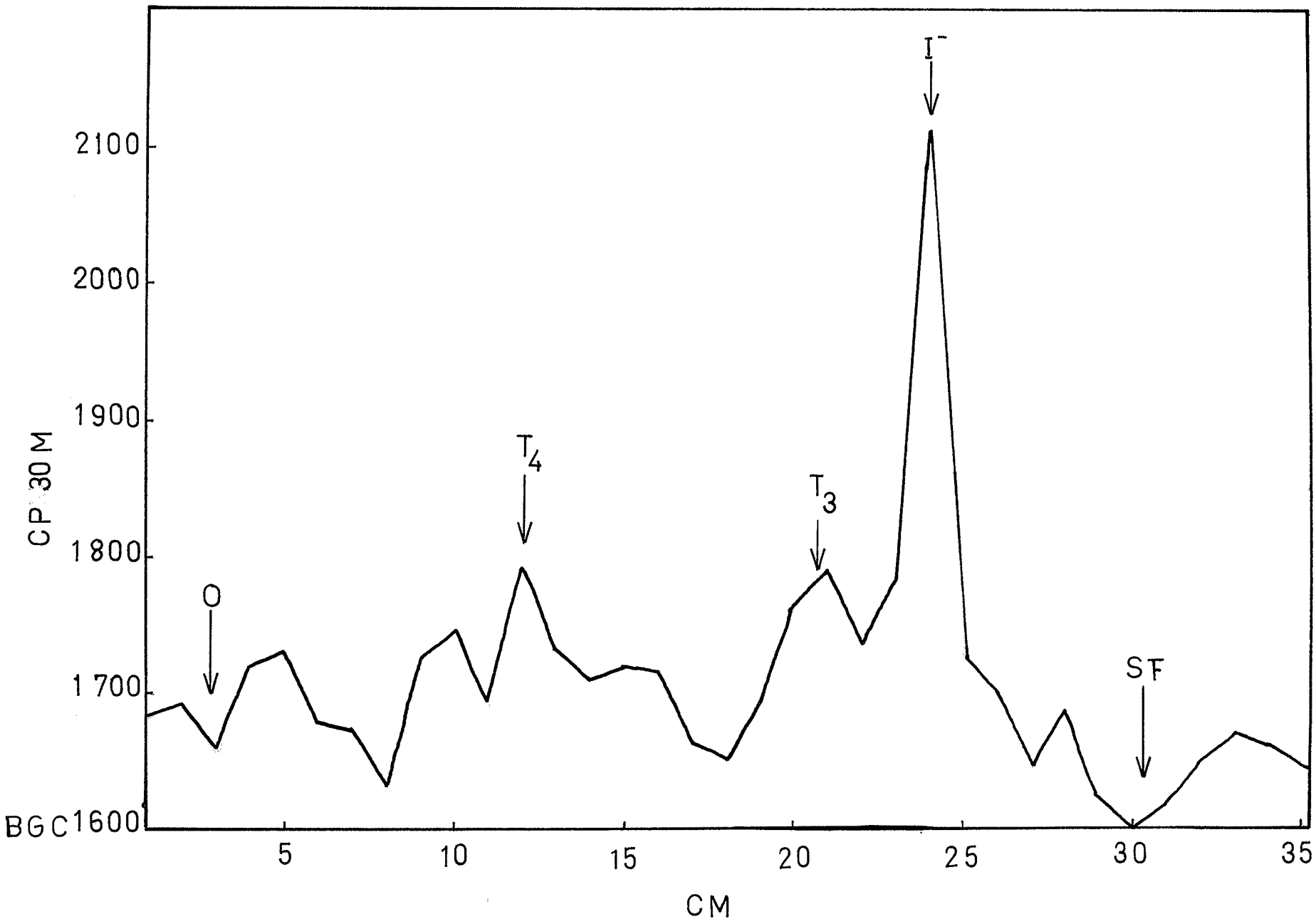
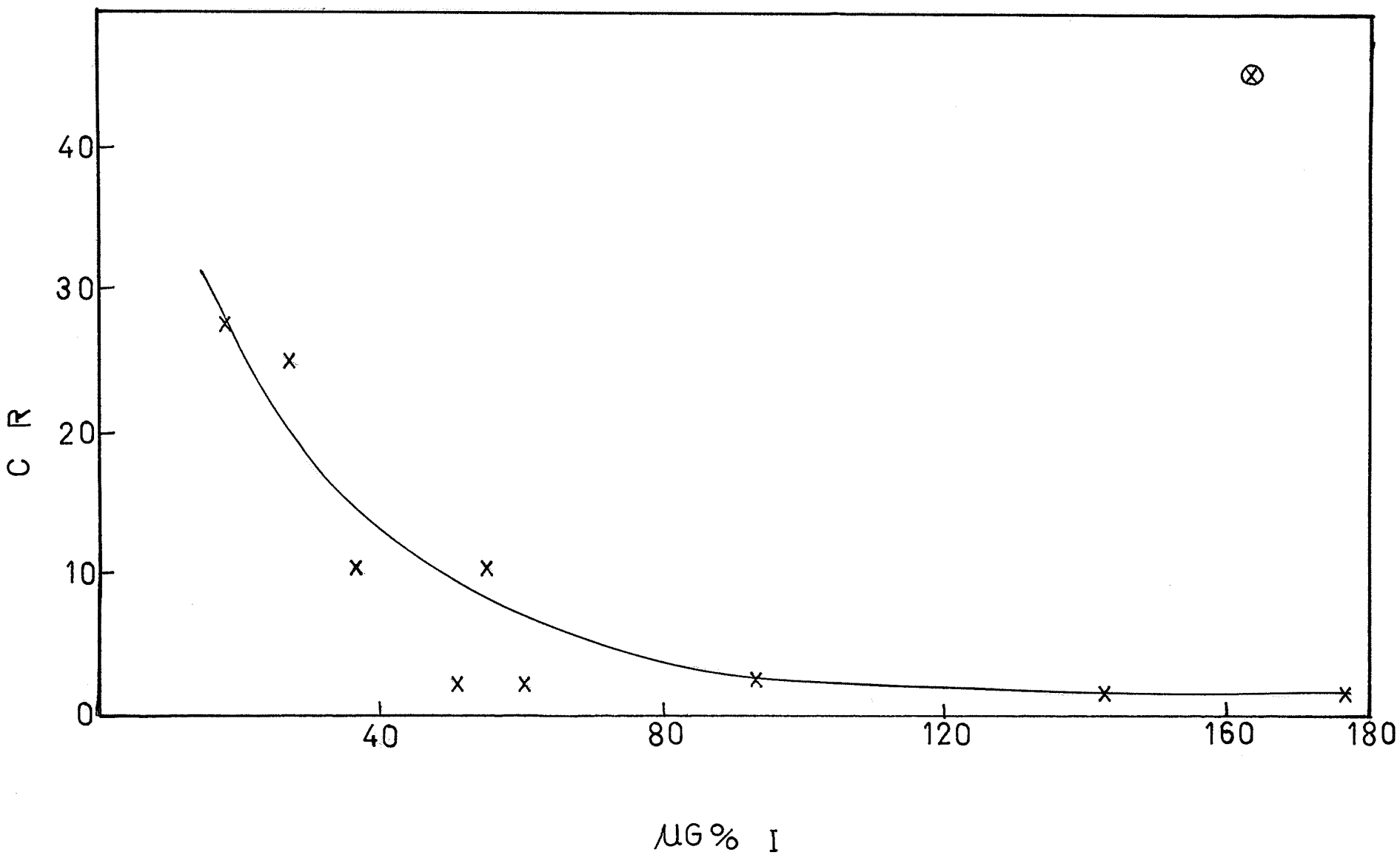

I⁻
2100
2000
1900
T4
T3
1800
O
1700
SF
BGC 1600
CP 30 M
5
10
15
20
25
30
35
CM

FIGURE 7. The relationship between the conversion ratio and μg % I in the serum of brook trout injected with ^{125}I.


40
30
20
10
0
C R
40
80
120
160
180
µG % I

DISCUSSION

A. Thiourea treatment

Thiourea in a concentration as low as 0.0001 % suppresses the function of the thyroid gland of brook trout (Salvelinus fontinalis), and a concentration 1000 x higher (0.10 %) gives approximately the same degree of suppression as measured by the radioactive counts. Apparently the thyroid was completely inhibited, however contamination of the gland with extrathyroidal tissues probably was responsible for some radioactivity.

Kinnear (1960) found that the minimum dose of thiourea effectively inhibiting thyroxine synthesis in the starry flounder was 0.0025 % and concentrations of 0.001 %, 0.0005 %, and 0.0001 % thiourea were not inhibitory. This difference in results could be due to different species used and also the period of treatment. Another difference was that Kinnear measured thyroxine synthesis and not thyroid uptake as in the present study. Fish in the present study were treated for 21 days at 10° C while Kinnear (1960) only kept his fish for 10 days in 0.0001 % thiourea at 10° C to 12° C. The low concentration of thiourea found effective in his study (0.0025 %) is not necessarily the minimum dose for all species of fish.

A high dose of thiourea is known to produce extrathyroidal effects and most of the concentrations used in the literature are quite high. Hoar and Bell (1950) maintained salmonids in a 0.36 % solution of thiourea but Massey and Smith (1968) had to reduce the concentration to 0.10 % to avoid excessive mortality. Even 0.10 % of thiourea is quite high compared with the minimum dose of 0.0001 %, and their observations,

suggesting that thiourea also raised the guanine content of the skin sections, are therefore dubiously related to thyroid activity itself. In the present study, 0.10 % of thiourea neither altered the purine levels in the skin nor the thickness of the skin.

Undoubtedly thyroxine synthesis was inhibited by the treatment of thiourea as shown in the decrease of thyroid activity using radiochemical methods, but thyroxine does not specifically control purine metabolism in fish though it may stimulate guanine production to some extent as suggested by Hoar and Bell (1950) and Hoar et al., (1951). Therefore the withdrawal of thyroxine from the blood for a short period of 21 days does not seem to affect the guanine and hypoxanthine levels already present in the skin Fortune (1953, 1955, 1956) in a series of studies on Phoxinus phoxinus, showed that temperature affects the action of thiourea on thyroid gland and at higher temperatures the increase in cell height and decrease in colloid took place more rapidly than at lower temperatures.

Though some workers (Massey and Smith, 1968) claimed that thiourea raised the guanine content in the skin of fish, the present study shows no such relationship. This could be due to the insufficient length of treatment (21 days) and low temperature (10° C).

B. Thyroid hormone treatment

The thyroxine or thyroid hormone level in the blood was raised by intraperitoneal injections of L-thyroxine or mammalian thyroid extract. Administration of thyroxine or thyroid extract to the fish presumably affects the pituitary-thyroid axis by depressing the output of TSH from

cells in the pars distalis with consequent decrease in activity of the thyroid gland. When comparing the inhibition of thyroxine and thiourea on thyroid gland in Experiment (iv) it is found that the magnitude of decrease in the thiourea-treated group was greater than in the thyroxine-treated group, and the thyroid did not seem to be completely inhibited by the thyroxine.

Though thyroxine inhibits the function of thyroid as does the thyroid extract, the former probably does not represent the whole constituent in the normal thyroid secretion. Robertson (1949) observed that when thyroxine alone was injected into the rainbow trout, there was no local pallor and no increase in silvering but after thyroid powder injection he was able to produce silvery trout. Triiodothyronine might be the active principle of the thyroid hormone.

In the present study both thyroxine and thyroid powder inhibit the function of the thyroid significantly but only the latter was able to increase the guanine and hypoxanthine level (mg/cm^2) in the skin of the fish to any noticeable amount. The thyroid powder-treated group was maintained at a higher temperature (15° C) and a longer period (35 days) than the thyroxine-treated group, these conditions could render the thyroid powder more effective in the nitrogen metabolism in the fish than the thyroxine besides their difference in constituents.

The conversion ratio of the thyroid powder-treated group was also measured and this is one of the best methods in determining the activity of the thyroid gland because it expresses the extent of conversion of administered radioiodine into protein-bound-(hormone)iodide (Baggerman, 1963) and thus the changes in the output of hormone from the thyroid gland

can be detected radioactively. In the thyroid powder-treated group the conversion ratio was significantly decreased which shows that the thyroid gland was suppressed.

LaRoche and Leblond (1952) found that after feeding thyroid extract to S. salar there was a thickening of the layers of the integument due to an enlargement of both epidermis and derma. They also injected 200 µg sodium thyroxinate into the salmon and observed similar results. However, the doses of thyroid extract employed by LaRoche and Leblond (1952) were probably greater than the amount of hormone normally secreted by the thyroid gland. In the present study the injection of 100 µg of L-thyroxine or 1 mg of thyroid powder did not alter the thickness of the skin significantly.

C. Thyrotropic hormone treatment

The administration of exogenous thyroid hormone may not be as effective as the action of the endogenous thyroid hormone produced by the fish themselves. Since the teleost thyroid is less responsive to exogenous TSH at low temperature than at higher temperature (Olivereau, 1955; M. Fontaine, Baraduc and Y.A. Fontaine, 1955), the fish in the TSH-treated group in the present study were kept at 15° C instead of at 10° C. The period of treatment was increased too, to ensure a maximum effect of the TSH on the thyroid gland of the fish.

The thyroid gland was greatly stimulated by the TSH and the high conversion ratio indicates an increase in the thyroid hormone output. The chromatography results show that probably both thyroxine and triiodothyronine were secreted by the thyroid gland.

Variation in the amount of stable iodide present in the iodide pool in the fish before the radioactive iodide was injected caused variation in the conversion ratio. The radioactive iodide when injected into the fish was evenly distributed in the iodide pool. If the level of stable iodide in the blood is high, the radioactive iodide will be diluted in the iodide pool and the chance of uptake by the thyroid gland will be small. The amount of radiohormones secreted depends on the radioiodine uptake.

The guanine and hypoxanthine levels in the skin are greatly increased though there is no increase in the skin thickness and G/H ratio. Since there is no report of extrathyroidal effect of TSH so far (Hoar and Eales, 1963) it is safe to think that the thyroid gland is secreting a physiological level of hormone in the experiment.

CONCLUSIONS

A. Thiourea treatment

Thiourea suppresses the function of the thyroid gland in a concentration as low as 0.0001 % and a concentration 1000 x higher (0.10 %) gives approximately the same degree of inhibition.

Thiourea treatment does not alter guanine and hypoxanthine levels measured both in mg/cm^2 skin and % in skin, the G/H ratio and the thickness of skin (mg/cm^2).

The concentrations used in the literature are far above the minimum effective levels for this species and may cause extrathyroidal effects in fish.

B. Thyroxine treatment

Thyroxine inhibits the secretion of TSH from the pituitary gland and the activity of the thyroid gland is reduced through a feedback mechanism. The thyroid uptake reduction after injection of 1 µg of L-thyroxine is as great as after an injection of 100 µg. Thyroxine does not suppress thyroid function as much as thiourea.

Guanine and hypoxanthine levels and G/H are not altered in Experiment (ii) which was treatments with 1 µg, 10 µg, and 100 µg of thyroxine, but G/H in Experiment (iv) which was treatment with 3.5 µg of thyroxine, increases significantly and this result is difficult to explain. The skin thickness is not altered after thyroxine treatment.

C. Thyroid powder treatment

Thyroid powder inhibits the thyroid function through the hypo-

thalamo-hypophyseal axis. This treatment was carried out for 5 weeks and at a higher temperature (15° C).

Thyroid powder is more effective in producing silvering than L-thyroxine alone this could be due to the presence of other hormones in the thyroid powder or due to the more prolonged treatment at the higher temperature. Both guanine and hypoxanthine levels measured in mg/cm^2 are significantly increased but the purine % in skin, G/H and skin thickness are not altered.

D. TSH treatment

Mammalian TSH is effective in stimulating the activity of the thyroid gland of the brook trout and this is reflected in all aspects of radiodide metabolism after TSH treatment for 5 weeks and at 15° C.

Endogenous thyroid hormone is more effective in increasing purine levels. Guanine and hypoxanthine levels (mg/cm^2) increase significantly at 0.01 % level, and % G also increases at 0.05 % level, but % H, G/H and skin thickness are not altered significantly though increased slightly.

Thyroid hormone stimulates the purine metabolism in the fish though the specific point in the biochemical cycle of purine production which the thyroid hormone may be effective is not clearly known. Endogenous thyroid hormone is more effective in producing silvering in the brook trout than the exogenous thyroid hormone.

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