THE UNIVERSITY OF MANITOBA

THYROXINE AND IODINE METABOLISM IN THE BROOK TROUT, SALVELINUS FONTINALIS (MITCHILL) DURING PERIODS OF SUSTAINED EXERCISE

by

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ABSTRACT

Thyroid activity of brook trout, forced to continually swim against a current for several weeks (current fish), was compared to that of fish in still water (calm fish).

Following radioiodide injection, % uptake of radioactivity by the thyroid, thyroid/serum ratio, serum protein-bound radioiodide, and conversion ratio, indicated a small but not significant increase in thyroid activity, due to exercise. Serum inorganic radioiodide loss was faster for current fish in two out of three experiments mainly because of greater extrathyroidal excretion.

Levels of serum stable iodide (^{127}I) increased for both groups during the experiments, possibly because the fish were starved. Current fish averages were consistently higher. The slow metabolism of radioiodide in the brook trout, and the considerable variability in radioiodide parameters between individual fish, were shown to be due to the high and variable serum stable iodide levels. T/S and CR were inversely related, while serum $I^{125}I$ was directly related to total serum iodide (^{127}I). % thyroid was often drastically increased when total serum iodide was low.

Following ^{125}I-l-thyroxine injection, rate constants for loss of serum protein-bound radioiodide were faster for current fish. Fish forced to swim for 5 days did not show this effect. Faster $PB^{125}I$ turnover rates for current fish were attributed to increased biliary loss of thyroid hormone.

The level of total serum thyronines was always slightly higher for current fish. For both groups it was extremely low.

Thin layer chromatography of sera from fish 25 hr after T_4* injection revealed substantial amounts of T_3*, radiotriiodothyronine, in addition to T_4*, radiothyroxine, and $I^{125}I$.

It is concluded that for the brook trout, under the conditions of this experiment, there is no clearcut relationship between metabolic stress, due to exercise, and thyroid function. Any changes in thyronine turnover could be attributed to alterations in biliary loss.

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INTRODUCTION

Although thyroid hormones are related to metabolic functions in homeotherms, this relationship is less predictable in poikilotherms, being particularly controversial for fish. There have been two main approaches to the problem in fish.

In the first approach, most studies have involved measuring changes in metabolic rate (O_2 consumption) following administration of thyroid hormones, TSH, thyroid inhibitors, or radiothyroidectomy. Unfortunately there has been little standardization in procedures (Smith and Everett, 1943; Hopper, 1959; Pritchard and Gorbman, 1960; and Mohsen and Godet, 1960). This is especially apparent in the manner by which thyroid hormones have been given (injection, in the food, and addition to the water) and the dose administered. In almost all instances, doses of thyroid hormones have been tried, without knowing the normal physiological level of the circulating hormone and its rate of turnover for the species under investigation. The importance of this one variable alone, in the type of response that might follow, cannot be overemphasized, since in mammals Tata (1964b) found completely different effects following the administration of excessive or small doses of thyroid hormones. Similar criticisms could apply to the administration of antithyroid compounds and methods of thyroidectomy, both of which could result in side effects. From the variety of experimental conditions used, the controversy surrounding the relationship between thyroid function and metabolic rate in fish is not

surprising, and it is often stated that positive results represent the exceptional cases (Gorbman and Bern, 1962). So far little has been concluded from the first type of approach.

The second approach is to follow changes in thyroid activity when the metabolic demands placed upon the fish are increased. This has been attempted in a preliminary fashion by Fontaine and Leloup (1959) and Eales (1963) in Salmo gairdneri; and Bonnet (1970) in Mugil auratus. In each case metabolic rate was increased by forcing fish to swim against a current and these fish were compared with controls, either in a reduced flow, or in still water. Although there were indications of increased thyroid activity, solely based on radioiodide metabolism, this was by no means clearly established. Within a particular study all radioiodide parameters of thyroid activity were not influenced. For example, uptake of radioiodide into the thyroid was not in all cases elevated for the current group. Eales (1963) obtained slightly higher uptakes for his control fish.

No attempt was made to replicate results and the maximum duration of any of the experiments was 18 days, with the total number of sample times only being 4. No attempt was made to study radiothyroxine turnover. However, certain results, which so far have not been verified by any research to date, were found:

(1) Both the level of total stable iodide and total hormonal iodide were increased for fish in the current (Fontaine and Leloup, 1959)

(2) Current fish were noted to have an increased rate of extrathyroidal iodide excretion (Eales, 1963)

(3) Intrathyroidal (iodide and iodotyrosines) were affected by swimming (Bonnet, 1970)

In the present study thyroid function and iodine metabolism have been compared for brook trout, Salvelinus fontinalis (Mitchill) held for prolonged periods in a continuous current, and in still water. Besides studying radioiodide metabolism, the peripheral metabolism of radiothyroxine was investigated to obtain indications of hormone turnover rates and changes in excretory pathways, with exercise. Also the influence of exercise on levels of circulating thyronines and stable iodide (^{127}I) was followed.

In this way it was hoped to obtain a more complete picture than that available from previous studies.

Brook trout were used because they could be obtained in large numbers, and because it is known that certain brook trout exhibit anadromous qualities and a definite migratory pattern, having to swim considerable distances. Sea-run fish have been observed off the eastern shore of Hudson Bay and they inhabit streams and therefore are naturally subjected to currents (MacCrimmon and Campbell, 1969).

LITERATURE REVIEW

This review encompasses four aspects of the study.

A. The Thyroid Gland and Metabolic Rate in Vertebrates

1. Homeotherms

The thyroid gland of homeotherms influences metabolic rate. According to Tata (1964), Magnus Levy in 1895 first discovered the correlation between metabolic rate and thyroid activity, in man. Since that time thyroid hormones have been shown to affect metabolic rate in other homeotherms (Pitt-Rivers and Trotter, 1964; Tata, 1964a). Metabolic response to thyroxine can occur at an early age, as has been shown for the rat (Tirri, Pantio, and Tarkkonen, 1968) and the pig (Kaciuba-Uscilko, Legge and Mount, 1970). The latter authors found a high sensitivity to thyroxine in the young pig, during its first week after birth, when the hormone was not bound to plasma proteins to the same extent, as in the older animal.

A correlation between the biological half-life of thyroxine (T_4) and metabolic rate was achieved in the thyroidectomized rabbit by Tonoue and Yamamoto (1967). They concluded that T_4 accumulation in peripheral tissues led to an increase in metabolic activity, which accelerated the T_4 disappearance rate by raising diffusion and, or, degradation rates of T_4.

The mechanism of thyroid hormone (T_4 and triiodothyronine (T_3)) action in mammals (reviewed in detail by Tata 1964 a,b and 1967)

is still not clear, although certain concepts are emerging. The response to thyroid hormones depends on the dose. Excessive or pharmacological doses reduce the latent period between hormone administration and the metabolic response, and also uncouple oxidative phosphorylation of mitochondria. This uncoupling leads to increased oxygen consumption, by a compensatory elevation of substrate oxidation, to overcome decreased efficiency of mitochondrial phosphorylation. In contrast, physiological doses of thyroid hormones are associated with long latent periods and produce anabolic actions. These actions include:

(1) acceleration of n RNA synthesis and turnover;

(2) increase in the incorporation of amino acids into protein by mitochondria and microsomes;

(3) increase in mitochondrial respiration and phosphorylation;

(4) depletion of hepatic glycogen.

From these and other observations, Tata concludes that the calorigenic action of thyroid hormones at physiological levels is secondary to a general stimulation of cytoplasmic protein synthetic activity. Inhibition of normal protein synthesis resulted in failure of physiological doses to stimulate basal metabolism.

Thyroid hormones are not the sole hormonal regulators of metabolic rate. For man and other animals both T_3 and T_4 potentiate the metabolic effect of catecholamines (Svedmyr, 1966; Tanche and Therminarias, 1969). In adrenalectomized mice, T_4 failed to augment metabolic rate. Small doses of adrenalin administered to adrenalectomized mice restored the effect of T_4 on oxygen uptake

(Subrahmanyam and Lockett, 1965).

2. Poikilotherms

The metabolic response to thyroid hormones in cold-blooded vertebrates has, so far, been inconsistent. Several reviews (Lynn and Wachowski, 1951; Pickford and Atz, 1957; Dodd and Matty, 1964; and Gorbman, 1969) indicate a division on this subject. For convenience most of the relevant literature has been tabulated (Tables I, II, III). In Table I, thyroid hormones administered by a variety of methods and in varying amounts, led to increased metabolic rate in some poikilotherms but not in others. Even within a species different responses have been obtained, for example, in Carassius auratus and Lebistes reticulatus. It can be seen from this table that animal age (weight) could influence the response (Smith and Matthews 1948; Müller, 1953) as well as the experimental temperature (Maher and Levedahl, 1959; Maher, 1961; Maher, 1965; Wilhoft, 1966; and McNabb, 1969), dosage of thyroid hormone (Hopping, 1931; Lewis and Frieden, 1959; Thornburn and Matty, 1964) and the method of hormone administration.

Antithyroid compounds (Table II) do not always depress oxygen consumption (Chavin and Rossmoore, 1956; Pritchard and Gorbman, 1960) and in one study actually increased metabolic rate (Picos, Schmidt, and Popovici, 1969). Thyroidectomy, in general, does not lead to decreased oxygen consumption (Table III). However, Maher and Levedahl (1959) and Maher (1965) stressed the importance of temperature in

Table I. Influence of thyroid hormones on whole body or tissue metabolic rate in poikilotherms

Species	Tissue	Length Volume or Weight	Temperature °C	Thyroid Hormone or Extract	Dosage	Method of Administration	Response and Remarks	Author
Alligator mississipiensis	blood	6-10 ml.	30	T_4	4.5×10^{-3}g. 3×10^{-3}g.	single injection "	150-190% above control 3 days after admin. No change.	Hopping (1931)
Rana temporaria				T_4		injection	No change	*Henschel and Steuber (1931) (P.T.)
Lebistes reticulatus		22 mm.	20-22	T_4	1:500,000	Added to H_2O, H_2O changed daily for 15 days	No change	*Drexler and Issekutz (1935) (S & E)
Cyprinus	muscle		30	T_4	1×10^{-15}g./ml.	in vitro	Respiration stimulated at optimum concentration	*Hearmann (1936) (P & A)
Opsanus tau		350g.	24	T_4 (disodium salt "Roche", 1mg/ml.)	Total dose ranged from 10 to 23.5mg.	injection 2-3 mg/day for 4-5 days. Controls distilled H_2O, pH11	No significant effect (4 out of 5 pairs). In 5th rise of 28%, 9th to 12th day after 1st injection	Root and Etkin (1937)
Caressius auratus			24-27	Desiccated thyroid tablet	1g. fresh gland substance or 2/5 gr. U.S.P. powder	Fed with food (1:1) every 2nd day for week or more. Controls fed every day.	No significant change even after 7-9 weeks of thyroid feeding	Etkin, Root, and Mofshin (1940)
Rana pipiens				Desiccated thyroid or T_4		Fed liver pulp 2x/wk with and without addition of desiccated thyroid or T_4	Increase in O_2 consumption	*Warren (1940) (P & A)
Caressius auratus		18-40g.	20	T_4 (Roche-Organon) and (Squibbs crystalline)	1-6 mg.	intreperitoneal injection	No significant change. Each fish its own control.	Hasler and Meyer (1942)
Lebistes reticulatus		22 mm.	24-25	Desiccated thyroid powder		Fed with food (1:1) Controls regular food. Feeding 7 days or more	Experimental group consumed 12.5% more O_2 on average. Difference not significant	Smith and Everett (1943)
Bathystoma		10-32g.	20-25	Parrot fish thyroid extract		single intreperitoneal injection	Significant rise in O_2 consump. in those fish 15g. or more within 24 hours after injection. No change in fish 16g. or less.	Smith and Matthews (1948)
Caressius auratus		3.5-83g.	15-22	T_4	1 mg.	single intreperitoneal injection	Increase over control. Animals over 15 grams had higher O_2 consump.	
				T_4	0.1-2.0 mg.	injection every 3 days	After 6 to 8 injections marked increase in O_2 consump. In another exp. old animals had x O_2 elevation of 103%, younger animals 43.6%.	Müller (1953)

Table I (cont'd)

Species	Tissue	Length Volume or Weight	Temperature °C	Thyroid Hormone or Extract	Dosage	Method of Administration	Response and Remarks	Author
Acipenser stellatus (larvae)				T_4	1:2,000,000	Added to H_2O	Reversed effect of thiourea. O_2 consumption* control level	*Zaks and Zamkova (1952) (P & A)
Rana pipiens	skin (stages IX-XIV)	350 mm.^{2}	24.8	T_4	1:10,000,000	53 larval animals placed in solution of T_4 (12.5ml. for 48 hrs.)	$\bar{x}$ O_2 consump. of T_4 treated skins significantly higher than controls in every stage except XIV	Barch (1953)
				T_4-cholesterol pellets (20% T_4)		5 pellets implanted subcutaneously on right side. Control pellets on left side. Left in 72 hrs. then O_2 consump. measured.	In 12 of 14 cases QO_2 of experimental side significantly higher than control side	
Salmo gairdneri		20-30g.		T_4		Fed to fish	No significant increase in metabolism	Baraduc (1954)
Carassius auratus			24-25	DL-T_4	0.5mg.	injected daily for 3 days. Saline injected controls	No significant change in O_2 consump. of intact fish	Chavin and Rossmoore (1956)
Pseudoscarus guacamaia		800-1400g.	20	Parrot fish thyroid extract	20mg.	intraperitoneal injection	No increase in O_2 consump. when either parrot fish thyroid extract or L-T_4 injected. Measured 10-12 hrs. after injection	Matty (1957)
				L-T_4	5mg.	controls - saline		
Carassius auratus		10-15g.	20	DL-T_4	1:2,500,000	fish immersed in solution (changed every 3rd day)	No significant change in standard or active metabolism. T_4 increased NH_3 nitrogen content of H_2O	Hoar (1958)
Lebistes reticulatus			not controlled	Mammalian thyroid powder	0.3g. (0.01%)	added to H_2O H_2O changed and hormone renewed 2x/wk for 45 days	Significant increase in O_2 consump. exp. $\bar{x}$ = 321 ml/kg/hr control $\bar{x}$ = 279 ml/kg/hr. Used mature females approx. same weight	Hopper (1959)
Rana grylio		1.0-1.5g.	23	L-T_3; L-T_4	Different dosage levels tried T_3 (50µl; 1.0×10^{-4}M 50µl; 2.0×10^{-2}M) T_4 (100µl; 2.0×10^{-3}M)	single intraperitoneal injection controls injected with saline	Both T_3 doses stimulated O_2 consump. of tadpole to same extent. Increase of 70% in terms of O_2 consump./g. tadpole. Larger dosage of T_4 than of T_3 required to obtain significant response	Lewis and Frieden (1959)
Anolis carolinensis		3-6g.	21-24	Na-L-T_4	20 gamma in 0.025ml. of a 0.9% NaCl soln. Also 10 gamma tried	intraperitoneal injection/day/animal for 4 wks. Controls same volume saline	Significant increase in one out of four experiments	
			30	"	10 gamma	intraperitoneal injection/day/animal for 3 wks. Controls same volume saline	After one week 30% increase over controls. Two weeks 70% greater. Temperature suggested as factor mediating response	Maher and Levedahl (1959)

Table I (cont'd)

Species	Tissue	Length Volume or Weight	Temperature °C	Thyroid Hormone or Extract	Dosage	Method of Administration	Response and Remarks	Author
Squalus suckleyi (embryos)			13-14	L-T_3, L-T_4	10 µg or 100 µg in 0.05 ml	intraperitoneal injection on alternate days Controls 0.7% NaCl Total number of injections given in two day intervals usually 8-10	T_4 (10µg) - In 2 out of 4 experiments elevated O_2 consump. above controls 10-20% T_4 (100µg) - In one test 12% increase above controls T_3 (100µg) - O_2 consump. 18-20% above controls Responses with larger doses observed 10-16 days after beginning injections	Pritchard and Gorbman (1960)
Protopterus		two fish 27.5g. 46g.		T_4 (Roche) 0.5ml.		single intramuscular injection each fish its own control	Increase in O_2 consump. one day after injection, maximal after 4 days.	Mohsen and Godet (1960)
Tilapia mossambica				½ grain thyroid tablets (BDH)		Fed	Increase in O_2 consump. on 1st day after feeding Maximal rate 2nd or 3rd day	Madanmohunrao (1961)
Lacerta muralis			30	L-T_4	0.2 gamma/g.b.w. in saline	daily injections controls same volume saline	20% increase after 1 wk; 2 wks 38%. Within 9 days at 20°C no difference. At 30°C increase of 45% after 1 wk. Suggests temperature influence	Maher (1961)
			20	"				
			30	"				
Necturus maculosus	Liver Kidney Intestine		4	L-T_4, L-T_3	240µg in 0.5 ml. 50% propylene glycol	intraperitoneal injection 3x/wk controls 50% propylene glycol	No significant difference after 4-7 weeks with T_4 and T_3 treatment (T) MIT and DIT no effect (4 wks) (T)	Galton and Ingbar (1962)
				DIT & MIT				
	Ventricle (homogenate)			L-T_4	10^{-4}-10^{-6}M	in vitro	No effect on oxidation of succinate (in vitro)	
Caressius auratus	isolated gills	10-15g. (W.B.*)	21	L-T_4 Na Salt	1:1,000,000	added to Warburg flasks or administered to H_2O	No change when T_4 added in vitro No change in O_2 consump.	Thornburn and Matty (1963)
Bufo bufo	Bladder Skin Kidney		25	T_4, T_3	10^{-5}, 10^{-6} and 10^{-7}M	in vitro Blank runs performed in which no hormone added	10^{-7} and 10^{-6}M T_4 and T_3 significantly increased. O_2 consump. of bladder 10^{-5}M T_4 less of an increase. T_3 slight effect on skin O_2 uptake of kidney diminished by T_4 and T_3	Thornburn and Matty (1964)
Eumeces obsoletus	Liver Skeletal muscle Heart Brain Lung	17-33g. (W.B.*)	30	Na-L-T_4	1 µg in 0.05ml of 0.06% Nacl per 5g b.w.	daily injection for 3 wks. Tissues then removed Controls same volume saline	All tissues except skeletal muscle showed significant rise in O_2 consump.	Maher (1964)

Table I (cont'd)

Species	Tissue	Length Volume or Weight	Temperature °C	Thyroid Hormone or Extract	Dosage	Method of Administration	Response and Remarks	Author
Eumeces fasciatus		4-10g	30 20 30	Na-L-T_4	1 ug in 0.05ml of 0.6% Nacl per 5 g. b.w.	daily intraperitoneal injection controls same volume saline	O_2 consump. significantly higher than control after 3 wks (+28%) 10 days at 20° abolishes T_4 effect (-3%) At 30° significant rise in O_2 consump. after one wk (+32%) Environmental temperature important for response	Maher (1965)
Lebistes reticulatus			26±1	T_4	1:2,000,000	fish immersed for 4 wks	T_4 no effect on respiration If O_2 consump. inhibited by goitrogen, T_4 would restore normal respiration. Suggested that there is an upper limit to the effect of T_4 on respiration	Sage (1965)
Xenopus (larvae)	Tail	0.8 mg dry body weight	25	L-T_4	1:2,000,000	maintained in culture with L-T_4	119% increase in respiration over control tissue 5-7 days after onset of treatment	Heinemann and Weber (1966)
Bufo bufo	Urinary bladder Skin Kidney		16 (W.B.*)	T_4, T_3	1 mg/kg b.w. dissolved in 0.25 ml of 0.9% NaCl solution	Some animals injected into dorsal lymph sac daily for 2 weeks	Pretreatment with T_4 in vivo usually lowered O_2 uptake Affect of T_3 pretreatment varied. T_4 and T_3 little effect in vitro.	Thornburn and Matty (1966)
			25 (T)*		in vitro T_4 and T_3 at 10^{-6}M	in vitro	Alanine in incubating medium resulted in increase in O_2 consump.	
					$1:10^7$-$1:10^{16}$ (for toad bladder) made with saline	Each animal its own control	Wide range of T_4 concentrations stimulated isolated toad bladder in vitro	
Sceleropus cyanogenys	Liver Kidney Brain	$\bar{x}$ 51.5g (W.B.*)	12-60 Thermal gradient 32 (T)	L-T_4	0.2 gamma/g. b.w. in 0.9% saline	Adults received daily intraperitoneal injections for 3 wks. Tissues then excised Controls - saline alone	T_4 caused significant increase for whole animals after 1 wk. All tissues showed significant increase in O_2 consump. with T_4	Wilhoft (1966)
		$\bar{x}$ 0.98g (N.B.)	32 (N.B.*) 16 (N.B.*) also 12-35 Thermal gradient O_2 consump measured at 32	"	0.2 gamma/g. b.w.	Newly born - daily injections (4 wks). Controls - saline alone	Significant increase in O_2 consump. at 32° for newborn lizards and in gradient, but not at 16°C.	

Table I (cont'd)

Species	Tissue	Length Volume or Weight	Temperature °C	Thyroid Hormone or Extract	Dosage	Method of Administration	Response and Remarks	Author
Bufo americanus " bufo " marinus	Ventral skin Urinary bladder Liver		25	Na-L-T_4	1.0-10µg/ml	in vitro	No increase in O_2 consump. of any tissue	Taylor and Barker (1967)
Salmo trutta	Liver mitochondria		10±5 (W.B.*)	T_4	3 ppm (3.75 µM)	introduced into H_2O every 2 days for 14 days	Treatment increased specific activity of oxidative enzyme systems of mitochondria Fall in P:O ratio or decreased phosphorylating efficiency.	Massey and Smith (1968)
	Liver mitochondria			T_4	$5x10^{-8}$ to $5x10^{-7}$M	in vitro	T_4 uncoupled oxidation and phosphorylation at concentration lower than those used for mammals in mitochondria from untreated fish. Succinate and glutamate used.	
Poecilia reticulata			26±1	Na-L-T_4	1 in $2x10^6$	immersion for 4 wks controls kept in tap H_2O	T_4 increased swimming activity but not O_2 consump. If respiration lowered by antithyroid compound would restore to normal level. Response might depend on level of thyroid activity in animal	Sage (1968)
Rana pipiens			30 18	T_4 sodium salt	100µg/100g b.w. in 0.2ml	daily injection into dorsal lymph sac for 16 days or more controls injected with saline	Significantly higher O_2 consump. by 14 days at 30°C. At 18°C, O_2 consump. on basis of means did not differ significantly	McNabb (1969)
Aequidens latifrons		1.2-6.2g.		T_4 (Roche)	0.001%	into the H_2O, renewed every 8 days	After 265 days of treatment O_2 consump. significant when compared to controls (+35%)	Ruhland (1969)

* P.T. (Pitt-Rivers and Trotter, 1964)

* S & E (Smithand Everett, 1943)

* P & A (Pickford and Atz, 1957)

* WB (whole body)

* T (tissue)
* NB (newly born)

Table II. Influence of antithyroid compounds on whole body or tissue metabolic rate in poikilotherms

SPECIES	WEIGHT (g)	TEMPERATURE °C	ANTITHYROID COMPOUND	DOSAGE	METHOD of ADMINISTRATION	RESPONSE AND REMARKS	AUTHOR
Fundulus heteroclitus	6.4-20.2	20.8-21.4	Thiourea	0,5-1.0mg/g	intreperitoneal injections for 5-6 days, daily or on alternate days controls untreated	Thiourea did not depress O_2 consump.	Matthews and Smith (1947)
Campostoma anomalium pullum			Thiouracil	12 grains/15l tap H_2O (517 p.p.m.) 9 grains/15l tap H_2O (388 p.p.m.)	continuous H_2O bath - in one out of three experiments renewed on 3rd and 7th day (2nd concen) controls - untreated H_2O	By day 2, O_2 consump. 20% below controls. Unless bath renewed metabolic rate gradually increased	Osborn (1951)
Acipenser stellatus (larvae)			Thiourea	0.033%	addition to medium for 9 days	O_2 consump. decreased as much as 18.8%	
Salmo salar (larvae)		9-12	"	0.033%	immersed for 16-150 hours	O_2 consump. decreased when compared to controls	*Zaks and Zamkova (1952) (P & A)
Carassius auratus	3.5-83		Methylthiouacil	0.05-0.1ml of a 5% sodium solution	injection	After Methylthiouacil injection, injection of TSH on next day ineffective in elevating O_2 consump. Inhibition by M.T.U. marked up to 4 days after injection	Muller (1953)
Rana pipiens	20-35		6-n-propylthiouracil	0.033mg/g. wet weight	injection via dorsal lymph sac 3x/wk for 5 wks. control uninjected	$\bar{x}$ O_2 consump. decreased exponentially with time, reaching a value of 40% below initial value	Calhoon (1955)
Carassius auratus		24-25	Thiouracil	0.05%		Thiouracil did not alter O_2 consump. after 11 and 26 days of treatment	Chavin and Rossmoore (1956)
Squalus suckleyi (embryos)		13-14	Propylthiouracil	50ug	intraperitoneal injection on alternate days total number, 8-10	No effect on O_2 consump relative to saline injected controls. This based on 4 experiments (approx. 18 days each)	Pritchard and Gorbman (1960)
Lebistes reticulatus		26±1	Thiourea	0.1%	immersion for 4 wks. controls in tap H_2O	Thiourea inhibited O_2 consump. when allowance made for size differences. Activity reduced. Thiourea acting via thyroid gland	Sage (1965)
Sceloporus cyanogenys (liver) (kidney) (brain)	51.5 (W.B.)	12-60 Thermal gradient 32 (T)	Thiourea	0.3mg/g.b.w in 0.9% saline	daily intraperitoneal injection for 3 wks controls - saline	Statistically significant reduction of O_2 consump. after 1 wk (in vivo) which continued for the experimental duration. Tissues after in vivo administration of thiourea showed a significant decrease in O_2 consump.	Wilhoft (1966)
(newly born)	0.98 (W.B.)	12-35 thermal gradient O_2 consump. measured at 32 (all experiments)	Thiourea	0.3mg/g.b.w.	injections every other day controls - saline	Significant reduction in O_2 consump. at 32°C	
Poecilia reticulata		26±1	Thiourea	1 in 10^3	immersion (4 wks.) controls in tap H_2O	Thiourea treated fish less active. Decrease in O_2 consump. (thiourea) Ratio of activity to the respiratory scope for activity of thiourea treated and control animals equal	Sage (1968)
Carassius auratus		7-9 5-7	Thiourea "	1g/l H_2O 2g/l H_2O	in H_2O treatment for 30 days	O_2 consump. increased on average by 61.23% O_2 consump. increased by 158.92% Hypermetabolic effect of thiourea began after 1st 3 days of treatment	Picos, Schmidt and Popovici (1969)
Aequidens latifrons	1.2-6.2		Thiourea	0.03%	in H_2O renewed every 8 days	Thiourea rapidly decreased O_2 consump. (-24%) but this chemical blocking not stable	Ruhland (1969)

* P & A (Pickford and Atz, 1957)

Table III. Influence of TSH and thyroidectomy on whole body metabolic rate in poikilotherms

SPECIES	LENGTH or WEIGHT	TEMPERATURE °C	TREATMENT	DOSAGE	METHOD of ADMINISTRATION	RESPONSE AND REMARKS	AUTHOR
Lebistes reticulatus		20-22	TSH		in H_2O, 1-28 days	No effect on O_2 consump.	Drexler and Issekutz (1935)*
Triturus torosus			Thyroidectomy		surgical	O_2 consump. declined to 72.5% of normal 2-3 wks. after operation	Taylor (1936)**
Carassius auratus	8.3-10.2g (young)		TSH	3-4mg.	single intraperitoneal injection	O_2 consump. increased; range 32.7-89.5% $\bar{x}$ = 56.5%	
	56-74g (old)	16	(in form of HVL dried powder)	5-6mg.	controls - saline	O_2 consump. increased; range 146-241% $\bar{x}$ = 186%	Muller (1953)
(young)				3mg.	injected every 3rd day for 48 days	Average increase 52.5%	
(old)				5mg.		Average increase 89.1%	
Scyllium canicula	60-70cm	16.2-17.6	Thyroidectomy		surgical controls - sham operation	No significant decrease in O_2 consump. of either group for 42 days after operation No difference between groups when compared every 3rd day.	Matty (1954)
Carassius auratus		24-25	TSH	0.3 U.S.P. units	injected daily for 3 days controls - untreated or injected with 0.05ml saline	Statistically significant increase in O_2 consump. (40.8%)	Chavin and Rossmoore (1956)
Salmo gairdneri	3.8-6.0g	15.5-16.5	Thyroidectomy	250 µCi	intraperitoneal injection of carrier free ^{131}I as NaI controls - not injected	Thyroidectomized $\bar{x}$ O_2 consump. = 0.21ml/g/hour control $\bar{x}$ O_2 consump = 0.20ml/g/hour Difference not significant	Fromm and Reineke (1956)
Pseudoscarus guacamaia	40-60cm.	22-26	Thyroidectomy		surgical each animal its own control	No decrease in O_2 consump. (measurements made up to 39 days at 3-9 day intervals) A few fish thyroidectomized for up to 124 days failed to reveal any change	Matty (1957)
Anolis carolinensis	3-6g	10 20 30	Thyroidectomy		surgical controls - unoperated or sham operation	In general not significant at low temperatures. At 30° thyroidectomy caused significant decrease in O_2 consump.	Maher and Levedahl (1959)
		20	TSH (thytropar)	4mg.	single injection/day controls - saline	No change in O_2 consump. at 20°c (TSH).	
		30				Significant difference after 2wks 30° (TSH)	
Eumeces fasciatus	4-10g	30	Thyroidectomy		surgical controls - not operated on	After 3 wks significant difference (O_2 consump. lowered)	Maher (1965)
		20				After 10 days at 20°C difference abolished	
		30				1 wk after returning to 30°C significant difference	

* Pickford and Atz.(1957)

** Lynn and Wachowski (1951)

mediating this response. In two separate studies, TSH (Table III) increased the oxygen consumption of Carassius auratus (Müller, 1953; and Chavin and Rossmoore, 1956) however, no effect was observed with Lebistes reticulatus (Drexler and Issekutz, 1935) and Anolis carolinensis at 20°C (Maher and Levedahl, 1959).

In the course of evolution, mammals could have developed a metabolic response to T_4, or a number of factors peculiar to poikilotherms could be hindering the solution to the problem and masking the response, for it is known that treatment with T_4 brings about increased locomotor activity in fish (Hoar, Keenleyside and Goodall, 1955 and Sage, 1968). Factors such as the difficulty in obtaining a basal metabolic rate, and cyclical behavior associated with reproduction and photoperiod, are considerable problems when investigating poikilotherms. Also temperature could definitely affect metabolic response, since as Matty (1954) pointed out, the temperature of poikilotherms is usually lower than that of homeotherms, as is the rate of oxidative metabolism. It has been estimated that the thyroid secretion rate of trout is also lower (10 to 19 times) than found for homeotherms (Hoffert and Fromm, 1959).

The level of thyroid activity in fish may be important in determining whether or not an increase or decrease in respiratory rate might follow the administration of T_4 (Sage, 1968).

Hoar (1958) refers to a paper by Mansfeld (1949) who suggested that the primary effect of thyroid hormone is one of accelerating the splitting of protein, and that these products lead secondarily to

increased oxygen consumption. In poikilotherms he considered the buildup of breakdown products insufficient to slowly call forth the stimulatory effect on oxidation processes.

It is possible a particular form of stress might be required to stimulate both metabolic and thyroidal activity, as indicated by Hickman's study on the starry flounder in 1959, where stress due to the fish being in salt water, increased both metabolic rate and thyroid activity.

A site of action for thyroid hormones on fish metabolism was claimed by Hochachka (1962). Both T_4 and T_3 increased the rate of gluconate oxidation by liver homogenates and slices by as much as 125%. Glucose metabolism was thought to proceed by the pentose phosphate pathway. Also Massey and Smith (1968) found that T_4 administered in vitro to mitochondria of brown trout uncoupled oxidation and phosphorylation at lower concentrations than those reported for mammals.

In summary, there is definitely sufficient evidence in the literature, so as not to exclude a possible role for the thyroid gland on poikilotherm oxygen consumption, and as suggested above, several factors could be masking the response.

B. Exercise

1. General Discussion of Exercise and Its Effect on the Physiology of Fish

Activity is one of the many factors, considered by Brett (1962) which influences oxygen consumption in fish (Beamish and Mookherjii, 1964; Brett, 1964; Brett and Sutherland, 1965; and Smit, 1965). Metabolic response to imposed exercise is regulated by factors such as fish weight (Job, 1955; Brett, 1965; Rao, 1968); temperature (Graham, 1949; Gibson and Fry, 1954; Job, 1955; Brett, 1964; Brett, 1967; and Dickson, 1968); oxygen and carbon levels (Graham, 1949; Basu, 1959; Davis et al, 1963; Kutty, 1968; Dalberg, Shumway and Doudoroff, 1968), and salinity (Rao, 1968; and Farmer and Beamish, 1969).

Intense muscular exercise taxes aerobic means of deriving energy to the limit and when oxygen is limiting fish must derive more energy via the anaerobic metabolism of glycogen, resulting in the accumulation of pyruvic and lactic acid and oxygen debt. During recovery from this debt fish undergo deep respiration, which aids the oxidation and conversion of accumulated lactic acid to glycogen.

Black (1955, 1957 a,b), Black et al (1960), Black et al (1966), and Stevens and Black (1966) have studied these changes extensively, monitoring blood pyruvate and lactate, and liver and muscle glycogen, for a number of fish species. Intense muscular exercise for 15 min in all instances significantly increased blood lactate over the unexercised condition. Recovery to control levels required 16 hr for

yearling Kamloops trout (Black, 1957a). Moderate exercise (40.7 ft/min) for 30 min produced changes, less marked, but still different from the controls. Pyruvate also increased following 15 min of strenuous exercise, but moderate exercise of 20 min duration, resulted in a value only slightly higher than the unexercised state.

As expected, muscle glycogen diminished rapidly (80% during 5 min of exercise) following severe exercise of short duration in 1½-year-old rainbow trout (Stevens and Black, 1966). With re-exercise a further decrease in muscle glycogen levels was brought about, and a correspondingly greater increase in muscle and blood lactate levels occurred.

The degree of influence of exercise on fish carbohydrate metabolism depends upon the experimental conditions. For example, severe exercise for 2 min coupled with starvation (over a period of 84 hrs) resulted in a greater decrease in muscle glycogen than for fed, exercised rainbow trout. However, liver glycogen values did not change significantly for either group and there was overlap for muscle lactate changes, between the two groups (Black, Bosomworth and Docherty, 1966). When a low temperature (0.2°C) was combined with starvation for 25 days, Salmo salar exercised to exhaustion, had slightly higher liver and muscle glycogen levels when compared to the mean values for fed, exercised fish. Blood lactate levels did not differ (Wendt, 1965). Therefore, temperature could modify the effect of starvation with exercise.

Dean and Goodnight (1964) showed that temperature, itself, can influence the effect of exercise on carbohydrate metabolism, depending

upon the species of fish under investigation. For example, Lepomis macrochirus at 5°C was the only species, out of 4 investigated, not to exhibit a decrease in muscle glycogen with exercise, at 5 and 20°C. Also, blood glucose increased with exercise at 5 and 20°C, for all species except L. macrochirus at 5°C.

Metabolic rate changes during the process of recovery from oxygen debt have been monitored by Heath and Pritchard (1962) for L. macrochirus and by Brett (1964) for the sockeye, Oncorhynchus nerka. L. macrochirus required 10 to 25 hr to return its metabolic rate to the pre-exercise level, while Brett estimated a time of 3.2 hr, independent of acclimation temperature, for sockeye to regain spontaneous activity.

The manner in which current is applied is most important when considering physiological changes. For example, physically training trout to a current, or conditioning them, brings about interesting results (Hochachka, 1961; Hammond and Hickman, 1966). Hochachka physically conditioned rainbow trout to a current (1 ft/sec) for 6 months at 4°C. Conditioned fish were found to sustain an oxygen debt three times that of unconditioned fish. Also the trained fish when fatigued were able to utilize more of the available glycogen in muscle, than the untrained fish. The amount of blood haemoglobin was greater for trained rainbow trout. Also, they had larger hearts. Hochachka considered that since greater buffering and oxygen-carrying capacity are consequences of the high haemoglobin concentrations, then the trained fish could tolerate more lactic acid accumulation.

Rainbow trout were conditioned to velocities of 20 cm/sec and 40 cm/sec for 16 days by Hammond and Hickman (1966). The trained

trout resisted fatigue better than the controls from still water, when all 3 groups were tested at a high current velocity (53 cm/sec). Significantly greater lactate concentrations appeared in the muscle of conditioned trout, but the rate of removal was fastest for these groups after cessation of activity. Similar trends were reported for plasma lactate. Plasma phosphate following exercise was significantly higher for the conditioned and control fish, but the differences between the three groups were not significant.

It may be concluded that exercise causes physiological changes within fish. The extent of these changes depends upon the form of exercise and on experimental conditions.

2. Exercise and Thyroid Activity in Fish

There are, to the authors' knowledge, three experiments designed to test the relationship between thyroid function and exercise in fish.

Fontaine and Leloup (1959) conditioned rainbow trout for 10 days to a current of 0.4 m./sec (11 hr/day) at 9 to 11°C. The fish were then injected intraperitoneally with 5 μCi of ^{131}I and killed 24, 96, and 188 hr after injection. They obtained a greater thyroid uptake of radioiodide for the current group, at the three sampling times. Labelled hormone iodide (PBI) was 3 times higher for the current fish 188 hr after injection, but there was no increase in conversion ratio. Total stable plasma iodide was greater for the current group as was the stable hormonal iodide bound to the proteins (2.7 mcg% versus 2 mcg% for the controls). Elevation of total iodide levels in the current group was attributed in part to loss in mineral iodide (inorganic)

from muscle to serum.

When determining the influence of exercise on ^{131}I metabolism of steelhead, Eales (1963) first injected the fish and then placed half of them in a fast current and the remainder (controls) in a slow current (20% of fast current). Sampling was continued up to 8 days, over a temperature range of 10 to 10.5°C. Increased swimming increased the loss of inorganic radioactivity from the whole body, slightly decreased the percentage uptake of ^{131}I into the thyroid, and slightly increased the conversion ratio.

Mugil auratus were exercised at 7 cm/sec for up to 15 days by Bonnet (1970) and were compared to a control group in still water. Both groups received an injection of ^{125}I, 48 hr before they were killed. Temperature was held at 12°C. After 7 days swimming the percentage of labelled MIT (monoiodotyrosine) plus DIT (diiodotyrosine) within the gland, diminished for the current group. However, the thyroid uptake factor (T.U.F.), the plasma protein-bound hormonal iodide ($PBH^{125}I$), and the percentage of T_3 plus T_4 within the gland, were not different from the control. At 15 days the current group had a higher T.U.F. (12.61% rather than 7.75%) and a greater amount of radioactive iodotyrosines (64.92% instead of 58.64%) within the gland. However, iodothyronines revealed little change from the control, as did the level of plasma $PBH^{125}I$.

Less direct evidence for a relationship between exercise and thyroid function has been suggested by Leloup and Fontaine (1960). They point out that high values of hormonal iodine (^{127}I) are especially characteristic of diadromous fish (salmon, sea trout and

shad). Also rates of hormonal secretion (mcg. hormonal iodine/100 gram body weight/day) are higher for fish engaging in upstream migration. In addition, secretion of T_4 by the lungfish is greatly decreased during estivation. These observations were correlated with greater oxygen consumption. It should be stressed that the high values of hormonal iodine obtained, could be the result of contamination, since the diadromous species also had the highest levels of plasma inorganic iodide (^{127}I).

Other conclusions have been reached concerning the basis for increased thyroid activity during migration. Most of the following studies deal with downstream migration, however, the conclusions reached, in all likelihood, could apply to upstream migration.

Increased thyroid activity (histological) in migrating Pacific salmon was thought by Hoar and Bell (1950) to be a function of the increased metabolic work of osmotic regulation and salt balance. Baggerman (1960) stressed photoperiod, as causing an increase in thyroid activity, at the onset of migration, which disturbs osmoregulation. She also pointed out that osmoregulation may increase the demands for thyroid hormones even further.

The seasonal factors of temperature and photoperiod were considered by Eales (1963) to be the possible causes for the increase in thyroid activity observed for yearling sockeye, and coho salmon at the time of migration. Both histological and radiochemical criteria showed similar trends. Besides these, the process of smoltification and the importance of the ambient level of ^{127}I, coupled with temperature effects on iodide excretion, were discussed by Eales as possibilities

that could contribute to heightened thyroid activity.

In contrast to the views of Leloup and Fontaine (1960) Woodhead (1959) considered that thyroid activity (histological) in the immature cod Gadus callarias L. initiated and sustained active and long migrations.

On evaluating the above literature and taking into account the drastic physiological changes which occur within fish at the time of migration, it would be a mistake to assume an effect of exercise, by itself, on thyroid activity.

3. Exercise and Thyroid Activity in Higher Vertebrates

A response by the thyroid in higher vertebrates to increased exercise has occurred in some instances, but not in others.

A flighty strain of chickens showed a thyroxine secretion rate (T.S.R.) (mcg. L-T_4/100 g.b.w./day) 67.6% higher than a docile strain. Docile birds, however, had a greater percentage thyroid uptake of ^{131}I (Srivastava and Turner, 1967).

An influence of exercise on some aspects of thyroid function has been claimed for the rat (Escobar del Rey and Morreale de Escobar, 1956b; Rhodes, 1967). In the former study thyroidectomized rats maintained on L-T_4, exercised for 12 hr in 24 hr, demonstrated a faster disappearance rate for ^{131}I labelled - L-T_4 than the controls. This contrasts with a previous study where the T_4 - disappearance rate was unaltered, when rats were forced to swim for 2 hr, and killed 3 hr after injection of T_4 (Escobar del Rey and Morreale de Escobar, 1956a).

Rhodes (1967) found a correlation between the amount of exercise and thyroidal storage of iodine. Non-exercising rats had approximately twice as much thyroidal iodine, as exercising rats. He concluded that this was due to a greater utilization of thyroid hormone during exercise, resulting in less storage of dietary iodine, and more rapid conversion to circulating hormonal iodine.

For partly- and fully-trained horses, Irvine (1967) obtained thyroxine secretion rates respectively 38% and 65% higher, than for the untrained horses. The combination of lower values for serum $PB^{127}I$, and greatly decreased serum half-lives of labelled T_4 from controls, was cited as the cause.

Conflicting results have been reported for man. Lashof et al (1954) could not alter the periperal utilization of thyroid hormone with exercise. A decrease in the circulating free-thyroxine level after exercise was found by De Nayer et al (1968). Thyroxine degradation (secretion rate) increased up to 75% for athletes in moderately severe training, when compared to those of resting non-athletes. Also, $PB^{127}I$ and free-T_4 levels did not differ significantly (Irvine, 1968). The increased peripheral degradation was thought to be due to increased peripheral deiodination of T_4.

From the data on fish and higher vertebrates, it seems evident that exercise does not accelerate all measurable indices of thyroid activity, in a single study. In some cases there is no effect of exercise whatsoever.

C. Stable Iodide

1. General Considerations of Stable Inorganic Iodide Levels and Their Effect Upon Radioiodine Metabolism in Fish

Blood iodide levels in fish are subject to considerable fluctuation which can be correlated to the following:

(1) movement from rich to poor iodide environments (Leloup and Fontaine, 1960);

(2) seasonal fluctuations in water iodide content (Hickman, 1962);

(3) elevated temperature resulting in greater iodide excretion (Leloup and Fontaine, 1960);

(4) diet iodide levels (La Roche, Johnson and Woodall, 1965);

(5) sexual maturation (Robertson and Chaney, 1953).

In general Hickman (1962) found that serum and thyroid inorganic iodide concentration in Platichtys stellatus changed seasonally, paralleling the changes in environmental iodine and salinity. Both Hickman (1962) and Leloup and Fontaine (1960) noted that certain fish, in a medium poor in iodine, are capable of a very high serum iodide concentration. Hickman speculated that active absorption from the water might be the mechanism. In contrast, Leloup and Fontaine suggested that diadromous teleosts have high levels of plasma iodide, due to their capacity for greater binding of iodides, subsequently verified by Huang and Hickman (1968).

Water iodine levels can drastically affect the thyroid gland when in low concentration, resulting in thyroid hyperplasia. Marine

and Lenhart (1910) were able to alleviate this condition by adding iodine (Lugols solution) to the water for up to 22 days. By daily feeding butter fish, Stromateus triacanthus, high in iodine, Marine (1914) eliminated and prevented return of thyroid hyperplasia in brook trout, previously fed on hog's liver and heart muscle. Robertson and Chaney (1953) also noticed this condition could be abolished in spawning Lake Superior trout, which had access to additional iodine in food, discharged from the holding ponds of a hatchery. Diets containing marine fish and sea weed were recommended by Radulescu et al (1968) for the treatment of thyroid tumors in brook trout.

There are a number of studies showing that the uptake of radio-iodine by the thyroid, can be modified through various iodide levels in the water, or in the diet (Gorbman and Berg, 1955; Hickman, 1959; Srivastava, 1960; La Roche, Johnson and Woodall, 1965). The general trend is one of greater thyroid uptake when fish are maintained on low iodine diets, or in water of low iodine content. Low environmental levels of iodine according to Hickman (1959), stimulate the activity of the thyroidal iodide trapping mechanism. Little attempt has been made to correlate thyroid uptake to blood iodine levels. This is briefly considered by Leloup and Fontaine (1960), when comparing thyroid uptake of the normal eel at 6.5°C, after treatment with TSH, to control eels. Apparently the TSH-treated eels had a greater level of plasma inorganic ^{127}I and this, by decreasing the specific activity of ^{131}I in the plasma, contributed to the apparent inhibition of the mechanism of iodine fixation. Robertson and Chaney (1953) obtained an inverse relationship between cellular thyroid follicle height and

blood iodine concentration. Also ripe and spent sea-run rainbow, and iodine-fed trout demonstrating no thyroid hyperplasia, had high blood iodine concentration, when compared to spawning Lake Michigan rainbow trout in similar condition, showing thyroid hyperplasia.

Taking into account variables, such as ^{127}I content in the interior and exterior environment, and rates of radioiodine excretion, Leloup and Fontaine (1960) made the statement that low thyroid uptake of radioiodine is not necessarily an index of thyroid hypoactivity. Both they, and Hickman (1959) go further and question the validity of thyroid uptake measures when these factors are not considered.

2. Effect of Stable Iodide Levels on Thyroid Function in Other Vertebrates

For the marine iguana, which feeds on algae rich in iodide, thyroidal iodide uptake was extremely slow (2½% in 25 days). Lowenstein and Stebbins (1969) suggested high plasma iodine levels were in part the cause. Feeding an iodine-deficient diet to chicks resulted in rapid uptake of injected ^{131}I (41.19% at 2 hr) as compared to the control maximum (4.52% at 8 hr) (Featherston, Boehm and Rogler, 1966).

Similar trends in ^{131}I uptake by the thyroid following administration of low- and high-iodine diets to cockerels and rats were reported by Rosenberg, Goldman, La Roche and Dimick (1964). These authors stated that high thyroidal ^{131}I uptakes in rats on low iodide diet were, due partly to the high specific activities of circulating iodide after ^{131}I injection.

Various doses of stable iodide administered to human subjects were found to alter the pattern of thyroid radioiodide uptake to the point where the gland could be blocked (Ramsden, Passant, Peabody, and Speight, 1967).

A number of studies on man and other mammals have considered the effects of iodide other than those due to alterations in the specific activity of administered radioiodide as an artefact in radioiodide metabolism. Iodide inhibition of thyroid function could conceivably occur at the pituitary, thyroid or peripheral levels (Ochi and DeGroot, 1969).

Inhibition at the pituitary level could not be demonstrated by Abbassi and Mckenzie (1967). Both DeGroot (1966) and Galton and Ingbar (1967) were unable to find any effect of excess iodide on the peripheral metabolism of T_4. This leads to a direct action of iodide on the thyroid. Buhler and DeGroot (1969) demonstrated a decrease of hormone release after KI administration, due to diminished thyroid degradation of thyroglobulin in the toxic human gland. Excess iodide was found to directly block the release of thyroid hormone caused by TSH or LATS, when administered 24 hr prior to their injection (Ochi and DeGroot, 1969).

Finally Burke (1970) was able to show that iodide inhibition of thyroid secretion occurred at one or more steps in glandular hormonogenesis, prior to adenyl cyclase activation and colloid droplet formation. Green (1966) adds supporting evidence for Burke's results. He noted that inorganic iodide (concentration 50 mM) modified thyroidal intermediary metabolism in vitro both by inhibiting glucose metabolism, involving both the hexosemonophosphate shunt and aerobic glycolysis,

and inhibiting Krebs-cycle oxidations.

Iodide deficiency results in functional changes of the thyroid. During sustained periods of dietary iodide deficiency, Greer, Grimm, and Studer (1968) concluded that rats preferentially synthesize and secrete, T_3 over T_4, because of the greater need to conserve iodide and the greater potency of T_3. Karmarkar et al (1969) found that goats from an area of iodide deficiency also had higher T_3/T_4 ratios in their thyroids, as well as higher MIT/DIT ratios, when compared to the control area. The T_3/T_4 ratio rather than being considered an adaptive mechanism was thought to be a direct consequence of iodide deficiency, based on the MIT/DIT ratio and the structure of thyroglobulin.

D. Some Effects of Starvation on Fish Physiology

Brett (1962) defined standard metabolism for fish as the least rate of metabolism commensurate with appropriate experimental techniques, while routine metabolism expresses the average oxygen consumption of fish, which are moderately active. It has been shown that both standard and routine metabolism can be depressed with starvation (Hickman, 1959; Beamish, 1964; Parvatheswararao, 1965, 1967; Glass, 1968; and Dickson, 1968).

According to Beamish (1964) this trend with starvation, can reflect both a reduction in the oxygen requirement for food assimilation, and a subsidence of spontaneous activity.

Active metabolism (the maximum rate consistent with the highest continued level of activity (Brett, 1962)) for rainbow trout was found

by Dickson (1968) to be independent of starvation during a 9-day period. As outlined by Dickson (1968) there would appear to be little evidence to suggest that active metabolism is influenced by starvation.

Starvation also can lead to some of the following physiological changes:

(1) an increase in the level of plasma free fatty acids (Bilinski and Gardner, 1968).

This was observed for rainbow trout after 5 and 14 days without feeding, but from then on levels of free fatty acids remained relatively constant up to 70 days.

(2) a decrease in liver glycogen and plasma glucose, an increase in plasma pH and hemotocrit (after 30 days) for the Atlantic cod (Kamra, 1966)

(3) a rise in the level of tissue water and a reduction in the level of serum proteins for the carp, with prolonged starvation. Serum proteins decreased by about a half with four to six months of starvation (Creach and Bouche, 1969).

The latter authors found that hematocrit in the carp progressively decreased at a slow rate after 15 days starvation in comparison to fed fish.

The effects of starvation combined with exercise, on some aspects of carbohydrate metabolism, have been considered under the general discussion of exercise in this literature review.

MATERIALS AND METHODS

A. Living Material

1. Maintenance of Stock Fish

Brook trout were obtained from the Province of Manitoba Trout Hatchery, West Hawk Lake, Manitoba, Canada. Most fish were from a common stock, which originated from the Ontario Department of Lands and Forests, Dorion Hatchery brood stock. Mixing on an extremely minor scale might have occurred with brook trout from Washington State, U.S.A. and Gods River, Manitoba.

The Hatchery fish were held either in large outdoor circular cement tanks, or in rectangular cement troughs, and were subjected to a slight current. They were fed Glencoe Minneapolis pellets, at least once, and sometimes twice a day. Its composition conformed to the U.S. Fish and Wildlife specifications.

As required fish were transported from the hatchery to the laboratory at the University of Manitoba. Here they were either held in a 560-l fibreglass tank, or transferred directly to the experimental tank, both having continuously running, aerated, dechlorinated water.

An almost natural outdoor photoperiod was experienced by fish in the holding tank, since this tank was situated in a room with several windows and had a top permitting light penetration. Superimposed on this illumination were the room fluorescent lights, and a small amount of light from an adjacent building at night. An attempt was made to regulate the room lighting from 8:00-8:30 a.m. to

5:00-5:30 p.m., approximately 9 hr/day. Water temperature in the holding tank varied with the time of year (Table IV).

The holding stock were fed, so that on the average, each fish (based on a 15-cm. fish) received a total, over three feedings of 4.7 grams of food (wet weight) per week. Size differences were taken into account, since both one- and two-year-old fish were used during the course of the experiments. The food consisted of a frozen homogenate of beef liver, ocean perch fillets (10:1 w/w) and a small amount of gelatin, the latter two constituents being eliminated from the diet after the end of November, 1969.

2. Maintenance of Experimental Fish

Stock trout, following transportation from the hatchery, were acclimated for a least 3 weeks prior to injection, at a temperature between 9.5-13°C. This temperature range was the most practical to maintain on a seasonal basis, and is not far removed from the optimum for the species. At times of year when the temperature of the holding tank fell within, or was very close to this range, the acclimation period in the experimental tank was appropriately reduced, with the total time of acclimation still maintained at not less than 3 weeks. Brett (1956) stated that some species require up to 20 days to gain resistance to low temperature. Since the experimental tank was in the same room adjacent to the holding tank, conditions of illumination were similar. However, the experimental tank was closer to a window and sufficient artificial low intensity light (from a nearby building)

Table IV. Water Temperature (°C) of the Holding Tank - June 1969-May 1970

	1969							1970				
MONTH	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY (1st half)
Average Monthly Temp °C	11.4	14.5	17.7	17.4	12.2	7.6	6.1	4.4	3.5	3.0	3.1	4.3
Temp °C at beginning and end of each month	9.8-12.1	11.1-15.6	15.8-18.9	17.1-15.3	15.0-9.1	8.8-6.4	6.9-6.3	5.5-3.5	3.6-3.1	3.2-3.7	2.7-4.2	4.0-4.4

passed through the clear fibreglass top to permit the fish in the current to maintain position.

Feeding of fish, totally acclimated in the experimental apparatus, was on the same basis as for those fish in the holding tank. In all experiments, feeding was discontinued for the experimental duration once the current was started, usually 10-14 days before radiochemical injection.

Starvation was felt to be necessary because excess food was not easily eliminated from the experimental tank, thereby increasing the risk of fungus infection. Also, as the diet contained thyroid hormones, it could not be assumed that each fish would consume the same quantity of food, and hence a similar amount of thyroid hormone. For fish in the current, appetite might have been influenced to a greater extent, as might have been the dominance behavior in feeding associated with size differences.

Only in 2 out of 7 experiments was it necessary to control fungus, this being accomplished through the addition of malachite green (1% solution; 6 ml added/200 l H_2O) to the water. The conditions of current and starvation proved to be very efficient in checking fungus infection, and never did fish have to be treated after the current was commenced.

Oxygen content of the water was kept above 8 ppm and for the most part was 85% saturated. This was well above the minimum safe concentrations for brook trout (4 to 5 ppm) and above 75% saturation, below which scope for activity is reduced (Graham, 1949).

When monitored the pH ranged from 6.9 to 7.2 in the experimental apparatus.

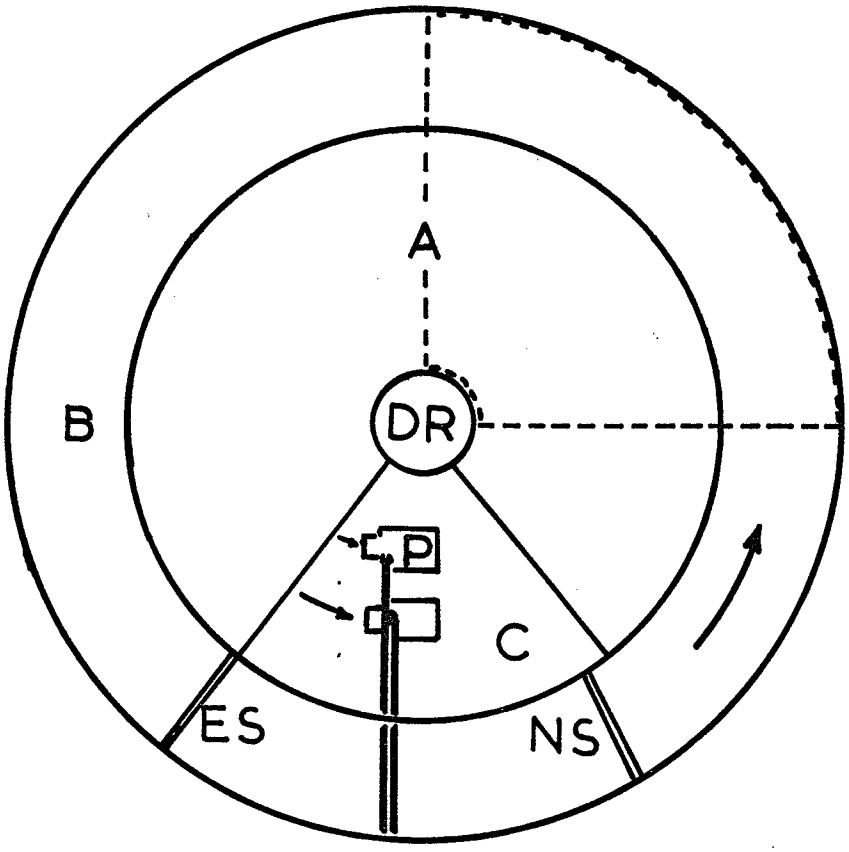
3. Experimental Tank

A 500-gallon fibreglass tank, 6 feet in diameter, was modified to provide a central calm water compartment and a peripheral compartment (flume) in which a current could be created by means of submersible pumps (Little Giant Pump Co.) in a third compartment (Fig. 1). All compartments were interconnected to ensure uniform temperature, by a narrow space at the bottom of the circular fibreglass hoop, and through a number of small holes. A similar apparatus has been described recently by Bonnet (1970). Water was forced counterclockwise around the flume out of a series of holes drilled in two parallel plastic pipes extending from the pumps, and set horizontally at 90° to the direction of current flow. The downstream screen (upstream of the pipes, but downstream of the fish) consisted of a number of vertical strands of stainless steel wire (#16 gauge) spaced $\frac{1}{4}$ to 3/8's" apart, in a fibreglass frame. Every alternate wire was positively charged, so that if a fish touched the grid, it would complete the circuit and receive a shock (3.5 to 6.5 volts D.C.). A variable auto transformer connected to a D.C. power supply regulated the voltage.

The nonelectrified screen was positioned sufficiently downstream of the pipes to prevent fish from resting in the variable current recorded in that vicinity.

The current profile was fairly uniform vertically, but did vary horizontally. The average velocity measured at three different positions, was 32 cm/sec (1.05 ft/sec) closest to the hoop, and 48 cm/sec (1.58 ft/sec) on the outside. The minimum and maximum currents

Figure 1. Experimental tank consisting of three compartments: calm water (A), current (B) and pump (C). The positions of the central drain (DR), pumps (P), electrified screen (ES) and nonelectrified screen (NS) are shown. Arrows indicate the direction of water flow, while the space enclosed by broken lines represents one section of the fibreglass cover.


A
B
DR
P
C
ES
NS

were 26 cm/sec (0.85 ft/sec) and 56 cm/sec (1.84 ft/sec). These were measured under ideal conditions, with no fish in the flume, and were probably slightly higher than under experimental conditions, when small amounts of fecal material adhered to the pump filters. These were cleaned periodically during an experiment. In practice, the fish stayed in the middle to inside of the current profile and probably swam close to 1 ft/sec. This was the current velocity used to train trout by Hochachka (1961).

For convenience in sampling, the clear fibreglass cover, for the apparatus, was subdivided into four sections.

Although the design of the apparatus had certain faults, for example, non uniformity in current velocity, it provided a good means for exercising large numbers of fish at a time and comparing samples of these with fish kept simultaneously under identical conditions, but in calm water. Fresh dechlorinated water at the appropriate temperature was led into the apparatus (2.84 l/min) and eventually left through the center drain, thereby reducing the likelihood of excreted radioactivity being absorbed into the fish.

B. Injection of Radioactive Materials

Immediately prior to injection all acclimated fish were removed from the calm water compartment and held in a 200-l tank at the acclimation temperature. Materials to be injected were either ^{125}I-L-thyroxine (T_4*) purchased from Nuclear Consultants Ltd. and Amersham/Searle or carrier-free Na^{125}I (Atomic Energy of Canada Limited,

Commercial Products, Ottawa, Canada). T_4* was diluted in 50% aqueous propylene glycol and ranged in specific activity from 35.0-135.0 mCi/mg., while radioiodide was diluted in distilled water. The fish were first anaesthetized (MS 222, tricaine methane sulphonate, 1:20,000) and then injected intraperitoneally with 0.1 ml of radioactivity, from a 1.0 ml tuberculin syringe (27-gauge needle). Injection was below the left lateral line directly beneath, or just in front of the dorsal fin, with the needle slanted at a 45° angle. At first, fish were injected through fine-mesh netting, however, in later experiments they were blotted at the site of injection with absorbent paper, injected, and inspected for leakage of the injected dose. This occasionally happened and the fish in question was eliminated. Following injection the fish were returned to their experimental compartment. The same procedure was repeated for fish in the current compartment, with time allowances being made for the total procedure, so that each group was treated in a similar manner.

Preparation of T_4* standards depended on the dose. Where 1 μCi was injected into fish, identical doses were injected into 100-ml flasks containing 2N NaOH and the volume was made up to the 100 ml mark, and the flasks well mixed. Four-ml aliquots from these flasks were counted and their radioactivity (cpm corrected for background radiation) multiplied by 25.

Lower doses (.2 to .25 μCi) were injected directly into glass counting tubes, and diluted to 4 ml with 2N NaOH. This represented 100% of the injected dose.

In all radioiodide experiments the dose (2 to 2.5 μCi) was prepared as for high doses of T_4*, except the dilutions were made up to 100 ml with distilled water.

C. Blood Collection and Removal of Thyroid and Enterohepatic Organs

A high dose of MS-222 (1:4,000) was used to kill the fish (approx. 1 min). They were then weighed to the nearest 0.1 g. and measured for total length (nearest 0.1 cm). The caudal artery was severed by removing the tail and the blood was allowed to flow freely, first of all into a heparinized hematocrit capillary tube, which was sealed at one end with seal-ease, and then into a heparinized aluminum dish. From this it was pipetted into 1-ml plastic centrifuge tubes and centrifuged (International Micro Capillary Centrifuge, Model MB with a slant head for 1-ml centrifuge tubes), at 10,000 to 15,000 xg for 3 minutes. The serum obtained was then stored immediately at -22°C in 2-ml disposable plastic beakers, sealed with parafilm. Following centrifugation under the same conditions as the blood, except a hematocrit head was used, the hematocrit was determined (RBC/total) for each fish.

The carcasses were labelled, packaged, and stored at (-22°C) until organ removal, usually several weeks later.

The thyroid region was considered to be between the first and fourth gill arches of the lower jaw, including the tongue, as illustrated by Drury (1967). Most of the thyroid tissue, according to Drury, is confined between the first and third gill arches. At

first all ventral muscle was taken off, however, some removal of thyroid tissue occurred, and therefore this muscle was left on. A correction factor for tissue removal was applied to those glands affected, based on the average percentage loss of radioactivity.

Removal of the enterohepatic organs (liver, gall bladder, foregut (esophagus and stomach) and post pyloric caecal intestine) has been described by Eales (1970). These organs were removed along with the thyroid, when the carcasses were partially thawed. Each frozen organ was removed in total and placed into an individual counting tube, whose volume was then made up to 4 ml with 4N NaOH. Both foregut and post pyloric caecal intestine (straight intestine after area of pyloric caecae) were measured, complete with contents, and the tubes were counted within 24 hr, allowing for partial tissue breakup.

The radioactivity of each enterohepatic organ, or thyroid, was expressed as a percentage of the injected dose as determined from the radioactive standards.

$$\% \text{ of injected dose} = \frac{\text{organ (c.p.m., corrected for background)}}{\text{"injected dose" (c.p.m. corrected for background)}} \times 100$$

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

Serum proteins, to which thyroid hormones bind in brook trout (Falkner, 1970) were precipitated by trichloroacetatic acid (TCA). The method was as follows. Serum (0.1 ml) was pipetted from a 0.1- ml

disposable pipet (diSPo micropipet calibrated to contain ± ½% accuracy) into 2 ml of 12.5% TCA, in a 12-ml thick walled centrifuge tube, resulting in the formation of a white precipitate. By stirring with a glass rod, the precipitate was broken up well, making sure to remove any adhering to the sides. A further 2 ml of 12.5% TCA was added and the resulting 4 ml shaken gently with a vortex stirrer (Deluxe Mixer Scientific Products) and centrifuged for 20 min (2900 to 3000 xg) in a Sorvall GLC-1 centrifuge. The supernatant (4 ml) was poured off into a test tube.

Resuspension of the precipitate was done by adding 2 ml of 2.5% TCA, and stirring with a glass rod. Another 2 ml was then added, and after gentle mixing, the 4 ml was centrifuged for 20 min. This washing procedure was performed to reduce protein contamination by inorganic radioiodide. Following centrifugation the second supernatant (4 ml) was added to the first, the resulting 8 ml thoroughly mixed, and a 4-ml aliquot transferred to a counting tube. The radioactivity (cpm) in the aliquot was doubled to provide the total inorganic iodide or nonhormonal iodide in the serum.

To the precipitate 1.9 ml of distilled water was added, and following resuspension by a glass stirring rod, 2 ml of 4N NaOH was added, which instantly dissolved the precipitate. The contents of the centrifuge tube representing the protein-bound or hormonal radioactive fraction in the serum, were transferred to a counting tube and counted. All radioactivity was counted using a well-type scintillation counter with a thallium-activated sodium iodide crystal, $2\frac{1}{4}$" x $2\frac{1}{4}$" (Nuclear Chicago DS202). Potassium iodide and L-ascorbic acid were added to all TCA solutions to provide final concentrations

approximately 1.2 x 10^{-3}M. This reduced protein contamination by inorganic radioiodide. This was also done by Yamamoto (1964).

The resulting serum fractions $PB^{125}I$ and $I^{125}I$ were expressed as

$$\frac{\% \text{ injected dose in serum fraction X body weight (g)}}{\text{ml of serum X 100}}$$

Where % injected dose

$$= \frac{I^{125}I \text{ or } PB^{125}I \text{ (c.p.m. corrected for background)}_X 100}{\text{"injected" dose (c.p.m. corrected for background when sample counted)}}$$

E. Chemical Determinations

1. Total Serum Iodide

The Hycell Cuvette P.B.I. method was used to determine the total iodide of the serum.

The major chemical reactions involved were as follows:

(1) release of protein-bound iodine by perchloric destruction of protein;

(2) oxidation of iodine to iodate by the addition of ceric reagent;

(3) conversion of iodate to iodide by addition of arsenious reagent;

(4) reduction of ceric ammonium sulphate by arsenious acid catalyzed by iodide, with the rate of catalysis being proportional to the iodide concentration at standard time and temperature.

A color reaction resulted and the cuvettes were measured photometrically with a B and L Spectronic "20" (420 mμ) in terms of optical density.

Owing to the much higher iodide levels in brook trout serum, than in human serum, it was necessary to dilute the fish serum from 20 to 40 times (for example 40x = 0.1 ml serum to 3.9 ml distilled, deionized water). One tenth of an ml of each of five standards of potassium iodide in distilled water (0, 5, 10, 15 and 20 mcg iodine/100 ml) was pipetted into five separate cuvettes (19 X 150 mm HYCEL certified cuvettes (PBI)) and 0.1 ml of the diluted serum for analysis was delivered into cuvettes. To each of these tubes 2 ml of digestion reagent (0.025% vanadic acid in 72% perchloric acid) was added in consecutive order, so that it slowly flowed down the inner wall of the cuvette. The resulting 2.1 ml was mixed gently with a vortex mixer, so that no undissolved serum remained on the wall. These cuvettes were then placed, in proper order, at timed 10 sec intervals into a Hycell Cuvette Thermal Block, preheated under a fume hood to 230° ± 5°C. The cuvettes were removed from the heating block exactly 6 min after placing the first one in the block, and in the same timed 10-second intervals. These were allowed to cool at room temperature for 15 to 20 minutes. Then 2.0 ml of ceric reagent (0.6% ceric ammonium sulfate in 27% sulfuric acid) was added in consecutive order and each tube was vigorously shaken, with a vortex mixer to assure complete mixing, because of the large difference in specific gravities between the ceric and digestion reagents. All tubes were left at room temperature for 15 to 20 minutes, to ensure temperature uniformity, as ceric addition results in a slight exothermic reaction.

The addition of 2 ml of arsenious reagent (0.9% arsenic trioxide in 8.2% sulfuric acid) was then done in timed sequence (30 sec intervals) to each tube in order. It was added 10-12 sec before the 30 sec mark, providing sufficient time for vigorous mixing (vortex mixer) and placing the cuvette in a water bath at 37°C. Exactly 20 minutes after the addition of arsenious reagent to the first cuvette, and in the same 30 sec intervals, each cuvette was removed from the water bath, slightly mixed, and dried and then placed into a B and L spectronic "20" (420 mμ; with a suitable adaptor) and the optical density was read against water (converted % transmission reading to optical density). On graph paper, the optical density of each standard was plotted on the y-axis against its respective concentration on the x-axis. From the standard curve so obtained, the values of the unknowns could be directly determined, which were then multiplied by the dilution.

2. Total Thyronine Levels ($T_4 + T_3$)

By killing 20 fish, 15 ml of serum was obtained, which was pooled, well mixed, and divided into three 5 ml aliquots. This was done for each condition (current and calm) at 3 different sampling times after the current was initiated. The resulting 6 vials (3 vials/condition) for each sampling time were packaged and sent to the Bio-Science Laboratories (California). They usually arrived within 2-3 days after mailing and were analyzed by the T_4-by-column test as outlined by the Handbook of Specialized Diagnostic Laboratory Tests 8th ed., 1969, published by the Bio-Science Laboratories.

The test employed an anion-exchange resin to remove T_4, T_3 and other iodoamino acids if present. Serum inorganic iodide was also removed by the resin column and remained on the resin. Proteins, including iodoproteins if present, passed through the column and were discarded. Mono- and diiodotyrosines, if present were removed through subsequent column washes, by solutions of decreasing pH. When the pH was lowered to 1.4 this caused T_4 and T_3 to be eluted from the column. Three separate fractions were collected, the first two containing the total thyronines present, with the content of each fraction determined by a non-incinerative technique. The third fraction served as a blank.

The method has several advantages. First of all serum inorganic iodine levels, to at least a 1000 µg%, did not interfere. Neither did abnormal iodoproteins, or increases in the levels of mono- and diiodothyronines. Also this test could have been performed on serum, which had been at room temperature for more than a week (in practice the test was performed approximately 3-4 days after sampling) since this latter advantage might only be applicable to human serum.

F. Chromatographic Procedure

All new shipments of T_4* were checked routinely for purity, by either descending paper chromatography, or thin-layer chromatography.

For paper chromatography, 10-25 µl of the stock solution of T_4* in 50% aqueous propylene glycol, was applied on Whatman #1 filter paper. Chromatograms were run descending in darkness (18 hr) with butanol : acetic acid : and water (4 : 1: 1 v/v). Rolled $\frac{1}{2}$-inch sections of the dried paper were counted in the bottom of counting

tubes, and the radioactivity occurring in peaks corresponding to T_4 and iodide, expressed as percentages of the total on the chromatogram.

In thin-layer chromatography plastic sheets (20 X 20 cm) precoated with silica gel, 0.1 mm thick (MN-Polygram Sil S-HR/uv254, Macherey-Nagel and Co. Düren Germany) were used. Three to five microlitres of T_4* diluted in 4% aqueous propylene glycol (higher concentrations of propylene glycol caused degradation of thyronines on the chromatogram) were spotted and dried in a warm air stream. T_3 and T_4 (sigma) standards dissolved in methanolic ammonia (99% methanol to 1% concentrated ammonia) were ran on either side. The sheet was run in butanol : ethanol and 6 N ammonia (5 : 4 : 1 v/v) for $2\frac{1}{2}$ hr, dried and the thyroxine standards localized under UV. A protective coating was sprayed on (Letraset protective coating) and the chromatogram sections (1/8" wide) counted in the bottom of glass counting tubes, and the radioactivity occurring in peaks corresponding to T_4 and iodide, expressed as percentage of the total on the chromatogram. This procedure was modified for the thin-layer analysis of serum radioiodo compounds. Twenty-five microlitres of serum were spotted in a line (13 cm) in the middle of a chromatogram sheet and T_3, T_4, MIT, DIT, and iodide standards spotted in paths on either side. Samples of the serum for analysis were spotted on top of the standards in the same density per unit length of origin, to ensure that any serum constituents influencing migration of the standards, would not interfere with identification.

Methods for Assessing Thyroid Activity

1. Radioiodide Metabolism

a) % Thyroid (percentage of the injected dose in the thyroid) - The extent to which the thyroid gland concentrates an injected tracer dose of radioiodide within the follicles, through active transport provides a measure of thyroid activity. It is expressed as follows:

$$\% \text{ thyroid} = \frac{\text{thyroid (c.p.m. corrected for background)}}{\text{"injected dose" (c.p.m. corrected for background)}} \times 100$$

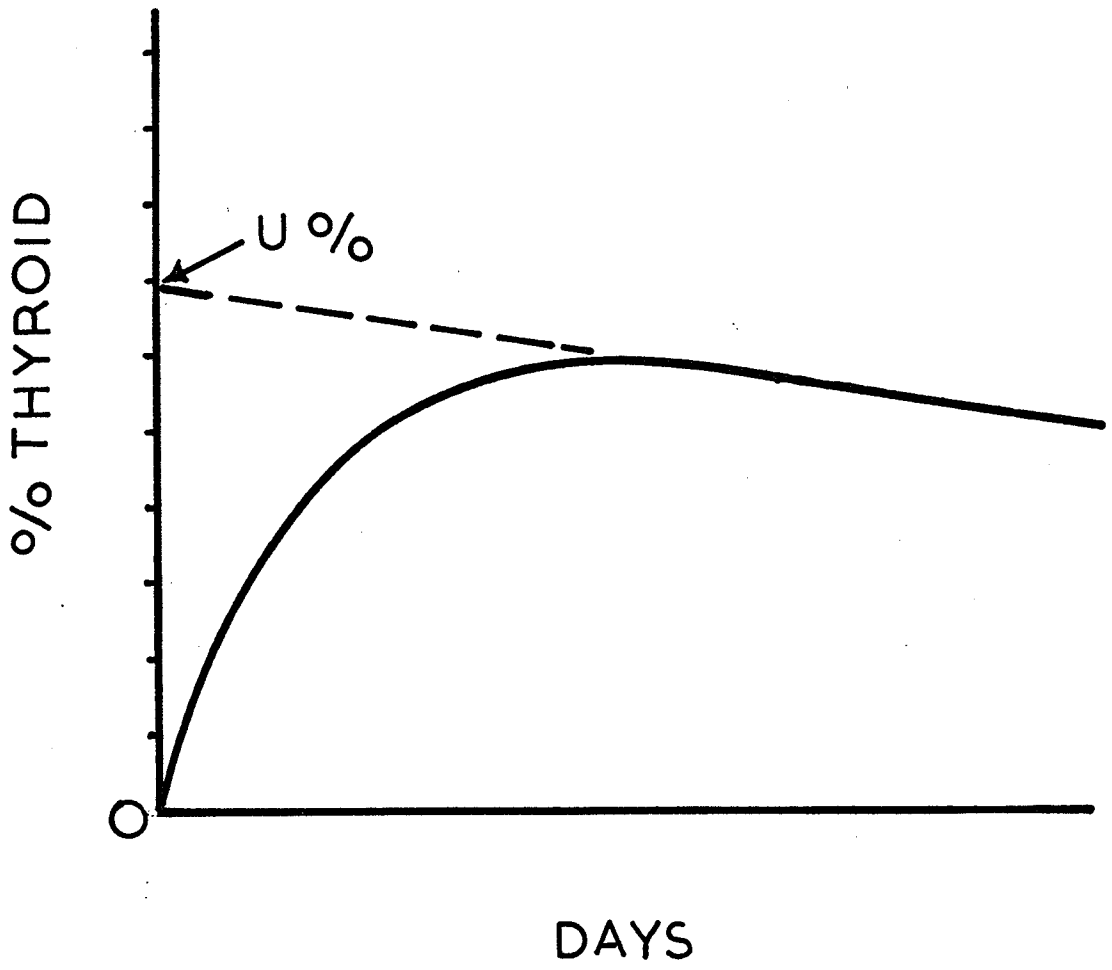
It is generally a poor indication of thyroid activity if:

(1) the level of stable iodide (^{127}I) does not remain constant in the external and internal environment (fish) over the sampling period, or between fish being compared;

(2) the extrathyroidal rate of radioiodide clearance from the blood is not the same under different experimental conditions.

To obtain the theoretical maximum uptake of radioiodide (U%) by the thyroid, which is later required for the calculation of the rate constant for thyroid uptake of radioiodide k_1, two procedures were followed. Either the line of best fit, in the stage of radioactive loss from the thyroid was extrapolated back to zero time (Fig. 2) (Dorey, 1970), or an average was calculated for the three highest % thyroids, at the time where thyroidal $I^{125}I$ accumulation had reached a plateau. The latter procedure was necessary because of the variability generally encountered with this measurement, during this study and also because of the slow loss of radioactivity from the gland, resulting in insufficient sample points for extrapolation back to zero time.

Figure 2. Method for obtaining the maximum uptake of radioiodide (U%) by the thyroid at zero time.


% THYROID
U %
O
DAYS

b) Rate Constant for Thyroid $I^{125}I$ Uptake (k_1) - By using the maximum thyroid uptake (U%) and by measuring the rate constant of radioiodide loss from the serum (K), it is possible to calculate the rate constants for thyroid $I^{125}I$ uptake, k_1, and for $I^{125}I$ excretion by all extrathyroidal pathways, k_2.

These rate constants have been determined in fish by Dorey (1970), who adapted the procedure of Robertson and Falconer (1961), for mammals.

By taking into account the serum radioiodide excretion rate the determination of (k_1) provides a superior method to % thyroid and T/S (discussed below).

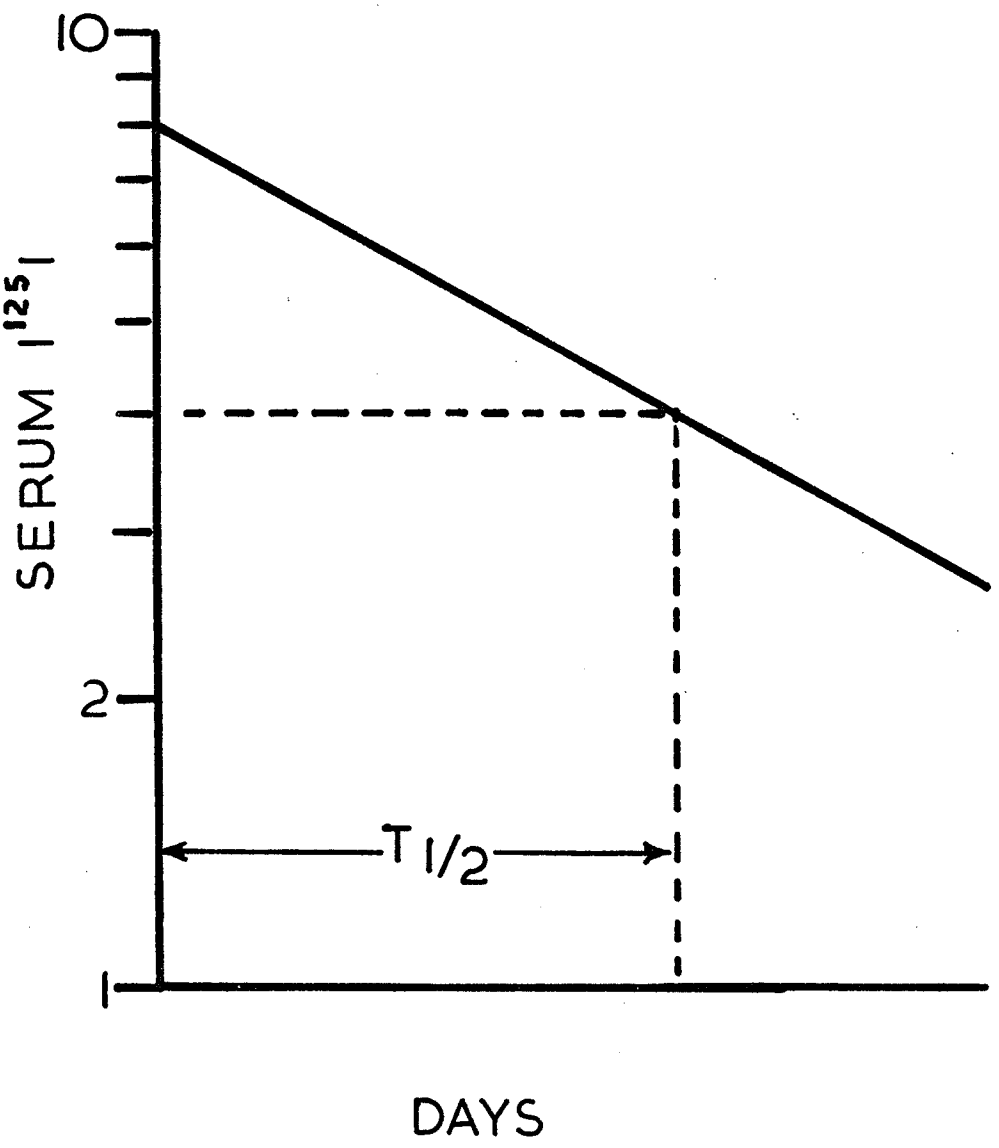
Radioiodide removal from the serum is exponential with time, becoming linear with a semilogarithmic transformation. It is possible from the transformed data to calculate the time taken for serum inorganic radioiodide to decrease by 50%, this being expressed as the biological half life ($t_{\frac{1}{2}}$) (Fig. 3). Knowing $t_{\frac{1}{2}}$, the fractional rate of loss of serum radioiodide by all routes (K) can be calculated by the equation:

$$t_{\frac{1}{2}} = \frac{\ln 2}{K} = \frac{0.693}{K} \qquad (1)$$

The rate constant (K) represents the combined total of the rate constants concerned with serum radioiodide removal, namely, k_1, the rate constant for thyroid radioiodide uptake and k_2, the rate constant for all extrathyroidal routes of excretion (gills, kidneys, skin, gut etc.). Hence $K = k_1 + k_2$ and

$$\text{Serum } I^{125}I \quad t_{\frac{1}{2}} = \frac{0.693}{k_1 + k_2} \qquad (2)$$

Figure 3. Method of determining the biological half life ($t\frac{1}{2}$) for serum inorganic radioiodide.


10
SERUM $I^{125}I$
2
1
T 1/2
DAYS

From this equation the total contribution of $k_1 + k_2$ can be determined, and by using the value obtained for maximum thyroid uptake (U%), the contribution of each rate constant can be derived.

$$\frac{U\%}{100} = \frac{k_1}{k_1 + k_2} \qquad (3)$$

This equation states that if the maximum uptake is 100% then $\frac{k_1}{k_1 + k_2} = 1$ and therefore there has been no contribution by k_2. This in reality does not occur.

c) Thyroid/Serum Ratio (T/S) - This measure has been discussed by Eales (1964). Like k_1 the T/S ratio is considered to be superior to the measurement of % thyroid. It compensates to some degree for changes in $I^{125}I$ excretion, which would influence the amount of radioiodide available for thyroid uptake. Eales (1964) stated, that it rests on the assumption that both thyroid radioiodide uptake and serum radioiodide removal rates do not alter between injection and sampling. The ratio in this study was measured as:

$$T/S = \frac{\% \text{ thyroid}}{\% \text{ injected dose } {}^{125}I/\text{ml serum}} \times \frac{\text{body weight}}{100}$$

Although inferior to k_1, which has a mathematical basis, it has the advantage that a time series of measurements is not required.

d) Conversion Ratio - This provides a measure of the radiohormone output by the thyroid gland, taking into account the radioiodide pool. It has been discussed and applied to fish studies by, for example, Hickman (1961), Hoar and Eales (1963), Eales (1963) and Wiggs (1963). It is expressed as:

$$CR = \frac{\text{serum } PB^{125}I \text{ (c.p.m. corrected for background)}}{\text{serum } PB^{125}I \text{ (c.p.m. corrected for background)} + \text{serum } I^{125}I \text{ (c.p.m. corrected for background)}} \times 100$$

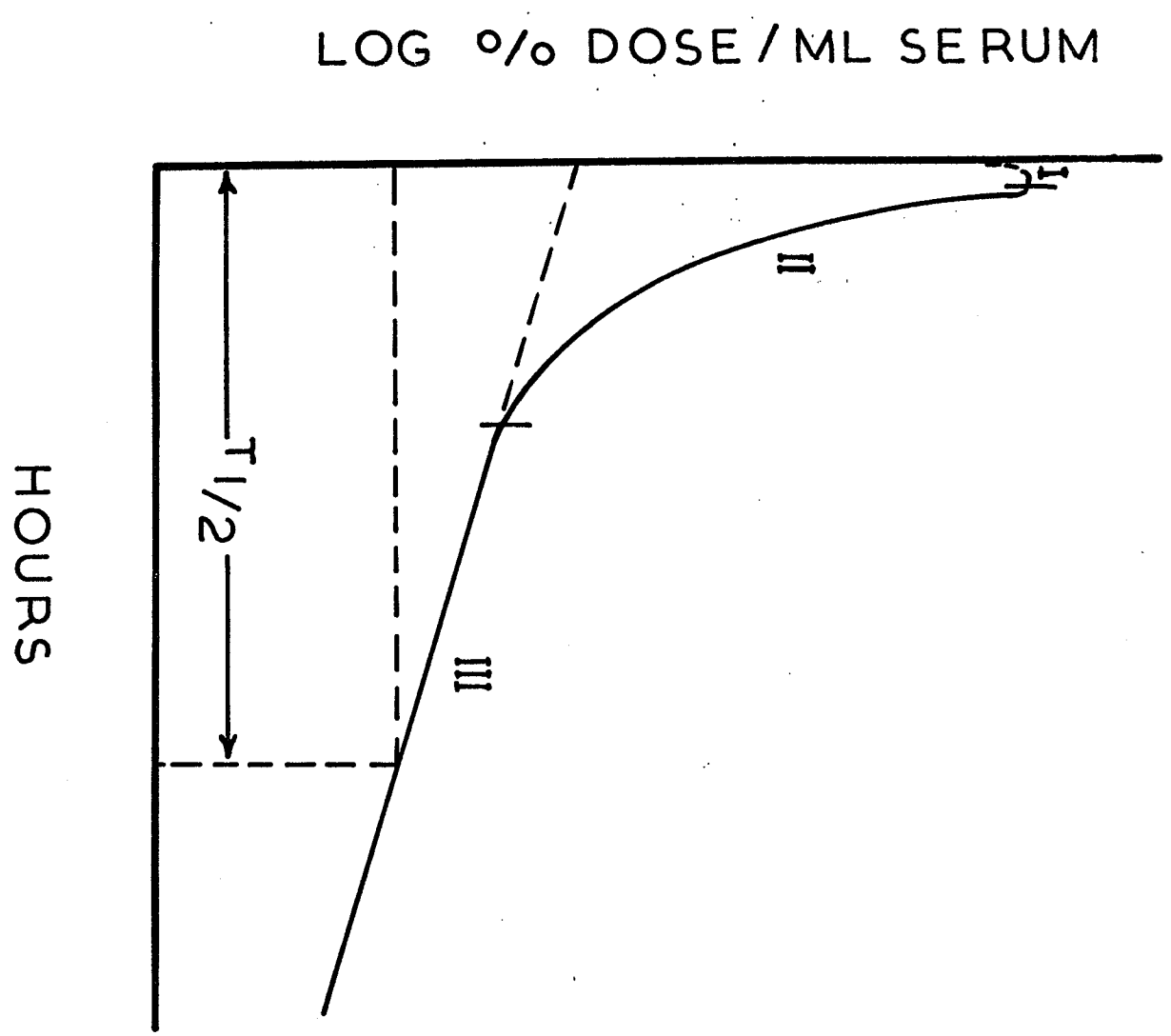
Its validity depends, according to Hoar and Eales (1963), on T_4* and T_3* being precipitated with the plasma proteins by TCA (established by Falkner (1970) for brook trout), and also on negligible peripheral loss of radiohormone over the experimental period. In addition both Hoar and Eales (1963) and Wiggs (1963) refer to the possibility of radioiodide binding directly to the plasma proteins, thereby influencing the protein-bound iodide determination. In this work this error was eliminated by the addition of KI to all TCA solutions.

2. Radiothyroxine Metabolism (Clearance of Serum $PB^{125}I$)

Following intraperitoneal injection of T_4* a characteristic pattern of $PB^{125}I$ decrease with time, involving up to 4 phases may be obtained for brook trout (Drury, 1967; Drury and Eales, 1968). Phase I involved the absorption of T_4*, and possible derivatives from the coelom into the blood stream where binding to proteins occurred. Therefore, in the blood, there is a peak for $PB^{125}I$ shortly after injection, following which there is a decrease (Phase II) due to distribution of T_4*, throughout the tissues. Together phase I and phase II constitute the equilibration period.

Assuming T_4* is cleared from the blood at a constant rate, the $PB^{125}I$ serum loss in phase III is exponential (Fig. 4). Over this phase biological half-lives ($t_{\frac{1}{2}}$) can be determined, and also the fractional rate of serum PBI turnover (radioactive or stable) by the

Figure 4. Graph showing a characteristic pattern for $PB^{125}I$ decrease with time following intraperitoneal injection of T_4* in fish. Phase I and II together constitute the equilibration period, while there is an exponential loss of serum $PB^{125}I$ over phase III. $T_{\frac{1}{2}}$ is the biological half-life of serum $PB^{125}I$.


LOG % DOSE / ML SERUM
HOURS
$T_{1/2}$
II
III

formula $k = \frac{0.693}{t\frac{1}{2}}$. From this value the thyroxine secretion/degradation rate can be estimated, knowing the total quantity of stable T_4 in the serum (volume of T_4 compartment x serum stable T_4 concentration) and relating this to the body weight.

In phase IV a second peak in serum $PB^{125}I$ sometimes occurs. Drury and Eales (1968) considered this to be a result of thyroid uptake of radioiodide, produced from the peripheral deiodination of radiothyroxine, its synthesis into hormone, and then release in the form of endogenous $PB^{125}I$.

Using thin layer chromatography it was shown that it would be more correct to refer to serum radiothyroxine clearance, following T_4* injection as radiothyronine clearance, because through deiodination the proportion of T_3* increases. T_3* has been shown by Eales, Welsh, and Chan (1970) (in press) to have a different serum clearance rate.

Drury and Eales (1968) considered fractional rates of turnover to be a valid index of serum PBI degradation rate and thyroid function, if it can be assumed that a steady state exists, and the extrathyroidal thyroxine pool does not differ markedly between conditions compared.

A summary of the parameters for measuring thyroid function is given in Table V.

Table V. Parameters for Measuring Thyroid Function

Injection	Parameter	Definition and Comments
^{125}I	% Thyroid	% of the injected dose taken up by the thyroid. Inferior to T/S and k_1 providing no indication of thyroid hormone output.
	T/S	Thyroid/serum ratio. Inferior to k_1 since it has no mathematical basis. Does not require time series of measurements.
	U%	Maximum thyroid uptake extrapolated to zero time or average of three highest % thyroids.
	K	Rate constant for loss of serum radioiodide via all routes.
	k_1	Rate constant for loss of serum radioiodide to the thyroid. Best radioiodide measure of thyroid activity.
	k_2	Rate constant for loss of serum radioiodide via all extrathyroidal routes.
	CR	Conversion ratio. Provides a measure of the radiohormone output by the thyroid gland.
T_4*	$t_{\frac{1}{2}}$	Biological half-life of serum PB^{125}I measured over Phase III.
	k	Fractional rate of serum PBI turnover either radioactive or stable.
	% dose in enterohepatic organs	% of the injected dose taken up by either the liver, gall bladder, foregut (esophagus and stomach) and post pyloric caecal intestine. Provides some indication of the peripheral metabolism of T_4* along with k.
---	Total Thyronine	Total circulating thyroid hormone (stable T_4 + stable T_3 in the serum). When used with k and an estimate for the volume of the T_4 and T_3 compartment can obtain the thyroid hormone secretion rate.[3] To a limited extent provides some indication of thyrometabolic status.
---	Total serum Iodide	Represents the total stable iodide in the serum (both inorganic and hormonal). This must be known when % thyroid is used as an index of thyroid activity.

RESULTS

Seven experiments, three dealing with exercise and iodine metabolism and the remainder with exercise and thyronine metabolism, are presented in this section.

A. Iodine Metabolism

1. Radioiodine Metabolism

The influence of exposure to a water current on indices of thyroid function following radioiodide injection was assessed in three separate experiments (I, II and III). (Summary, Table VI; Regression Equations Table VII.)

a) Experiment I (July-August 1969; Fig. 5, A, B, C, D) - From the stock tank, 72 fish (ave. weight 221.57 g ± 38.83 (S.D.)) were assigned at random to the current chamber, while 72 fish (ave. weight 229.09 g ± 41.97 (S.D.)) were assigned to the calm chamber of the apparatus. Water temperature was 13°C. Current fish swam continuously for 11 days and then all fish were injected with 2 μCi of radioiodide and 8 fish from each condition killed at 1, 3, 6, 9, 12, 15, 18, 21 and 24 days post injection (p.i.).

The maximum % thyroid was 14.0% for both groups although this value was attained at 12 days p.i. by current fish and at 15 days p.i. by calm fish. Both groups then lost radioactivity, either as organic or inorganic radioiodide (Fig. 5B). U% was 20.6% for current fish and 21.0% for calm fish.

Table VI. Summary of Radioiodine Metabolism

	Current			Calm		
	Experiment I (July-August 1969)	Experiment II (Oct.-Nov. 1969)	Experiment III (March-April 1970)	Experiment I (July-August 1969)	Experiment II (Oct.-Nov. 1969)	Experiment III (March-April 1970)
Temp °C	13	13	11	13	13	11
Length of exp. (days p.i.)	24	27	30	24	27	30
Average fish weight (g)±1 s.d.	221.57±38.83	70.96±19.62	106.64±21.72	229.09±41.97	67.54±17.29	106.97±22.43
Length (cm)±1 s.d.	26.43±1.53	18.08±1.67	21.92±1.69	26.57±1.59	17.83±1.55	21.86±1.47
U%	20.6	17.2*	14.4*	21.0	19.2*	17.5*
$t_{\frac{1}{2}}$ (serum $I^{125}I$) days	10.80	12.00	14.25	10.80	17.25	15.75
K	0.064	0.058	0.049	0.064	0.040	0.044
k_1	0.013	0.010	0.007	0.013	0.008	0.008
k_2	0.051	0.048	0.042	0.051	0.032	0.036
Max T/S ratio at 24 days	7.01(15d.) 4.59	11.50(18d.) 5.35	9.42(28d.) 7.07	7.09(24d.) 7.09	10.59(27d.) 6.82	8.17(30d.) 7.11
Max % CR at 24 days	1.20(24d.) 1.20	4.00(18d.) 3.34	1.72(24d.) 1.72	1.44(24d.) 1.44	5.03(27d.) 1.75	2.54(30d.) 0.88
Max PB ^{125}I at 24 days	0.019(24d.) 0.019	0.076(24d.) 0.076	0.035(24d.) 0.035	0.020(24d.) 0.020	0.112(27d.) 0.052	0.057(30d.) 0.021

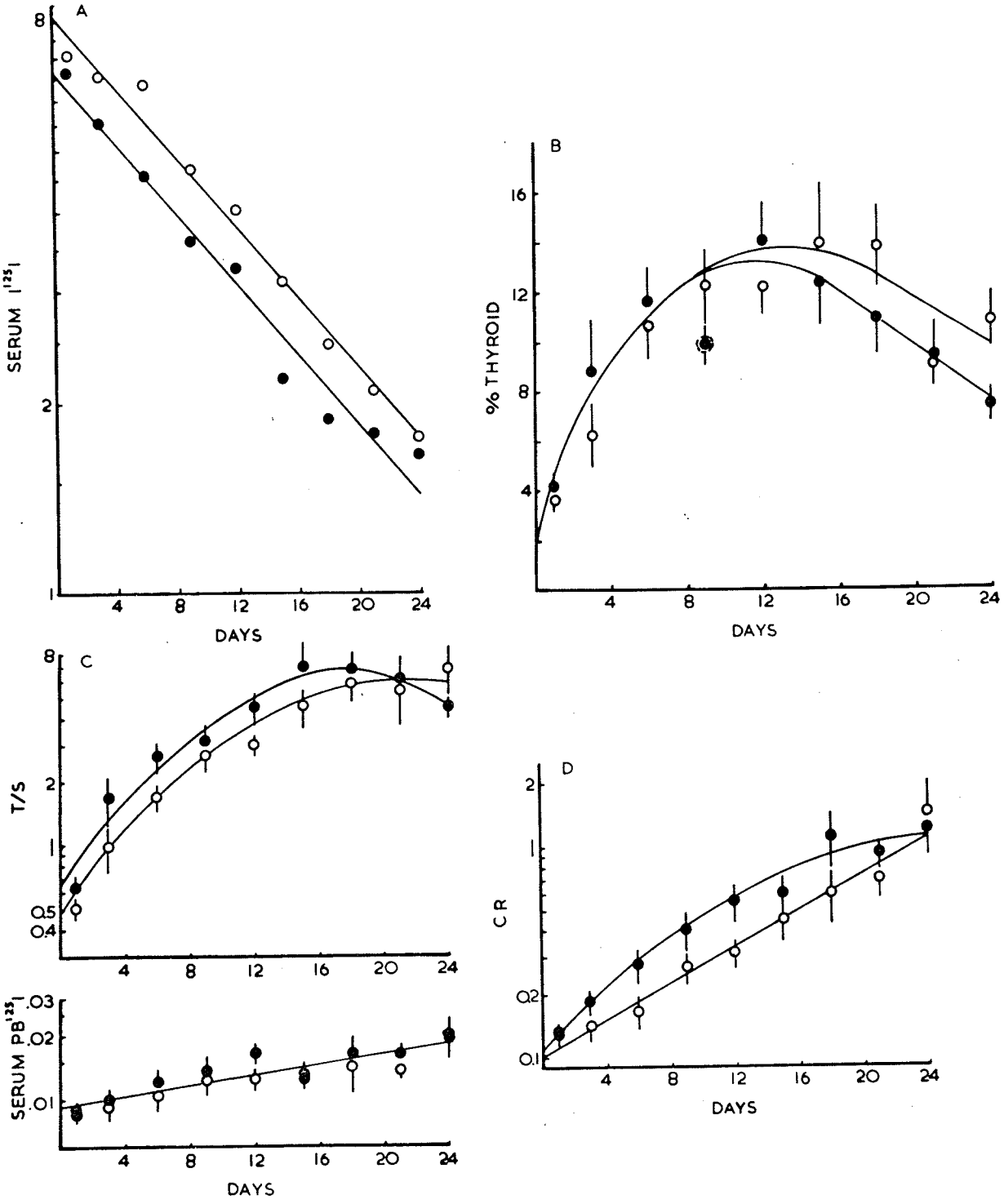
* U% (ave.)

Table VII. Equations for straight line regressions used in figures. (Radioiodine metabolism, Serum $I^{125}I$)

	Current			Calm		
Date	No. of Figure	T°C	Equation of the line*	No. of Figure	T°C	Equation of the line
July-August 1969	5	13	logy = 0.8233 - (0.0277)X	5	13	logy = 0.9155 - (0.0278)X
October-November 1969	6	13	logy = 0.7929 - (0.0248)X	6	13	logy = 0.7811 - (0.0174)X
March-April 1970	7	11	logy = 0.7746 - (0.0210)X	7	11	logy = 0.8947 - (0.0191)X

* logy = a + bX

Figure 5. Sustained swimming and radioiodine metabolism (current fish •, calm fish o) during July to August 1969. The indices of thyroid function, namely serum $I^{125}I$, % thyroid, T/S, serum $PB^{125}I$, and CR are plotted against time (days). Points are means of eight fish. Each mean for serum $I^{125}I$ is plotted as the antilogarithm of the logarithm average for the sample, since the straight lines were fitted by the method of least squares. Standard errors of the means are indicated except for serum $I^{125}I$.

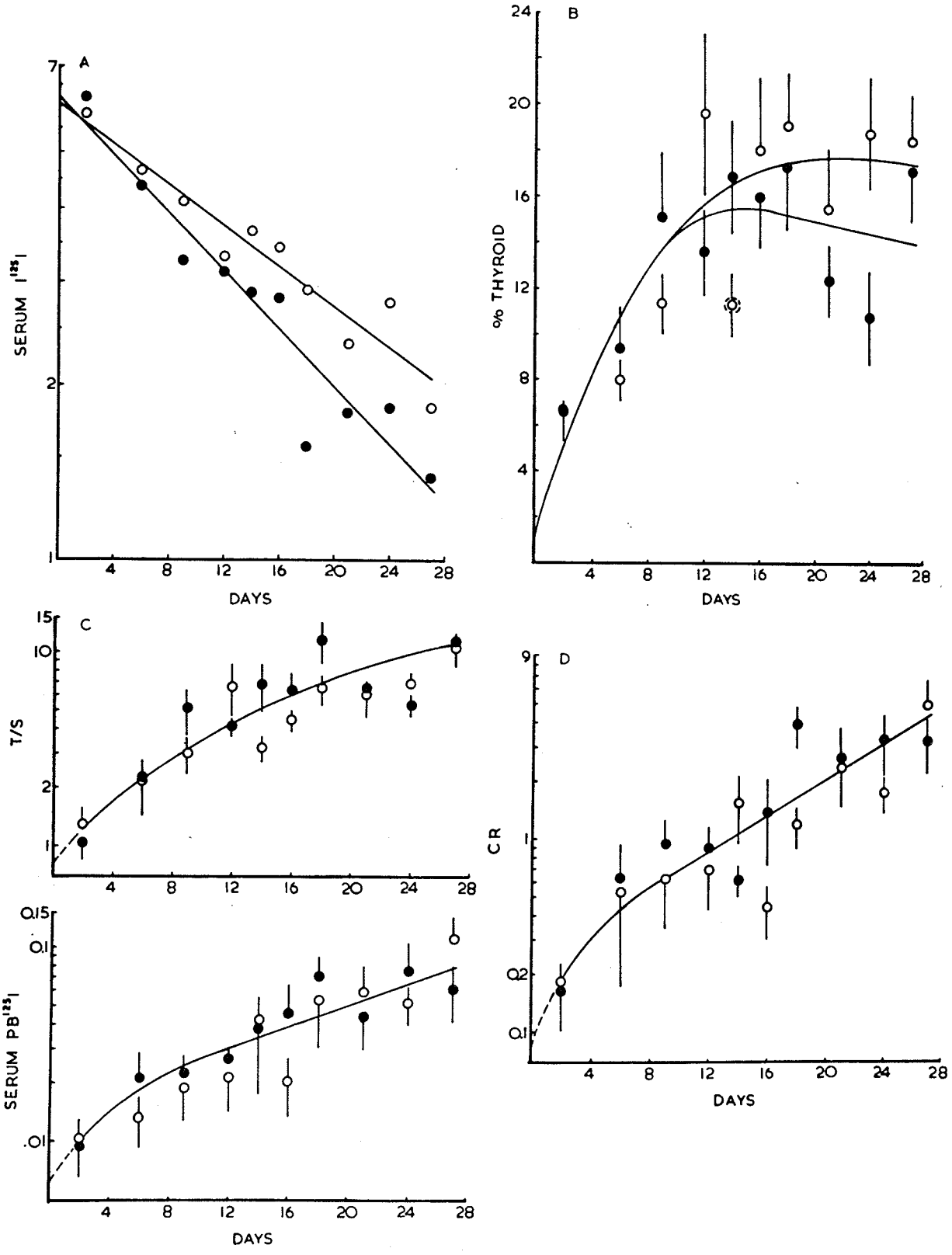

A
SERUM $I^{125}I$
8
2
1
4
8
12
16
20
24
DAYS
B
% THYROID
16
12
8
4
4
8
12
16
20
24
DAYS
C
T/S
8
2
1
0.5
0.4
4
8
12
16
20
24
SERUM $PB^{125}I$
.03
.02
.01
4
8
12
16
20
24
DAYS
D
C R
2
1
0.2
0.1
4
8
12
16
20
24
DAYS

Both groups lost serum radioiodide exponentially at an identical rate ($t\frac{1}{2}$ = 10.80 days, K = 0.064) and hence k_1 or k_2 did not differ between the calm or current conditions. However, the difference in intercept on the semilogarithmic plot of serum radioiodide, with time, indicated a difference in radioiodide distribution space between the two groups (Fig. 5A). The T/S CR and possibly serum concentration of $PB^{125}I$ tended to be slightly higher for the current fish (Fig. 5C, D). The maximum T/S was 7.01 (15 days p.i.) for current fish and 7.09 (24 days p.i.) for calm fish. Maximum CR of 1.20% (current) and 1.44% (calm) were attained at 24 days p.i.

In conclusion, sustained swimming produced little alteration in longterm radioiodide metabolism of brook trout. Where differences did appear they suggested that fish in a current might have a slightly greater thryoid activity.

b) Experiment II (October-November 1969, Fig. 6, A, B, C, D) - In this experiment smaller fish were used in order to determine if increased swimming by each fish (number of lengths/second) had any effect on thyroid activity. From the stock tank, 99 fish (ave. weight 70.96 g ± 19.62 (S.D.)) were assigned at random to the current chamber, while 99 fish (ave. weight 67.54 g ± 17.29 (S.D.)) were assigned to the calm chamber of the apparatus. Water temperature was 13°C. Current fish swam continuously for 11 days and then all fish were injected with 2 μCi of radioiodide and 9-10 fish from each condition killed at 2, 6, 9, 12, 14, 16, 18, 21, 24, and 27 days p.i.

Figure 6. Sustained swimming and radioiodine metabolism (current fish •, calm fish o) during October to November 1969. The indices of thyroid function namely, serum $I^{125}I$, % thyroid, T/S, serum $PB^{125}I$ and CR are plotted against time (days). Points are means of nine to ten fish. Each mean for serum $I^{125}I$ is plotted as the antilogarithm of the logarithm average for the sample, since the straight lines were fitted by the method of least squares. Standard errors of the means are indicated, except for serum $I^{125}I$.


A
SERUM I^{125}
7
2
1
4
8
12
16
20
24
28
DAYS
B
% THYROID
24
20
16
12
8
4
4
8
12
16
20
24
28
DAYS
C
T/S
15
10
2
1
4
8
12
16
20
24
28
SERUM $PB^{125}I$
0.15
0.1
.01
4
8
12
16
20
24
28
DAYS
D
C R
9
1
0.2
0.1
4
8
12
16
20
24
28
DAYS

Calm fish had a higher percentage uptake of radioiodide into the thyroid (Fig. 6B). For the calm fish U% was 19.2%, while for current fish this was 17.2%. Loss of thyroidal radioactivity was slow for both groups, although for fish in the current, this appeared to be slightly faster. There was increased serum radioiodide removal for current fish ($t\frac{1}{2}$ = 12 days; K = 0.058) than for the calm fish ($t\frac{1}{2}$ = 17.25 days; K = 0.040).

This resulted in a difference in the rate of thyroidal uptake of radioiodide for current fish (k_1 = 0.010 rather than k_1 = 0.008 for calm fish) and also in the rate of extrathyroidal removal of serum radioiodide (k_2 = 0.048 instead of 0.032 for calm fish). No alteration in the radioiodide distribution space was observed (Fig. 6A).

Mean T/S ratios and CR for the current fish were higher at 7 sample times out of 10 (Fig. 6C, D). Although the maximum T/S and CR respectively 11.50 and 4.00% both occurred at 18 days p.i. for current fish, instead of 10.59 and 5.03% at 27 days p.i. for calm fish, standard errors of the means often overlapped and there was no clear separation of sample means according to condition. This was also the case for serum PB^{125}I (Fig. 6C).

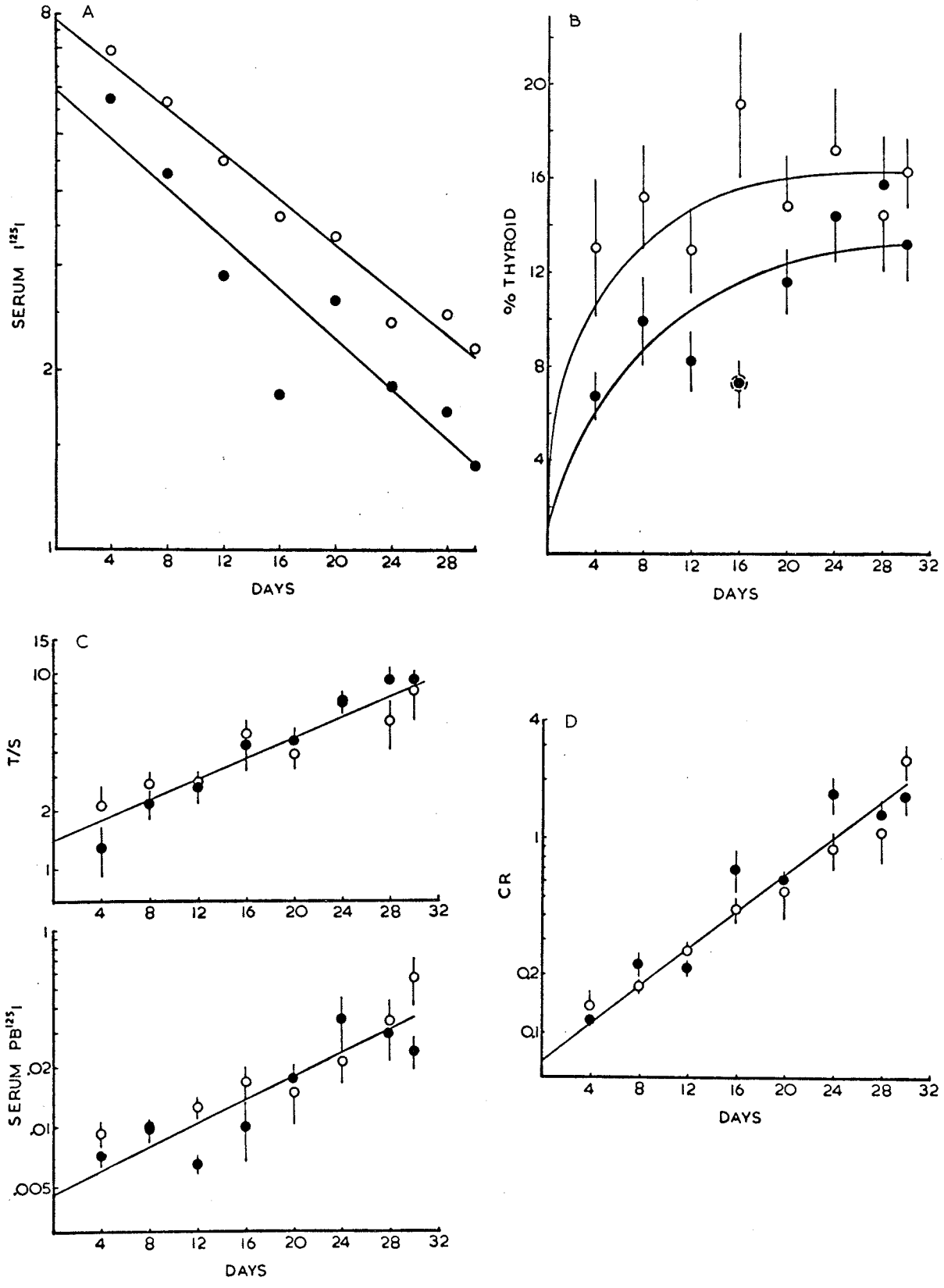
From this experiment it was concluded that serum inorganic radioiodide removal was accelerated in fish subjected to a continuous current, primarily through extrathyroidal pathways, which contributed in part to a reduced maximum thyroid uptake, since less radioiodide was available. A slight but very inconclusive increase in thyroid hormone production probably occurred for fish in the current.

c) Experiment III (March-April 1970; Fig. 7A, B, C, D) - A lower acclimation temperature was tested in this third experiment. From the stock tank 80 fish (ave. weight 106.64 g ± 21.72 (S.D.)) were randomly assigned to the current chamber, while 80 fish (ave. weight 106.97 g ± 22.43 (S.D.)) were assigned to the calm chamber of the experimental apparatus. Water temperature was 11°C. Current fish swam continously for 13 days, after an initial period where both groups had been held at the acclimation temperature for 12 days and then all fish were injected with 2.5 μCi of radioiodide and killed 4, 8, 12, 16, 20, 24, 28 and 30 days p.i.

As indicated in Fig. 7B calm fish had noticeably higher % thyroids, with both groups showing no significant decrease in thyroid radioactivity even after 30 days p.i. U% for calm fish was 17.5%, while current fish were lower at 14.4%. Fish undergoing sustained swimming had faster removal of serum radioiodide ($t\frac{1}{2}$ = 14.25 days; K = 0.049) than for calm fish ($t\frac{1}{2}$ = 15.75 days; K = 0.044). This was the result of an accelerated extrathyroidal removal of serum radioiodide by current fish (k_2 = 0.042 in contrast to 0.036 for calm fish). Rate of thyroid serum radioiodide removal was less for current fish (k_1 = 0.007) than for calm fish (k_1 = 0.008). Current fish had an altered radioiodide distribution space (Fig. 7A).

No difference between groups could be detected in the T/S ratio, CR, or serum PB^{125}I concentration (Fig. 7C, D). The maximum values for T/S, CR, and PB^{125}I for current fish were 9.42 (28 days p.i.), 1.72 (24 days p.i.), and 0.035 (24 days p.i.), while for calm fish these were respectively 8.17 (30 days p.i.), 2.54 (30 days p.i.), and 0.057

Figure 7. Sustained swimming and radioiodine metabolism (current fish •, calm fish o) during March to April 1970. The indices of thyroid function, namely, serum $I^{125}I$, % thyroid, T/S, serum $PB^{125}I$ and CR are plotted against time (days). Points are means of nine to ten fish with the exception of current fish thirty days p.i. (n=12). Each mean for serum $I^{125}I$ is plotted as the antilogarithm of the logarithm average for the sample, since the straight lines were fitted by the method of least squares. Standard errors of the means are indicated except for serum $I^{125}I$.


A
SERUM $I^{125}I$
8
2
1
4
8
12
16
20
24
28
DAYS
B
% THYROID
20
16
12
8
4
4
8
12
16
20
24
28
32
DAYS
C
T/S
15
10
2
1
SERUM $PB^{125}I$
.02
.01
.005
4
8
12
16
20
24
28
32
DAYS
D
CR
4
1
.2
.1
4
8
12
16
20
24
28
32
DAYS

(30 days p.i.).

In conclusion, as in Experiment II, continuous swimming increased the rate of extrathyroidal removal of $I^{125}I$ from the serum, and reduced thyroid uptake. No effect of current on hormone production was found.

Tables VIII, IX, X summarize the coefficients of condition and hematocrits for all radioiodide experiments. In general, there was little difference, either in coefficients of condition or hematocrits between groups of an experiment.

All three radioiodide experiments together indicate that continuous swimming under these experimental conditions results in (1) either slight increase (Experiment I) or no increase (Experiment II and III) in thyroid activity, (2) an increase in the rate of serum radioiodide removal mostly through extrathyroidal pathways (Experiment II and III) and, (3) a reduction in U% (Experiment II and III) and an alteration in radioiodide distribution space (Experiment I and III).

Also these experiments indicated that both radioiodide metabolism, generally, and radiohormone production, in particular, seemed very slow in this species.

2. Stable Iodine (^{127}I Metabolism) (Fig. 8)

Several workers (see literature review) have implied that serum stable iodide levels can influence thyroid uptake of radioiodide. Since preliminary observations on the brook trout revealed a high, and in many cases variable ^{127}I level, it was felt that an investigation of the relationship between serum ^{127}I levels, and various radioiodide parameters, was necessary to interpret the radioiodide data.

Table VIII. Coefficients of condition and hematocrits for July-August 1969 (Radioiodide Experiment)

	Coefficient of Condition* (mean of 8 fish) ± 1 S.E.		Hematocrit ^{RBC}/Total (means of 8 fish) ± 1 S.E.	
Days p.i.	Current	Calm	Current	Calm
1	1.199 ± 0.040	1.183 ± 0.037	42.56 ± 1.15	41.44 ± 1.68
3	1.137 ± 0.030	1.126 ± 0.026	41.56 ± 1.05	41.56 ± 1.17
6	1.140 ± 0.039	1.127 ± 0.038	39.43 ± 1.29	40.19 ± 1.82
9	1.142 ± 0.025	1.099 ± 0.027	37.83 ± 0.42	39.13 ± 1.33
12	1.093 ± 0.034	1.112 ± 0.032	40.25 ± 1.25	39.63 ± 1.92
15	1.117 ± 0.032	1.123 ± 0.024	42.00 ± 1.83	34.06 ± 1.79
18	1.048 ± 0.018	1.139 ± 0.021	38.50 ± 2.16	38.44 ± 2.19
21	1.078 ± 0.027	1.149 ± 0.022	44.14 ± 2.34	36.36 ± 1.81
24	1.101 ± 0.015	1.087 ± 0.037	41.00 ± 2.36	37.00 ± 1.97

* Coefficient of condition = weight (g) X 100/fork length ^{3}(cm)

Table IX. Coefficients of condition and hematocrits for October-November 1969 (Radioiodide Experiment)

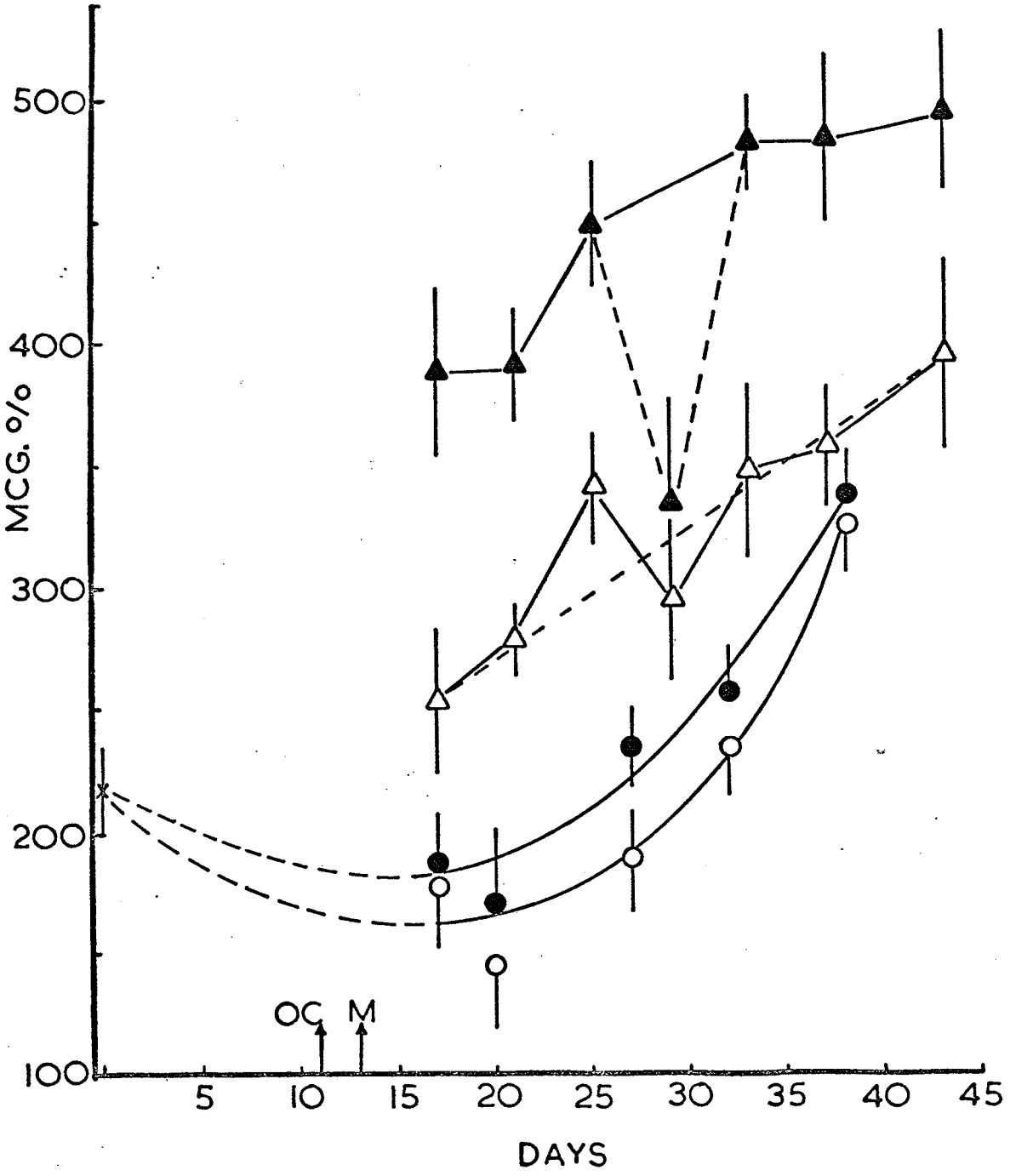
	Coefficient of Condition (mean of 10 fish) ± 1 S.E.		Hematocrit $^{RBC}/_{Total}$ (mean of 8-10 fish) ± 1 S.E.	
Days p.i.	Current	Calm	Current	Calm
2	0.992 ± 0.018	1.000 ± 0.019	33.61 ± 1.43	34.39 ± 1.57
6	1.018 ± 0.018	1.034 ± 0.023	33.70 ± 1.77	32.67 ± 2.19
9	0.987 ± 0.019	1.041 ± 0.028	36.70 ± 1.69	34.38 ± 1.54
12	0.991 ± 0.016	1.081 ± 0.025	33.55 ± 1.39	35.30 ± 2.07
14	1.003 ± 0.016	0.986 ± 0.015	31.69 ± 1.51	30.00 ± 0.73
16	1.026 ± 0.025	0.936 ± 0.020	34.50 ± 2.77	29.00 ± 0.67
18	1.020 ± 0.030	1.012 ± 0.032	33.50 ± 1.26	33.00 ± 1.51
21	0.958 ± 0.027	0.991 ± 0.013	31.67 ± 1.65	34.25 ± 2.00
24	0.954 ± 0.009	0.953 ± 0.013	34.83 ± 1.40	32.88 ± 1.69
27	0.959 ± 0.023	0.953 ± 0.023	31.71 ± 1.61	32.30 ± 0.79

Table X. Coefficients of condition and hematocrits for March-April 1970 (Radioiodide Experiment)

	Coefficient of Condition (mean of 9-12 fish) ± 1 S.E.		Hematocrit RBC/Total (mean of 9-11 Fish) ± 1 S.E.	
Days p.i.	Current	Calm	Current	Calm
4	1.060 ± 0.019	1.072 ± 0.029	38.20 ± 0.81	39.20 ± 1.31
8	0.992 ± 0.033	1.026 ± 0.020	37.28 ± 0.72	36.45 ± 1.19
12	1.018 ± 0.021	1.060 ± 0.019	37.78 ± 1.36	36.40 ± 1.16
16	1.007 ± 0.020	1.022 ± 0.022	37.61 ± 1.56	38.90 ± 1.47
20	0.990 ± 0.023	0.983 ± 0.017	37.33 ± 1.29	38.35 ± 1.62
24	1.024 ± 0.024	0.973 ± 0.024	38.60 ± 1.32	38.50* ± 1.16
28	0.965 ± 0.017	0.968 ± 0.019	37.55 ± 0.75	36.75 ± 0.67
30	0.964 ± 0.015	0.990 ± 0.016	34.86 ± 1.18	33.88 ± 1.35

* n = 7

Figure 8. Total serum stable iodine (^{127}I) levels for sera from experiment II (current ●, calm o) and experiment III (current ▲, calm Δ). Arrows indicate time at which fish were injected in October (CC) and March (M) after the initiation of the experiment. Points in experiment II are means of eight to ten fish, while those in experiment III are means of ten to twelve fish. Standard errors of the means are shown.


500
400
300
200
100
MCG. %
OC
M
5
10
15
20
25
30
35
40
45
DAYS

A limited number of determinations were performed on the individual sera of some fish from Experiment I. A sample of 10 fish, killed before the start of the experiment, had a mean total iodide concentration of 126.8 mcg% ± 15.4 (S.E.). After swimming for 26 days total serum iodide increased to a mean (n=8) of 178.7 mcg% ± 20.6 (S.E.). The calm fish also increased, but to a lesser extent (ave. 143.1 mcg% ± 27.6 (S.E.); n=8). Swimming for 32 days resulted in total iodide increasing further to an average of 273.5 mcg% ± 31.2 (S.E.) with n=8.

Based on these data it seemed that serum ^{127}I did not remain constant throughout an experiment and was also influenced by exposure to current. To verify these conclusions more extensive determinations were performed on the sera from Experiment II and III.

For Experiment II, apart from an initial decline from an average of 217.0 mcg% ± 17.0 (S.E.), n=7, at the beginning of the experiment, both groups sharply increased their average serum total iodides, reaching maximum values of 337.3 mcg% ± 18.9 (S.E.) for current fish and 325.9 mcg% ± 19.6 (S.E.) for calm fish. At all sample times current averages were higher.

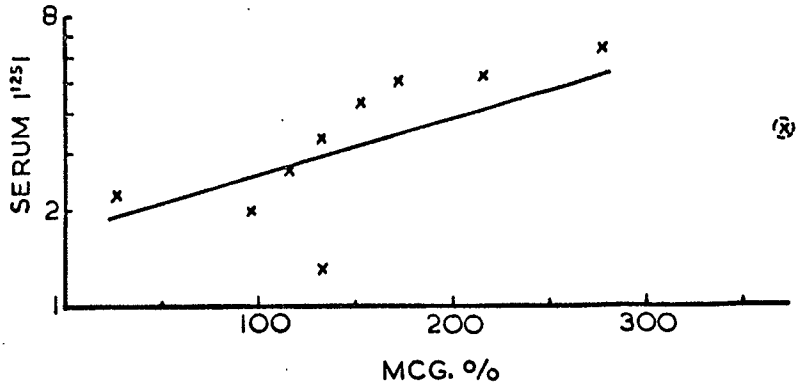
For Experiment III an even greater separation of current fish, averages from calm fish occurred, except at 29 days where the difference was smaller. This was caused by 3 fish out of 10, which had substantially lower total serum iodide. Current fish increased from 388.6 mcg% ± 34.1 (S.E.) at 17 days to 495.8 mcg% ± 32.6 (S.E.) at 43 days, while the level for the calm fish increased from 253.5 mcg% ± 29.5 (S.E.) to 395.8 mcg% ± 38.4 (S.E.).

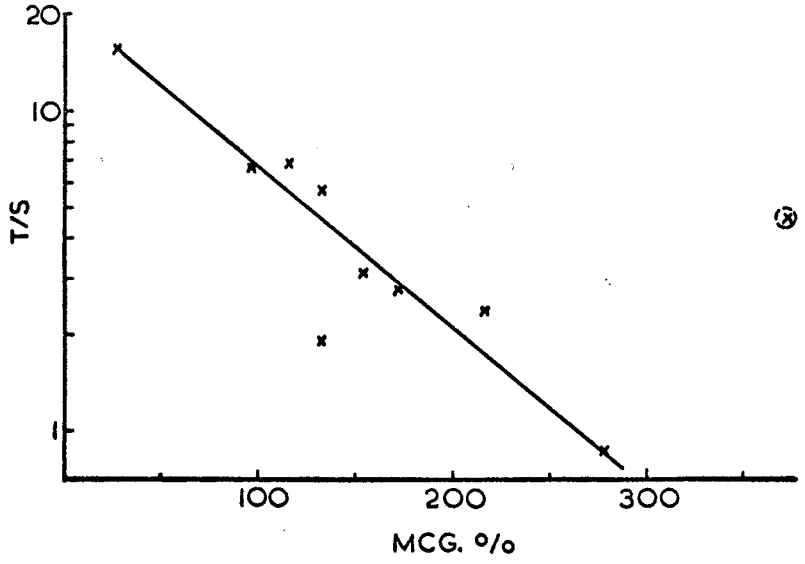
Since the internal serum iodide environment had not remained constant, but increased, it was decided to see whether or not the changes mentioned above in stable ^{127}I could influence radioiodide indices of thyroid activity. The two sample times with the highest standard errors of the mean for stable iodide levels, and therefore having the greatest potential scope for change in ^{125}I parameters, were chosen for both Experiment II and III. The T/S ratio, C.R., and serum $I^{125}I$ for each fish were plotted against the corresponding total serum iodide for that same fish.

An inverse relationship was found in all cases for the T/S ratio and the conversion ratio (Figs. 9, 10, 11, 12) with the exception of one sample from Experiment III (Fig. 12) where there was no relationship whatsoever between total serum iodide and C.R. A direct relationship was found between serum $I^{125}I$ and serum stable iodide, suggesting that greater removal of ^{127}I is accompanied by a corresponding greater removal of radioiodide. In the four samples examined % thyroid was not noticeably affected by total iodide levels, however, in one extreme case of low ^{127}I % thyroid was drastically increased, presumably because of increased specific activity of the injected radioiodide. This observation was particularly evident at other sample times.

Several conclusions were reached. First of all serum stable iodide did not remain constant under these experimental conditions. Second, current fish seemed capable of accumulating greater levels of serum stable iodide, although this difference might not have continued with a longer experiment. This was indicated in a later experiment,

Figure 9. Relationship between radioiodide indices of thyroid activity (serum $I^{125}I$, T/S, and CR) and total serum ^{127}I for current fish from experiment II (9 days p.i.). Each X represents an individual fish.


8
2
1
SERUM I^{125}
100
200
300
MCG. %


20
10
1
T/S
100
200
300
MCG. %

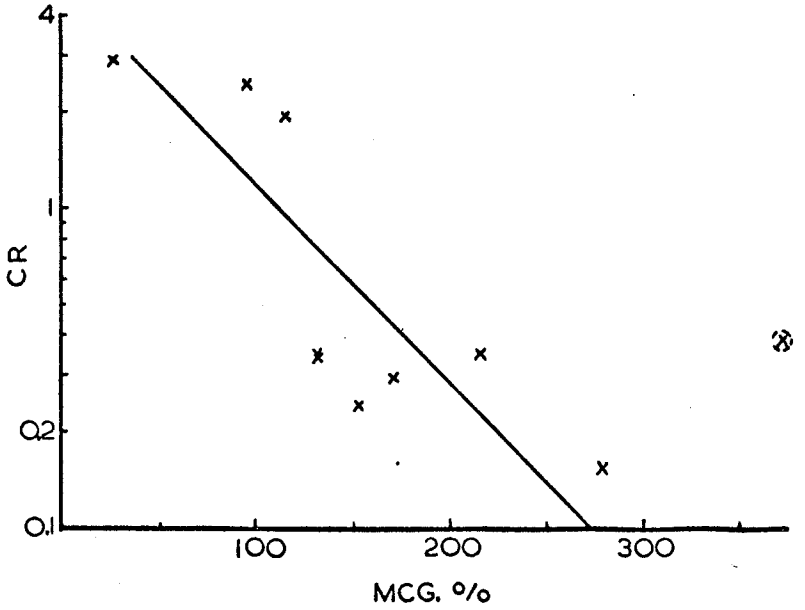
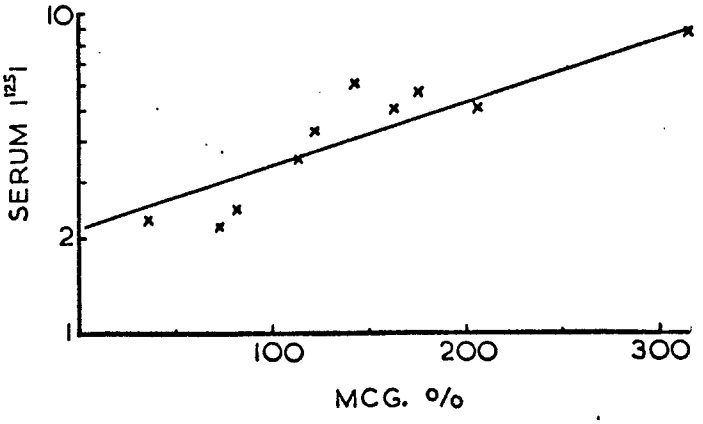
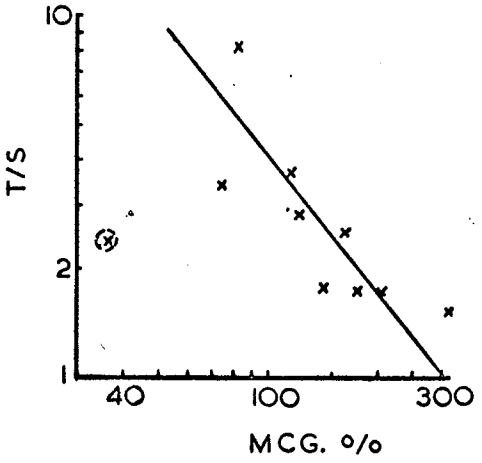

4
1
0.2
0.1
CR
100
200
300
MCG. %

Figure 10. Relationship between radioiodide indices of thyroid activity (serum $I^{125}I$, T/S, and CR) and total serum ^{127}I for calm fish from experiment II (9 days p.i.). Each X represents an individual fish.


10
2
1
SERUM I^{125}
100
200
300
MCG. %


10
2
1
T/S
40
100
300
MCG. %

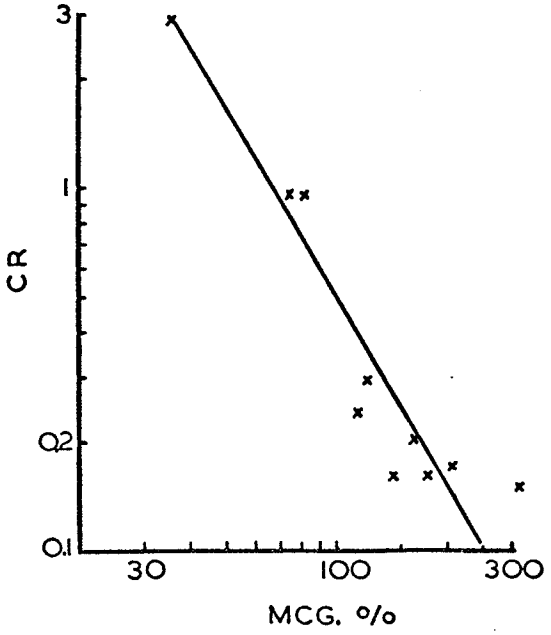
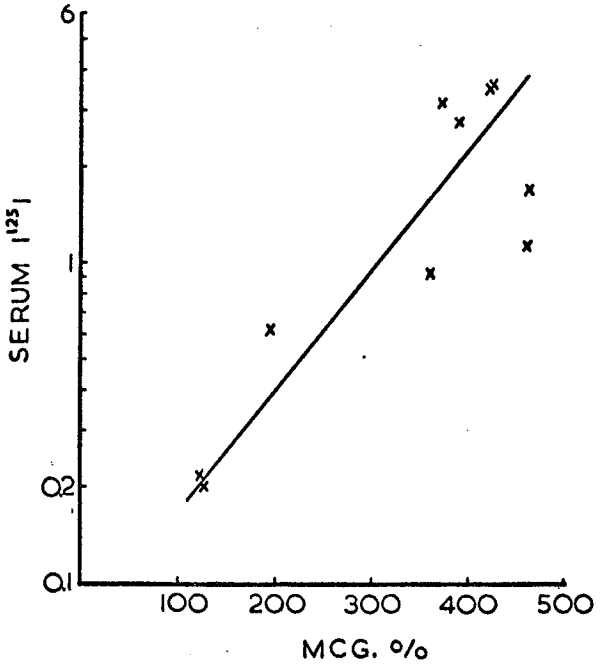
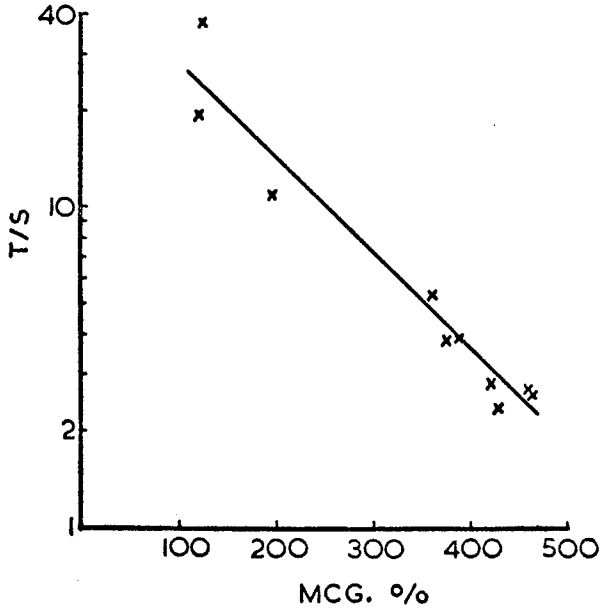

3
1
0.2
0.1
CR
30
100
300
MCG. %

Figure 11. Relationship between radioiodide indices of thyroid activity (serum $I^{125}I$, T/S, and CR) and total serum ^{127}I for current fish from experiment III (16 days p.i.). Each X represents an individual fish.


SERUM I^{125}
6
1
0.2
0.1
100
200
300
400
500
MCG. %


T/S
40
10
2
1
100
200
300
400
500
MCG. %

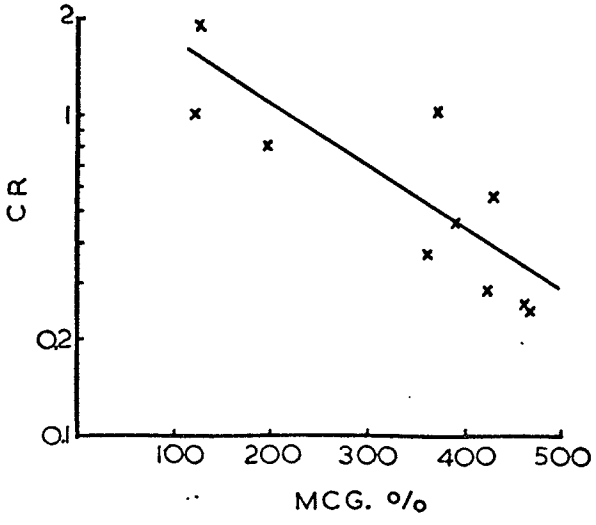
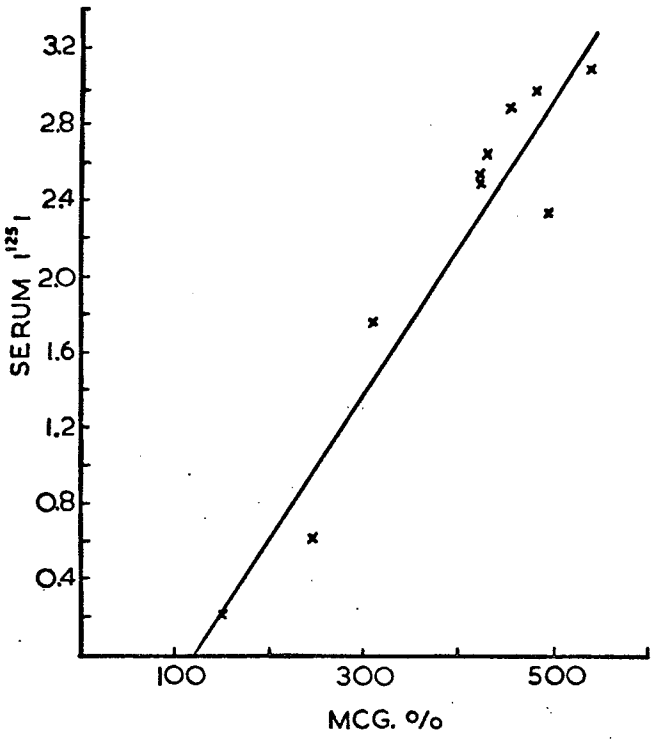
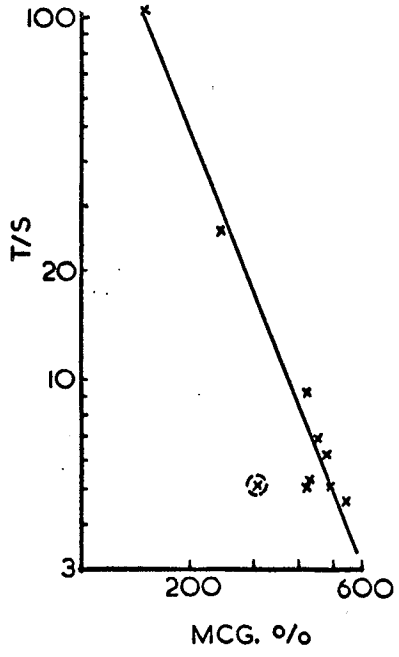
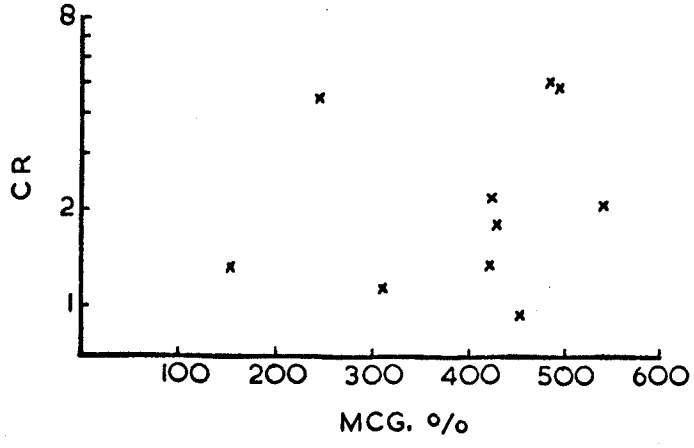

CR
2
1
0.2
0.1
100
200
300
400
500
MCG. %

Figure 12. Relationship between radioiodide indices of thyroid activity (serum $I^{125}I$, T/S, and CR) and total serum ^{127}I for calm fish from experiment III (30 days p.i.). Each X represents an individual fish.


SERUM $I^{125}I$
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
100
300
500
MCG. %


T/S
100
20
10
3
200
600
MCG. %


CR
8
2
1
100
200
300
400
500
600
MCG. %

where a serum sample, taken from a serum pool from 20 fish, which had been subjected to a current for 53 days, had a value of 394.8 mcg%, whereas the calm fish were slightly higher, having 411.6 mcg%. Finally, serum stable iodide levels were capable of influencing, markedly, radioiodide parameters of thyroid activity.

Thyronine Metabolism

1. Radiothyroxine Metabolism

To provide insight into the possible effect of current on the peripheral metabolism of thyroid hormones, three experiments (IV, V, and VI) were conducted from June 1969 to February 1970. (Summary, Table XI; Regression equations, Table XII.)

a) Experiment IV (June-July 1969; Figs. 13, 14) - From the stock tank, 67 fish (ave. weight 170.68g ± 35.66 (S.D.)) were assigned at random to the current chamber, while 67 fish (ave. weight 154.70 g ± 28.87 (S.D.)) were assigned to the calm chamber of the experimental apparatus. Water temperature was 13.5°C. Current fish swam continuously for 10 days, and then all fish were injected with 0.2 μCi of T_4*, specific activity 43.6 mCi/mg and 8-9 fish killed at 3, 6, 12, 24, 48, 72, 96 and 120 hr p.i.

The patterns of change with time for serum $PB^{125}I$, $I^{125}I$ and the % of total serum radioactivity that is $PB^{125}I$ are shown in Fig. 13A, B. Three hours after injection serum $PB^{125}I$ was highest as the result of absorption of T_4* from the coelom into the blood stream.

Table XI. Summary of Radiothyroxine Metabolism

	Current			Calm		
	Experiment IV June-July 1969	Experiment V December 1969	Experiment VI* February 1970	Experiment IV June-July 1969	Experiment V December 1969	Experiment VI February 1970
Temp °C	13.5	12	9.5	13.5	12	9.5
Days starved before injection	10	16	12	10	16	12
Average fish weight (g)± 1 S.D.	170.68±35.66	92.75±21.28	98.88±22.49	154.70±28.87	90.67±19.52	104.52±21.10
Length (cm) ±1 S.D.	25.35±1.65	20.61±1.51	20.96±1.48	24.78±1.53	20.32±1.59	21.43±1.49
Specific activity (T_4*) (mCi/mg)	43.6	135.0	35.0	43.6	135.0	35.0
$t\frac{1}{2}$ (serum PB^{125}I) (hours)	30.4	67.2	99.6	37.6	82.8	92.4
k	0.023	0.010	0.0070	0.018	0.0084	0.0075
max. % liver	18.4 (3hr)	9.0 (6hr)	6.7 (25hr)	16.6 (6hr)	10.2 (6hr)	5.0 (25hr)
max. % gall bladder	19.7 (12hr)	20.2 (72hr)	20.8 (73hr)	17.4 (72hr)	22.4 (72hr)	25.4 (97hr)
max. % intestine	17.2 (48hr)	10.2 (96hr)	8.5 (97hr)	14.6 (24hr)	9.02 (72hr)	9.0 (73hr)
max. % total enterohepatic	39.2 (12hr)	31.3 (24hr)	29.7 (97hr)	34.4 (72hr)	34.5 (72hr)	38.4 (97hr)

* Current turned on 50 hr p.i.

Table XII. Equations for straight line regressions used in figures (Radiothyroxine metabolism, Serum $PB^{125}I$)

	Current			Calm		
Date	No. of Figure	T°C	Equation of the line*	No. of Figure	T°C	Equation of the line
June-July 1969	13a.	13.5	$\log y = \bar{1}.8377-(0.0099)X$	13a.	13.5	$\log y = \bar{1}.8691-(0.0080)X$
December 1969	15a.	12	$\log y = \bar{1}.3958-(0.0045)X$	15a.	12	$\log y = \bar{1}.4493-(0.0036)X$
February 1970	17a.	9.5	$\log y = \bar{1}.5166-(0.0030)X$	17a.	9.5	$\log y = \bar{1}.5253-(0.0033)X$

*$\log y = a+bX$

Figure 13a. Influence of sustained swimming (current fish (● ▲) calm fish (○ △)) on the disappearance of $PB^{125}I$ and $I^{125}I$ from the serum following intraperitoneal injection of T_4*. Each point is plotted as the antilogarithm of the logarithm average for the sample, which was composed of eight to nine fish.

13b. Graph showing the per cent of total serum radioactivity that is $PB^{125}I$ in relation to time (hours). Standard errors of the means are indicated.

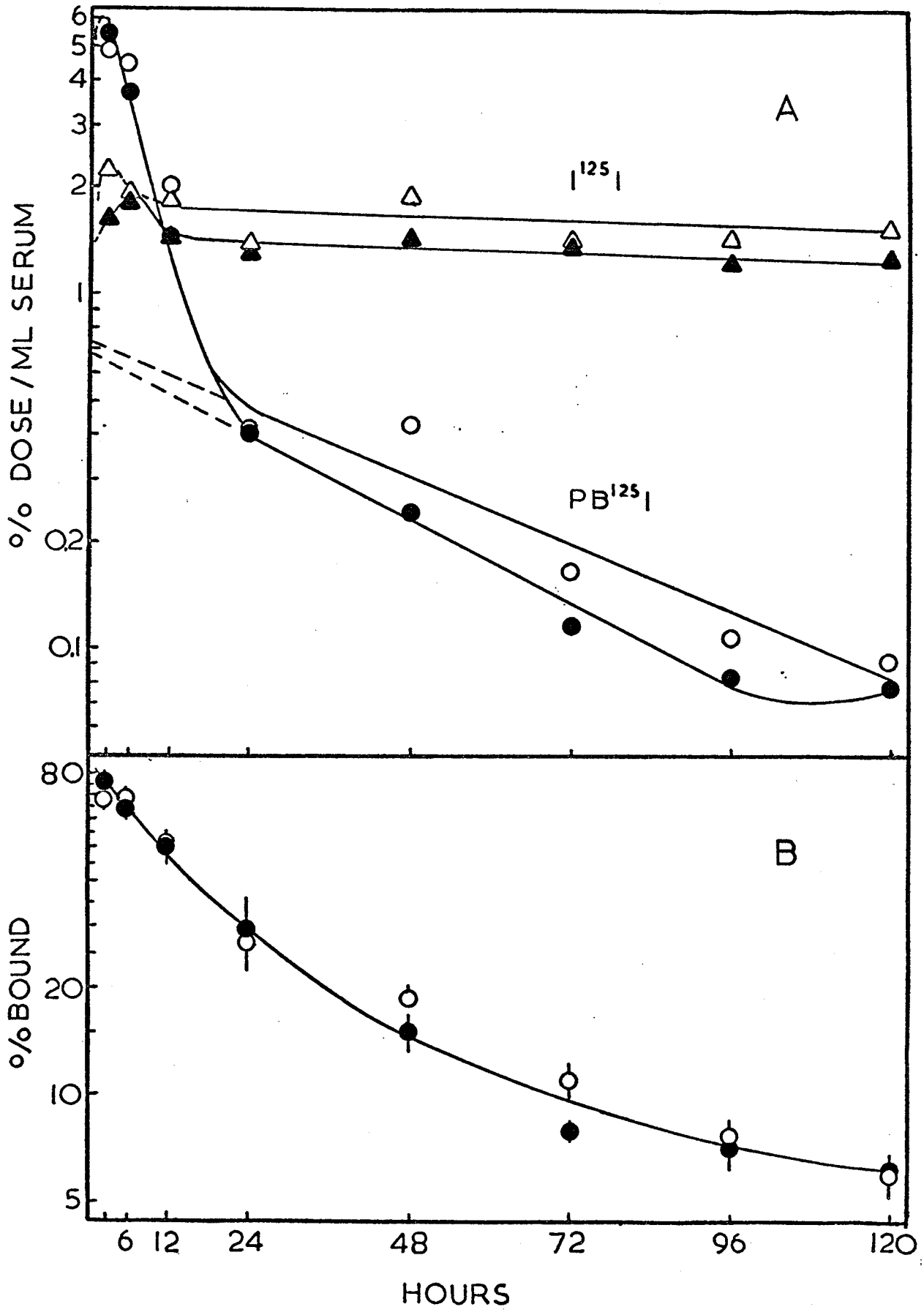
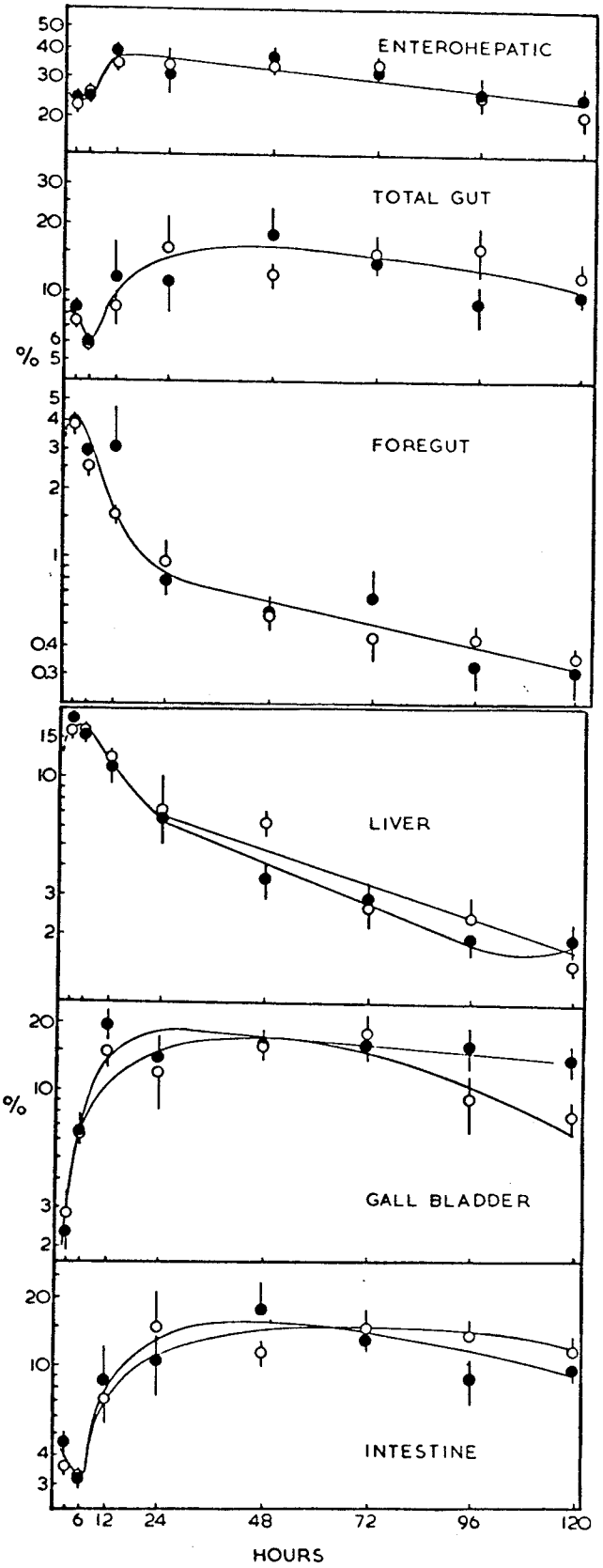

A
$I^{125}I$
$PB^{125}I$
% DOSE / ML SERUM
6
5
4
3
2
1
0.2
0.1
B
% BOUND
80
20
10
5
6
12
24
48
72
96
120
HOURS

Figure 14. Per cent of the injected dose found in enterohepatic tissues of current fish • and calm fish o at certain times (hours) following intraperitoneal injection of T_4*. Each point represents a mean of 8-9 fish. Standard errors of the means are indicated.


ENTEROHEPATIC
TOTAL GUT
FOREGUT
LIVER
GALL BLADDER
INTESTINE
%
%
HOURS
6 12 24 48 72 96 120

A rapid phase of $PB^{125}I$ reduction then followed at the same rate for both groups, which occurred up to 24 hr p.i. This has been attributed in the past to a distribution of the radioactivity into one or more extravascular compartments. After 24 hours there was an exponential phase being slower for calm ($t\frac{1}{2}$=37.6 hr, k = 0.018) than for current fish ($t\frac{1}{2}$ = 30.4 hr, k = 0.023). A slight increase in serum $PB^{125}I$ was noted for current fish at 120 hr p.i., possibly the result of reabsorption of hormone from excretory pathways back into the blood stream.

Serum $I^{125}I$ levels increased rapidly 3-6 hr p.i. and the quantity was maintained at nearly a constant level over the period of sampling.

Nearly 70% for calm fish and about 80% for current fish of serum radioactivity was $PB^{125}I$ 3 hr p.i. (Fig. 13B). This decreased at a similar rate for both groups reaching 6% bound 120 hr p.i.

Accumulation of radioactivity by enterohepatic organs is shown in Figure 14. All enterohepatic tissues (liver, gall bladder and post pyloric caecal intestine combined) accumulated up to 39.2% of the injected dose at 12 hr for current fish, while maximum enterohepatic accumulation was 34.4% at 72 hr for calm fish. However, at 12 hr calm fish had taken up 34.2% and the line connecting all sample means indicated peak uptake of radioactivity should have been seen at this time. No difference was detected in the total enterohepatic loss between the two groups. The liver and gall bladder patterns indicated slight differences and perhaps the intestine. Maximum liver uptake was 18.4% 3 hr p.i. for current fish, while that for calm fish was 16.6% at 6 hr p.i. Both groups declined rapidly until 24 hr when

they entered an exponential phase similar to serum PB^{125}I with current fish indicating a slightly faster clearance.

Maximum gall bladder radioactive accumulation was 19.7% 12 hr p.i. for current fish, with calm fish requiring 72 hr to reach their maximum of 17.4%, after which radioactive loss was faster than for current fish. Maximum intestinal radioactive accumulation was at 48 hr for current fish (17.2%) following which there was a decline.

Although maximum radioactive uptake was at 24 hr (14.6%) for calm fish, there was little loss of radioactivity from this maximum over the period of sampling (Fig. 14). Foregut radioactivity reached a maximum of 4% at 3 hr and declined rapidly to less than 1% at 24 hr and continued to decrease exponentially to 120 hr at the same rate for both groups (Fig. 14). Total gut (foregut and intestine) was of similar pattern to the intestine (Fig. 14).

It was concluded that exercise could increase the T_4* degradation rate, possibly as the result of increased removal of thyroid hormone from the circulation by the liver and gall bladder.

b) Experiment V (December 1969; Figs. 15, 16) - This experiment was performed at a lower acclimation temperature (12°C). From the stock tank 95 fish (ave. weight 92.75 g $\pm$ 21.28 (S.D.)) were randomly assigned to the current chamber, while 95 fish (ave. weight 90.67 g $\pm$ 19.52 (S.D.)) were assigned to the calm chamber of the experimental apparatus. Current fish swam continuously for 14 days out of 16 days and then all fish were injected with 0.25 µCi T_4*, specific activity 135 mCi/mg and 9-10 fish killed at 6, 12, 18, 24, 48, 72, 96, 120, 144,

Figure 15a. Influence of sustained exercise (current fish (●▲) calm fish (○△)) on the disappearance of $PB^{125}I$ and $I^{125}I$ from the serum following intraperitoneal injection of T_4*. Each point is plotted as the antilogarithm of the logarithm average for the sample, which was composed of nine to ten fish.

15b. Graph showing the per cent of total serum radioactivity that is $PB^{125}I$ in relation to time (hours). Standard errors are indicated.

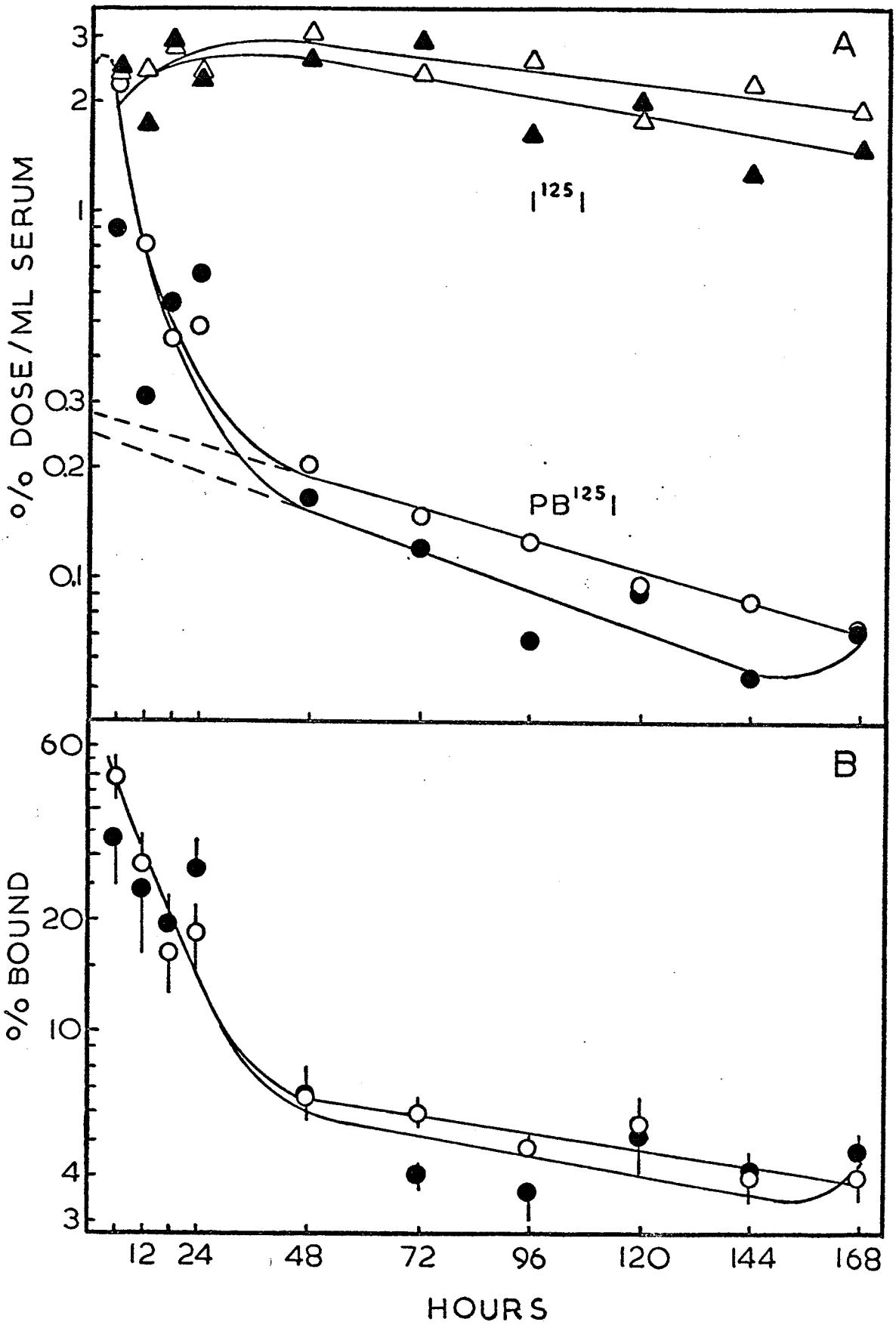
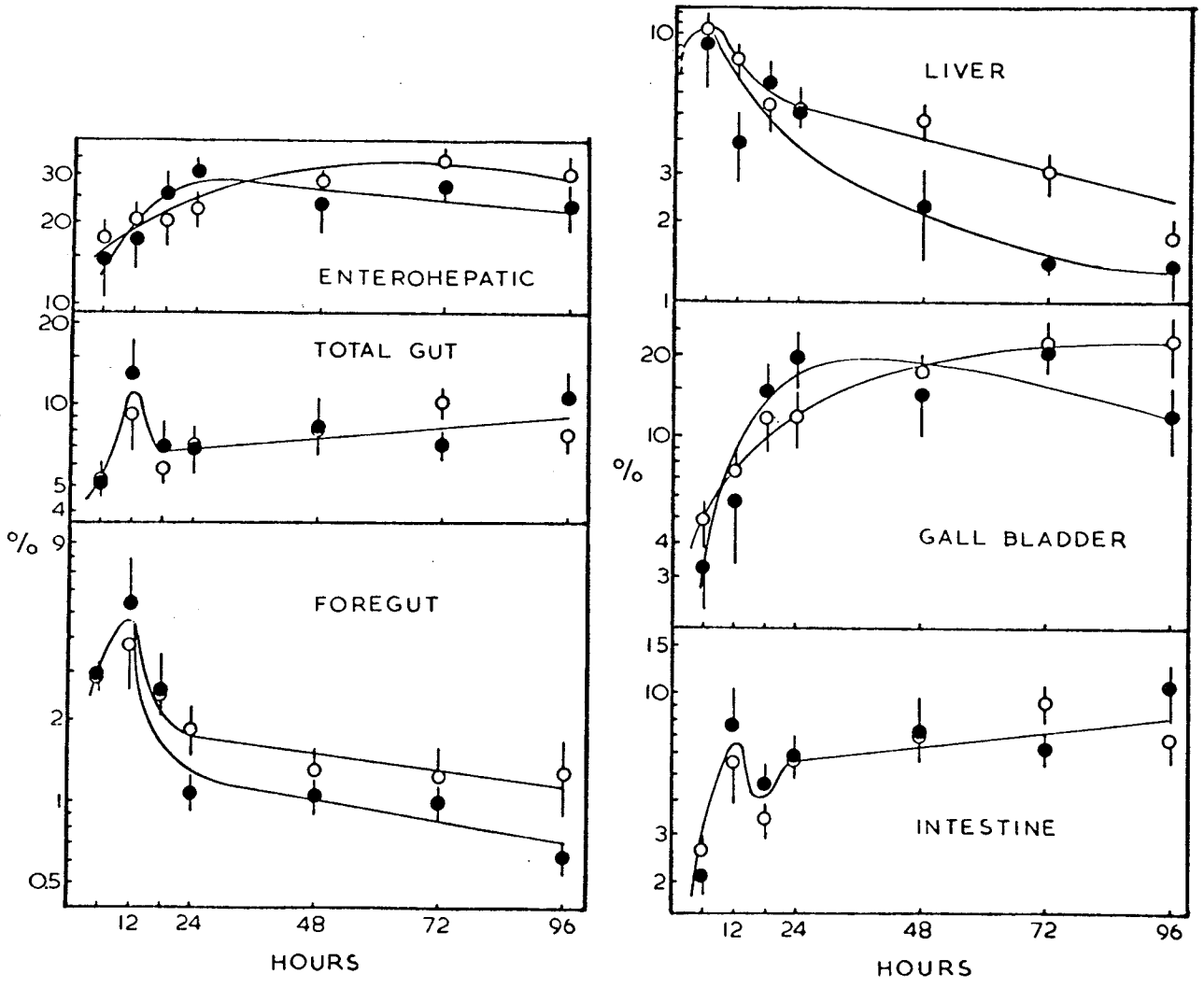

A
B
% DOSE / ML SERUM
% BOUND
$I^{125}I$
$PB^{125}I$
3
2
1
0.3
0.2
0.1
60
20
10
4
3
12
24
48
72
96
120
144
168
HOURS

Figure 16. Per cent of the injected dose found in enterohepatic tissues of current fish • and calm fish o at certain times (hours) following intraperitoneal injection of T_4*. Each point represents a mean of 9-10 fish. Standard errors of the means are indicated.


ENTEROHEPATIC
TOTAL GUT
FOREGUT
%
HOURS
LIVER
GALL BLADDER
INTESTINE
%
HOURS

and 168 hr p.i.

Figure 15A, B, illustrates serum $I^{125}I$, $PB^{125}I$ and % bound at the above sample times. Serum $PB^{125}I$ decreased rapidly to 48 hr, at which time both groups entered into an exponential loss which was slower for the calm fish ($t\frac{1}{2}$ = 82.8 hr, k = 0.0084) than for the current fish ($t\frac{1}{2}$ = 67.2 hr, k = 0.010). There was some indication at 168 hr p.i., that $PB^{125}I$ was beginning to increase.

Serum $I^{125}I$ rose up to 48 hr, after which there was a slow decline for both groups. The per cent of the radioactivity that was $PB^{125}I$ was about 50% for calm fish and 34% for current fish 6 hr p.i. and decreased rapidly until 48 hr when both groups had approximately 6.5% bound. An exponential decrease of similar pattern to serum $PB^{125}I$ then followed.

Current fish total enterohepatic organs accumulated maximum radioactive uptake of 31.3% at 24 hr p.i. and then declined, while calm fish maximum accumulation was 34.5% at 72 hr and then there was a decrease (Fig. 16).

Clearance of radioactivity taken up by the liver was faster for current fish (Fig. 16) with both groups having attained a maximum uptake of radioactivity of between 9-10% at 6 hr p.i. Current fish also demonstrated more rapid accumulation and loss of radioactivity in the gall bladder (Fig. 16).

Intestine and total gut did not differ between groups, steadily increasing over the period of sampling.

Maximum foregut radioactive uptake occurred at 12 hr p.i. for both groups, reaching an average of 5.4% for current fish and 3.8% for calm fish, with a more rapid decline after this for the former group.

The conclusions from this experiment were the same as those from Experiment IV. Thyroid hormone degradation rate was elevated in exercised fish probably as the result of increased loss or excretion of hormone particularly from the liver to the gall bladder.

c) Experiment VI (February 1970; Figs. 17, 18) - To investigate the effect on radiothyroxine metabolism, of suddenly forcing fish to swim, a third experiment was carried out.

From the stock tank 68 fish (ave. weight 98.88 g ± 22.49 (S.D.)) were assigned at random to the current chamber, while 68 fish (ave. weight 104.52 g ± 21.10 (S.D.)) were assigned to the calm chamber of the experimental apparatus. Water temperature was 9.5°C. All fish were injected after 23 days with 1 μCi T_4*, specific activity 35.0 mCi/mg and were sampled 25, 49, 73, 97, 117, 142, and 167.5 hr p.i. At 50 hr p.i. the current was turned on.

The hormonal and inorganic serum fractions as well as % bound are illustrated in Figure 17A, B. Rapid loss of serum $PB^{125}I$ continued until 49 hr, after which there was an exponential decrease. No effect of exercise was observed for current fish which had a decreased rate of $PB^{125}I$ removal ($t_{\frac{1}{2}}$ = 99.6 hr, k = 0.0070) than for calm fish ($t_{\frac{1}{2}}$ = 92.4 hr, k = 0.0075). Serum $I^{125}I$ increased up to 73 hr for both groups then slowly declined.

At 49 hr p.i. both groups had about 11% bound from which time there was an exponential decline to between 3 and 4% at 167.5 hr p.i. (Fig. 17B).

Figure 17a. Graph showing the disappearance of serum $PB^{125}I$ and $I^{125}I$ before and after sudden exposure to a water current at fifty hours after intraperitoneal injection of T_4* (current chamber fish •, calm chamber fish o). Each point is plotted as the antilogarithm of the logarithm average for the sample, which was composed of nine to ten fish.

17b. Graph showing the per cent of total serum radioactivity that is $PB^{125}I$ in relation to time (hours) before and after exposure to an imposed current. Standard errors of the means are indicated.

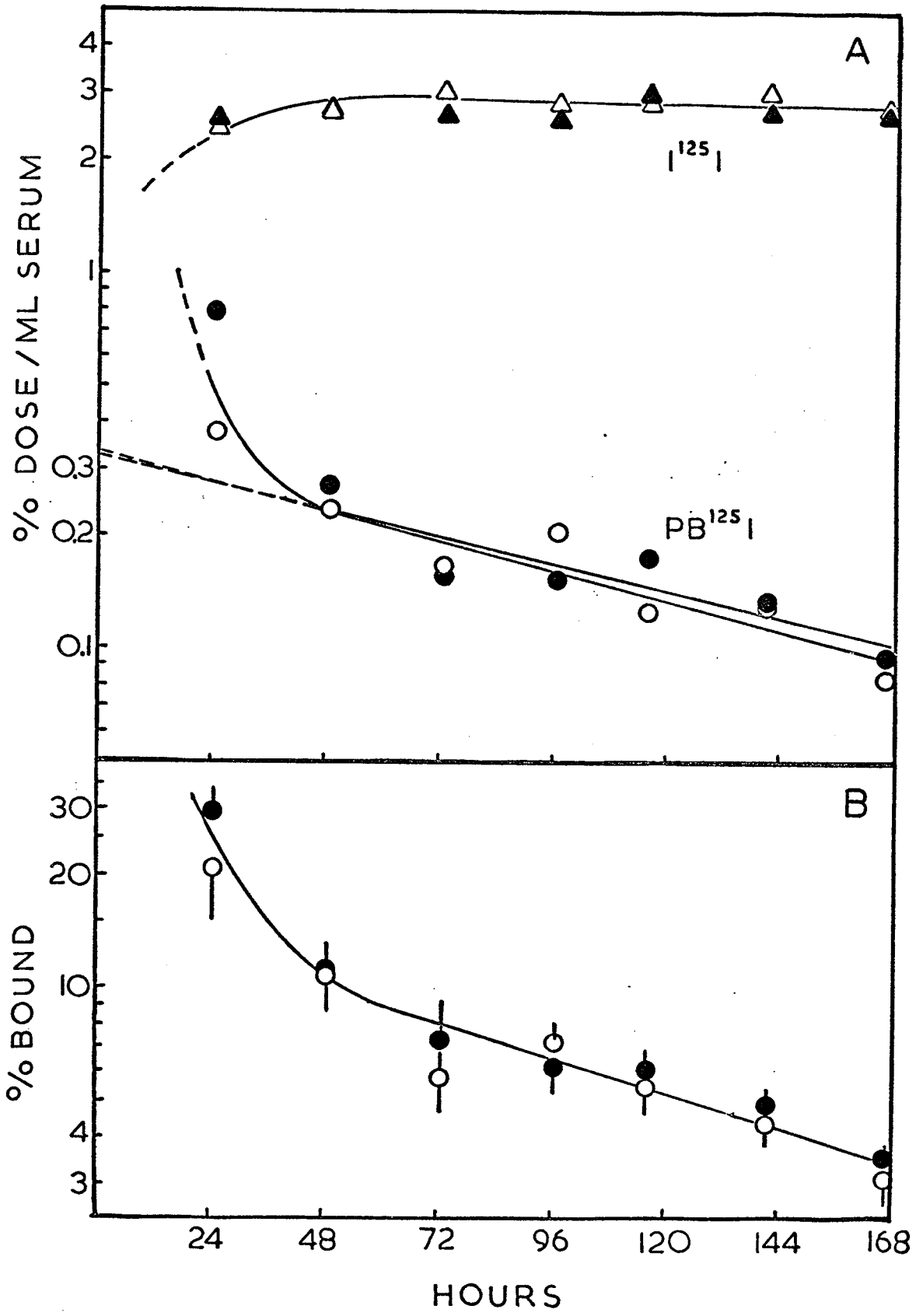
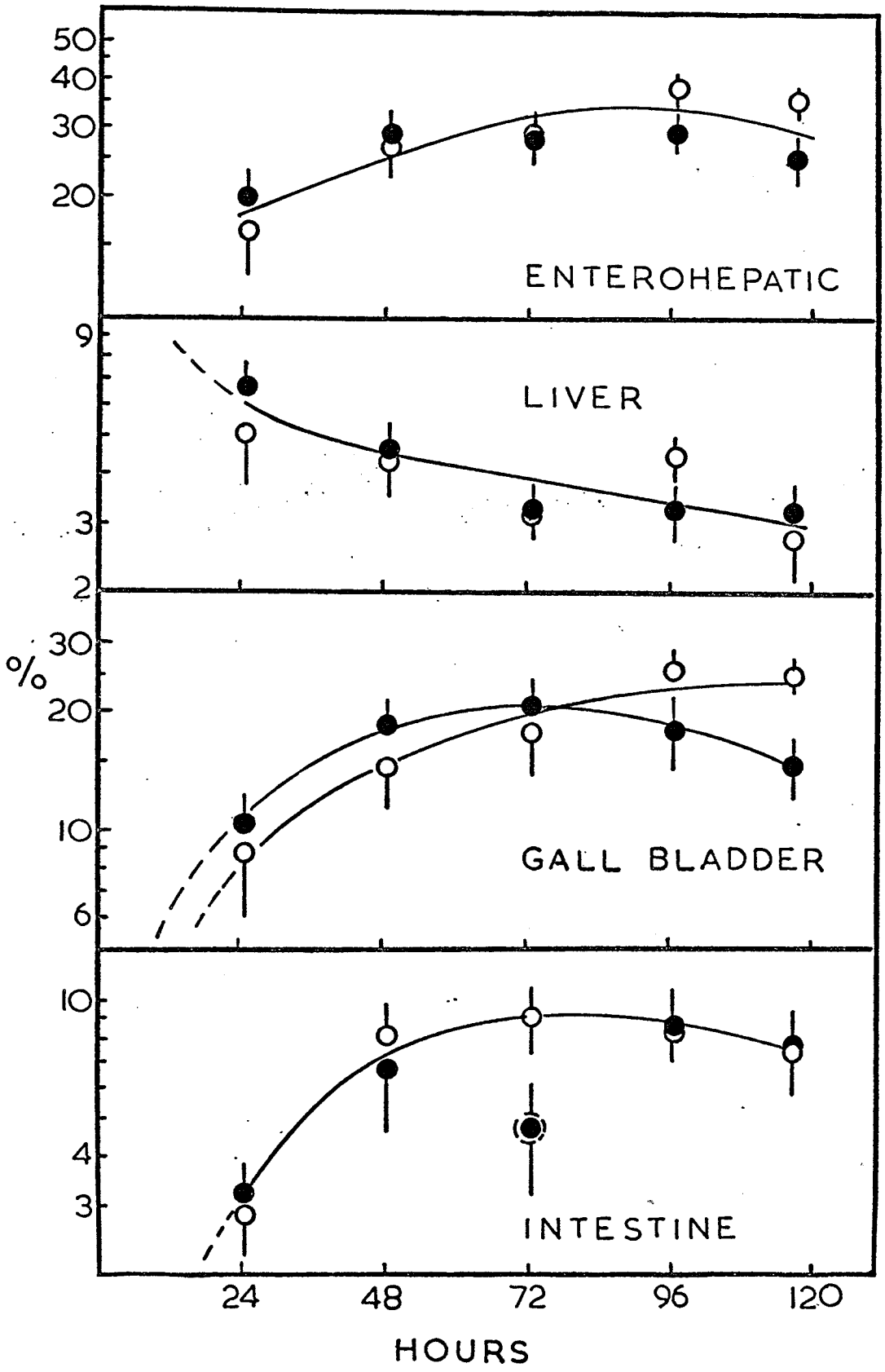

A
B
4
3
2
1
0.3
0.2
0.1
% DOSE / ML SERUM
$I^{125}I$
$PB^{125}I$
30
20
10
4
3
% BOUND
24
48
72
96
120
144
168
HOURS

Figure 18. Per cent of the injected dose found in enterohepatic tissues of current chamber fish • and calm chamber fish o before and after exposure to a water current at fifty hours after intraperitoneal injection of T_4*. Each point represents a mean of ten fish. Standard errors of the means are indicated.


50
40
30
20
ENTEROHEPATIC
9
3
2
LIVER
%
30
20
10
6
GALL BLADDER
10
4
3
INTESTINE
24
48
72
96
120
HOURS

Generally, no differences were noted in the enterohepatic accumulation and loss of radioactivity between groups, except possibly in the gall bladder where current fish reached a maximum accumulation of 20.8% at 73 hr, which subsequently declined. Calm fish continually accumulated radioactivity in their gall bladder up to 120 hr (Fig. 18).

(1) Thin Layer Chromatography - Since considerable deiodination was noticed throughout all the T_4* experiments (high levels of serum $I^{125}I$), it was decided to identify the serum radioactive materials at 25 hr p.i. by thin layer chromatography.

Most of the serum radioactivity was identified either as, iodide, T_4, or most probably T_3 or a T_3 like substance, since the migration distance was equal to the applied T_3 standard. For the fish in the calm water chamber, the ratio of T_3 to T_4 (T_3/T_4) was, on the average of 6 fish, 0.502 or there was twice as much T_4 as T_3. Fish randomly swimming in the current chamber had a T_3/T_4 ratio of 0.904 (mean of 8 fish) or there were almost equal amounts of T_3 to T_4. A predictable relationship was obtained between total radiothyronines determined by chromatography and the % of radioactivity as $PB^{125}I$ (Table XIII).

Tables XIV, XV, and XVI summarize the coefficients of condition and hematocrits, for all T_4 experiments. Generally, coefficients of condition did not differ within experiments, to any extent, between groups. In Experiment IV average hematocrits slowly declined during the experiment.

Table XIII. Chromatography of the sera from fish 25 hr after T_4* injection

Condition	No.	I	Thyronines %				TCA Precipitation % Bound
			T_4	T_3*	T_3/T_4	Total	
	3	53.9	18.7	8.1	0.433	26.8	25.1
	4	34.1	33.7	13.0	0.386	46.7	44.1
Fish in Calm	5	28.6	36.3	10.4	0.286	46.7	47.4
	6	82.1	3.7	2.6	0.703	6.3	5.5
Water Chamber	7	64.5	6.0	4.5	0.750	10.5	5.2
	8	44.2	22.6	10.3	0.456	32.9	26.6
Mean ± 1 S.E.		51.23 ± 8.15	20.17 ± 5.55	8.15 ± 1.61	0.502 ± 0.075	28.32 ± 7.07	25.65 ± 7.39
	1	35.4	20.3	11.9	0.586	32.2	31.2
	2	46.9	26.3	19.1	0.726	45.4	37.3
	3	68.2	4.1	6.8	1.659	10.9	3.6
	4	24.2	22.2	12.2	0.550	34.4	43.7
Fish in Current	5	48.7	14.9	15.8	1.060	30.7	24.8
Chamber Without	6	45.8	34.2	9.4	0.275	43.6	35.6
Current at This	7	73.8	7.0	15.1	2.157	22.1	19.9
Time	8	27.4	31.0	6.8	0.219	37.8	42.1
Mean ± 1 S.E.		46.30 ± 6.28	20.00 ± 3.82	12.14 ± 1.55	0.904 ± 0.242	32.14 ± 4.01	29.78 ± 4.72

*Similar migration as T_3 standard

Table XIV. Coefficients of condition and hematocrits for June-July 1969 (T_4* Experiment)

	Coefficient of Condition (mean of 8-9 fish) ± 1 S.E.		Hematocrit ^{RBC}/Total (mean of 8-9 fish) ± 1 S.E.	
Hr p.i.	Current	Calm	Current	Calm
3	1.007 ± 0.029	1.025 ± 0.018	45.63 ± 1.76	45.88 ± 2.01
6	1.034 ± 0.024	1.003 ± 0.014	45.38 ± 1.64	45.50 ± 1.73
12	1.056 ± 0.034	1.013 ± 0.026	44.38 ± 1.45	45.00 ± 2.76
24	1.008 ± 0.031	0.998 ± 0.011	38.59 ± 1.35	45.88 ± 2.77
48	1.030 ± 0.027	1.063 ± 0.030	40.94 ± 1.29	41.13 ± 0.84
72	1.027 ± 0.028	1.023 ± 0.028	37.72 ± 1.89	39.44 ± 1.62
96	1.032 ± 0.017	0.941 ± 0.022	34.81 ± 1.62	38.83 ± 0.86
120	1.061 ± 0.023	0.994 ± 0.016	39.25 ± 1.28	36.75 ± 2.13

Table XV. Coefficients of condition and hematocrits for December 1969 (T_4* Experiment)

	Coefficient of Condition (mean of 9-10 fish) ± 1 S.E.		Hematocrit ^{RBC}/Total (mean of 7-10 fish) ± 1 S.E.	
Hr p.i.	Current	Calm	Current	Calm
6	1.045 ± 0.017	1.009 ± 0.015	36.82 ± 1.73	36.19 ± 1.13
12	1.065 ± 0.035	1.047 ± 0.020	39.37 ± 1.92	36.22 ± 1.40
18	1.042 ± 0.022	1.019 ± 0.020	36.75 ± 0.97	35.79 ± 1.41
24	1.092 ± 0.035	1.033 ± 0.010	35.21 ± 2.40	34.63 ± 1.51
48	1.031 ± 0.036	1.021 ± 0.020	35.72 ± 1.63	34.57 ± 1.88
72	1.013 ± 0.030	1.031 ± 0.020	33.07 ± 0.54	35.90 ± 0.75
96	1.036 ± 0.019	1.023 ± 0.022	37.06 ± 1.85	- -
120	1.044 ± 0.016	1.035 ± 0.015	35.34 ± 1.13	33.90 ± 1.79
144	1.045 ± 0.018	1.030 ± 0.009	31.75 ± 1.38	36.33 ± 1.97
168	1.048 ± 0.022	1.010 ± 0.022	35.00 ± 1.28	34.07 ± 1.87

Table XVI. Coefficients of condition and hematocrits for February 1970 (T_4* Experiment)

	Coefficient of Condition (mean of 9-10 fish) ± 1 S.E.		Hematocrit ^{RBC}/Total (mean of 8-10 fish) ± 1 S.E.	
Hr p.i.	Current	Calm	Current	Calm
25	1.106 ± 0.051	1.044 ± 0.014	40.55 ± 1.38	44.06 ± 1.25
49	1.028 ± 0.019	1.042 ± 0.014	44.28 ± 1.65	44.65 ± 1.89
73	1.071 ± 0.025	1.057 ± 0.024	46.94 ± 1.21	43.40 ± 1.69
97	1.052 ± 0.021	1.033 ± 0.021	40.36 ± 1.41	41.94 ± 1.32
117	1.052 ± 0.020	1.060 ± 0.022	44.15 ± 0.92	42.22 ± 1.07
142	1.065 ± 0.018	1.046 ± 0.021	39.28 ± 0.89	44.17 ± 1.55
167.5	1.039 ± 0.024	1.081 ± 0.027	39.28 ± 1.79	43.88 ± 1.26

2. Total Serum Thyronines

a) Experiment VII (May-July 1970; Table XVII) - This experiment was conducted to determine if current resulted in any changes in the stable quantities of thyronines (T_4 and T_3) in the serum. Fish were acclimated at 13°C and 20 fish from each condition were sampled and 3 aliquots (each 5 ml) of pooled sera compared to 3 aliquots of pooled calm fish sera at 14, 26, and 53 days after exposure to current. Current fish (ave. weight 106.97 g ± 23.77 (S.D.)) at 14 days averaged 1.30 mcg% as compared to 1.07 mcg% for calm fish (ave. weight 121.16 g ± 24.32 (S.D.)), an increase of 21.5%.

This difference was reduced to 5.2% above the controls at 26 days, where current fish (ave. weight 110.80 g ± 19.76 (S.D.)) averaged 2.03 mcg% as opposed to 1.93 mcg% for calm fish (ave. weight 113.10 g ± 24.68 (S.D.)). At 53 days current fish (ave. weight 115.62 g ± 25.29 (S.D.)) averaged 1.17 mcg%, while that for calm fish (ave. weight 108.58 g ± 23.03 (S.D.)) was 1.03 mcg%,a rise of 13.6% over the controls.

It was concluded that exposure to a current under these experimental conditions could increase stable amounts of thyronines as high as 21.5% above the controls.

Table XVII. Total thyronines May-July 1970

Days After Current Initiated	Replicates No.	Total Thyronines mcg./100 ml Current (each sample pool of 20 fish sera)	Calm (each sample pool of 20 fish sera)	% Change from Calm Condition
14	1	1.4	1.0	
	2	1.4	1.1	
	3	1.1	1.1	
Mean ± 1 S.E.		1.30 ± 0.10	1.07 ± 0.03	+ 21.5
26	1	1.9	1.8	
	2	1.8	1.9	
	3	2.4	2.1	
Mean ± 1 S.E.		2.03 ± 0.19	1.93 ± 0.09	+ 5.2
53	1	1.1	0.8	
	2	0.9	1.2	
	3	1.5	1.1	
Mean ± 1 S.E.		1.17 ± 0.18	1.03 ± 0.12	+ 13.6

DISCUSSION

A. Iodine Metabolism

1. Radioiodine Metabolism and Exercise

The indices of thyroid activity used in this study indicate that radioiodine metabolism in the brook trout is apparently a slow process, which is in accordance with results obtained by Drury and Eales (1968) and Dorey (1970).

Maximum uptake of radioiodide into the thyroid (U%) ranged from 14.4% (current fish, Experiment III) to 21.0% (calm fish, Experiment I). Calm fish in experiments II and III had higher % thyroids. Eales (1963) found that steelhead in a fast current had slightly lower radioiodide uptake than fish in a slow current, over the period of sampling (8 days). In contrast, exercised rainbow trout up to 188 hr p.i. had higher % thyroids (Fontaine and Leloup, 1959).

In two experiments (II and III) current fish had a more rapid removal of radioiodide from the serum by extrathyroidal pathways. This supports the result obtained by Eales (1963) where fish in the fast current had faster extrathyroidal loss of radioiodide from the body. Fontaine and Leloup (1959) did not observe greater extrathyroidal removal of serum radioiodide for their current fish, which may partly explain why their fish had higher % thyroids.

Rate constant values for loss of serum radioiodide to the thyroid, k_1 were generally higher than those reported by Dorey (1970)

except in her experimental series at 16°C where k_1 was 0.008. At 13°C k_1 ranged from 0.008 to 0.013 in the present study, however, seasonal factors in combination with experimental conditions probably influenced the results.

The maximum T/S ratio, C.R. and PB^{125}I for this study were respectively 11.50 (at 18 days for current fish, Experiment II), 5.03 (at 27 days for calm fish, Experiment II), and 0.112 (at 27 days for calm fish, Experiment II). It can be seen that all of these maximum values occurred during October to November in fish of the lowest mean weight. Maximum values in Experiment I and III were considerably lower.

Influence of exercise on T/S ratio, C.R. and PB^{125}I was very inconclusive. No effect of exercise on C.R. was found by Fontaine and Leloup (1959), while Eales (1963) reported only a slight increase in C.R. Bonnet (1970) did not observe any alteration in serum PB^{125}I after 15 days of exercise (total length of experiment) in M. auratus, although Fontaine and Leloup (1959) did find PB^{125}I to be three times higher for current fish 188 hr p.i.

Due to lack of consistent evidence, in previous work and in the present study, it is doubtful, based on radioiodine parameters of thyroid activity, if increased metabolic activity is accompanied by a concomitant rise in thyroid activity for fish. This is in agreement with a good portion of the work on thyroid function and fish metabolism, which indicates no effect of thyroid hormones, T.S.H., antithyroid compounds, or radiothyroidectomy on oxygen consumption (see literature review).

2. Stable Iodine (^{127}I) Metabolism

Throughout all radioiodide experiments total stable serum iodide did not remain constant, but tended to increase over the experimental duration, influencing all radioiodide parameters. Both the T/S ratio and C.R. were inversely related to the level of total serum iodide, while there was a direct relationship found with serum $I^{125}I$. % thyroid was often high when total serum iodide was low. The variability found in the radioiodide indices of thyroid activity was probably largely caused by the variable levels of stable iodide between fish. Also high levels of serum stable iodide in brook trout, in comparison to other species of fish, could explain why radioiodine metabolism in this species is a slow process.

Levels of stable iodide were higher in current fish which (1) verified data reported by Fontaine and Leloup (1959) and, 2) explained, on the basis of specific activity (see literature review), in combination with the faster rate of radioiodide removal from the serum, lower thyroid uptake of current fish in Experiment II and III.

The mechanism of serum iodide build-up for current fish was thought by Fontaine and Leloup (1959) to involve loss of iodide from muscle. Other possibilities might be (a) greater extraction of iodide from the water by the gills, as the result of increased water flow across the gill surface and, (b) greater deiodination of stable hormone, since labelled thyronines disappeared from the blood more rapidly in current fish. In any case since radioiodide loss from the serum increased for fish in a current, it is apparent that the rate of addition of stable iodide to the serum was greater for current fish,

than for calm fish, although the pathways of addition remain obscure.

Starvation could have contributed to the increased serum iodide in both groups, since by reducing biliary excretion of thyroid hormone, it probably led to increased deiodination as the main alternative pathway for thyroid hormone degradation and excretion.

Support for this hypothesis has recently been obtained by Eales (1970) (unpublished data) where starved fish following T_4* injection had higher levels of inorganic iodide, higher % thyroids and reduced biliary loss of T_4* in comparison to fed fish.

Since the brook trout belongs to the Order Clupeiformes it is capable of significant binding of inorganic iodide by the plasma proteins (Huang and Hickman, 1968). Falkner (1970) has provided evidence for this, when he demonstrated that by dialysis 90% of serum iodide was bound to protein in brook trout.

Leloup and Fontaine (1960) present maximum values of total plasma iodide for several species in mcg%: 257 for Salmo salar, 576 for Salmo trutta, and 2300 for Alosa alosa. So far a maximum of 752 mcg% has been recorded for brook trout, examined in this laboratory by Dorey (1970). This occurred in April (1969). The highest levels of total serum iodide for this study also occurred in April (1970), suggesting a possible seasonal change.

In conclusion, it cannot be overemphasized that serum stable iodide should be determined during any radioiodine experiment in order to properly assess the data.

B. Thyronine Metabolism

It is suggested that exercise elevates thyronine degradation rate. This conclusion is based on (1) the greater fractional turnover rates observed for current fish (Experiment IV and V), (2) increased levels of total serum thyronines (stable) (Experiment VII) and, (3) little alteration in the thyronine distribution space (Experiment IV and V). Thyronine degradation rate is the product of the thyronine distribution space, the serum stable thyronine concentration and the fractional turnover rate, k. In view of the results obtained from thin layer chromatography (Experiment VI) where T_3 was present along with T_4 25 hr after T_4* injection, the term thyronine degradation rate is used rather than thyroxine degradation rate. No effect of exercise was found when fish were suddenly exposed to a current. Thus any increase in thyroid hormone degradation rate is a response to prolonged metabolic stress.

Results in this study are similar to those reported by Irvine (1968) who investigated the effects of exercise on thyroxine degradation in man. Athletes engaged in moderately severe training had the greatest thyroxine degradation rate, while nonathletes taking daily muscular exercise, had less of an increase above resting nonathletes. Elevation in T_4 degradation was attributed to increased deiodination, a more important pathway in humans than biliary loss. It is possible that increased deiodination occurred in Experiment IV and V, for as discussed earlier, stable serum iodide levels were always higher for current fish.

Studies on the enterohepatic excretory route for T_4, first elucidated in brook trout by Eales (1970) indicate an additional

possible explanation. It is known from the work of Gorman, Flock, Owen, and Paris (1966) that an equilibrium exists between blood and liver T_4. For example, in double isotope experiments, they showed the simultaneous flow of T_4 in opposite directions across the cell membranes in the liver. Also, by increasing the competition for binding sites in the blood, through addition of stable T_4, they were able to increase the net flux of T_4 from the blood to the liver. From this and other mammalian studies (Myant and Osorio, 1964; Osorio and Myant, 1965) it is now generally believed that only the free T_4 in the plasma is taken up by the liver cells. Radioactivity in the enterohepatic organs, mainly in the Experiment IV and V livers and gall bladders, suggested that fish in the current had greater loss of radioactivity from the liver to the gall bladder. Possibly there was a higher percentage of free T_4 in exercising fish, which from the above discussion, led to greater accumulation of glucuronide conjugates of T_4 in the bile and hence caused reduction in serum $PB^{125}I$. Glucuronide conjugates of T_4 have been identified in the brook trout by Sinclair (1970) (unpublished data). Irvine (1968) found that exercising athletes had a higher percentage of free T_4 in serum.

Several mammalian studies dealing with cold-acclimated rats have shown increased loss of thyroid hormone, above controls at normal temperature, to be the result of greater fecal excretion (Intoccia and Van Middlesworth, 1959; Kassenaar et al, 1959; Cottle, 1964; and Galton and Nisula, 1969). Galton and Nisula concluded that there was no increase in the amount of T_4 reaching the peripheral tissues in rats exposed to cold, and they thought that

increased thyroid activity observed for these rats was not due to increased heat production, but to greater loss of T_4 through the gastro-intestinal tract. Increased bile flow in cold-acclimated animals was related partly to their increased food consumption. It is, therefore, quite possible that the enterohepatic excretory system for thyroid hormones can be a delicate control mechanism for regulating blood levels of hormone, in addition to the central thyroid-pituitary axis.

The idea that biliary flow can be modified by food consumption is important for interpreting the gall bladder patterns obtained in the study, with those found by Eales (1970). In addition it is known that dietary constitutents can affect T_4* excretion (Van Middlesworth, 1957). It follows that starvation probably was the cause of the very slow loss of gall bladder radioactivity observed in all T_4* experiments. In contrast, Eales observed that almost 20% of the injected dose was in the gall bladder by 24 hr, after which there was a rapid decline (less than 2% of the dose remained in the gall bladder at 72 hr). The importance of starvation in regulating biliary flow has been verified in a controlled experiment by Eales (1970) (unpublished data).

The initial rapid phase of serum $PB^{125}I$ loss following T_4* injection has been explained by distribution of thyroid hormone throughout the body's extrathyroidal pool, for mammals (Sterling, 1964) and fish (Drury and Eales, 1968 and Eales, 1970). However, Eales (1970), and data from this study implied, that this could be the result of extensive uptake of radioactivity by enterohepatic tissues, rather than a general dispersion throughout the body. Another possibility mentioned by Tata (1964) is deiodination. In all T_4* experiments

high levels of inorganic iodide occurred shortly after injection as well as high ratios of T_3 and T_4 (25 hr p.i., Experiment VI).

There was some suggestion that serum $PB^{125}I$ began to increase following the phase of exponential loss for fish in the current. This was probably caused by absorption of biliary-excreted T_4 from the intestine, a well documented phenomenon in mammals (Myant, 1957; Chung and Van Middlesworth, 1964; 1967). A more evident increase in serum $PB^{125}I$ was reported in brook trout by Drury and Eales (1968) although they thought this represented endogenously produced hormone, the thyroid using the inorganic iodide produced by deiodination. In the present study radioiodide metabolism is so slow that this explanation almost certainly did not apply.

Comparison between experiments reveals noticeable differences in biological half-lives for $PB^{125}I$, in direct relation to the experimental temperature. Such a relationship was found in the eel by Leloup (1965) and in the brook trout by Drury and Eales (1968). In the eel at 24°C $t_{\frac{1}{2}}$ of injected T_4* was about 1.4 days with this increasing to 2.5 days at 10.5°C. Brook trout $t_{\frac{1}{2}}$ values for serum $PB^{125}I$, after T_4* injection, were 18.0 days at 5°C, 3.2 days at 12°C and 1.5 days at 15°C. Values for control fish in the present study were 3.9 days at 9.5°C, 3.5 days at 12°C and 1.6 days at 13.5°C.

C. General Conclusions on the Effect of Exercise Upon Thyroid Function

(1) Exercise does not increase thyroid hormone secretion above controls when assessed by radioiodide indices of thyroid activity.

(2) Radioiodide removal from the serum after $Na^{125}I$ injection can be accelerated by exercise.

(3) Exercise causes an increase in the level of serum stable iodide (^{127}I) which, in combination with faster radioiodide removal from the serum, can lead to reduced % thyroids in comparison to control fish.

(4) Increased swimming over a long duration causes elevation of thyronine degradation rate primarily through greater enterohepatic uptake of hormone from the serum. Since radioiodide data do not support a conclusion for increased thyroid activity with exercise, it is very doubtful that increased thyronine degradation, observed in two T_4* experiments, is of metabolic significance.

BIBLIOGRAPHY

Abbassi, V. and J.M. Mckenzie. 1967. Lack of iodide effect on serum and pituitary thyrotropin in vivo. Endocrinology, 81:871-876.

Baggerman, B. 1960. Salinity preference, thyroid activity and the seaward migration of four species of Pacific salmon (Oncorhynchus). J. Fish. Res. Bd. Canada, 17:295-322.

Baraduc, M.M. 1954. Influence de la thyroxinisation de jeûnes Truites arc-en-ciel (Salmo gairdneri) sur la teneur en lipides viscéraux et periviscéraux. Compt. Rend. Acad. Sci. 238: 728-730.

Barch, S.H. 1953. Oxygen consumption of normal and thyroxin stimulated Rana pipiens skin. Physiol. Zool. 26:223-231.

Basu, S.P. 1959. Active respiration of fish in relation to ambient concentrations of oxygen and carbon dioxide. J. Fish. Res. Bd. Canada, 16:175-212.

Beamish, F.W.H. 1964. Influence of starvation on standard and routine oxygen consumption. Trans. Amer. Fish Soc. 93: 103-107.

Beamish, F.W.H., and P.S. Mookherjii. 1964. Respiration of Fishes with special emphasis on standard oxygen consumption. I. Influence of weight and temperature on respiration of goldfish, Carassius auratus L. Can. J. Zool. 42:161-175.

Bilinski, E., and L.J. Gardner. 1968. Effect of starvation on free fatty acid level in blood plasma and muscular tissues of rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Canada, 25:1555-1560.

Black, E.C. 1955. Blood levels of hemoglobin and lactic acid in some freshwater fishes following exercise. J. Fish. Res. Bd. Canada, 12:917-929.

Black, E.C. 1957a. Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. I. Kamloops trout, Salmo gairdneri. J. Fish. Res. Bd. Canada, 14:117-134.

Black, E.C. 1957b. Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. III. Sockeye salmon, Oncorhynchus nerka. J. Fish. Res. Bd. Canada, 14:807-814.

Black, E.C., N.J. Bosomworth, and G.E. Docherty. 1966. Combined effect of starvation and severe exercise on glycogen metabolism of rainbow trout, Salmo gairdneri. J. Fish. Res. Bd. Canada, 23:1461-1463.

Black, E.C., G.T. Manning, and K. Hayashi. 1966. Changes in levels of hemoglobin, oxygen, carbon dioxide, pyruvate, and lactate in venous blood of rainbow trout (Salmo gairdneri) during and following severe muscular activity. J. Fish. Res. Bd. Canada, 23:783-795.

Black, E.C., A.C. Robertson, A.R. Hanslip, and W.G. Chiu. 1960. Alterations in glycogen, glucose and lactate in rainbow and kamloops trout, Salmo gairdneri following muscular activity. J. Fish. Res. Bd. Canada, 17:487-500.

Bonnet, B. 1970. Thyroide et milieu interieur chex Mugil sp. Influence de la nutrition et de l'environnement. Ph.D. Thesis, Université d'Aix-Marseille.

Brett, J.R. 1956. Some principles in the thermal requirements of fishes. Quart. Rev. Biol. 31:75-87.

Brett, J.R. 1962. Some considerations in the study of respiratory metabolism in fish, particularly salmon. J. Fish. Res. Bd. Canada, 19:1025-1038.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Canada, 21:1183-1226.

Brett, J.R. 1965. The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Bd. Canada, 22:1491-1501.

Brett, J.R. 1967. Swimming performance of sockeye salmon (Oncorhynchus nerka) in relation to fatigue time and temperature. J. Fish. Res. Bd. Canada, 24:1731-1741.

Brett, J.R., and D.B. Sutherland. 1965. Respiratory metabolism of pumpkinseed (Lepomis gibbosus) in relation to swimming speed. J. Fish. Res. Bd. Canada, 22:405-409.

Buhler, U.K., and L.J. DeGroot. 1969. Effect of stable iodine on thyroid iodine release. J. Clin. Endocrinol. 29:1546-1552.

Burke, G. 1970. Effects of iodide on thyroid stimulation. J. Clin. Endocrinol. 30:76-84.

Calhoon, T.B. 1955. Adrenal cortical extract on the oxygen consumption of propylthiouracil treated frogs. Endocrinology, 57:70-75.

Chavin, W., and H.W. Rossmoore. 1956. Pituitary-thyroid regulation of respiration in the goldfish, Carassius auratus L. Anat. Record, 125:599.

Chung, S.J., and L. Van Middlesworth. 1964. Absorption of thyroxine from the small intestine of rats. Endocrinology, 74:694-700.

Chung, S.J., and L. Van Middlesworth. 1967. Absorption of thyroxine from the intestine of rats. Amer. J. Physiol. 212:97-100.

Cottle, W.H. 1964. Biliary and fecal clearance of endogenous thyroid hormone in cold-acclimated rats. Amer. J. Physiol. 207:1063-1066.

Creach, Y., and G. Bouche. 1969. Influence du jeûne prolongé sur la composition du sang de la carpe (Cyprinus carpio L.). Rech. Hydrobiol. contin. 1:51-60.

Dalberg, M.L., D.L. Shumway, and P. Doudoroff. 1968. Influence of dissolved oxygen and carbon dioxide on swimming performance of largemouth bass and coho salmon. J. Fish. Res. Bd. Canada, 25:49-70.

Davis, G.E., J. Foster, C.E. Warren, and P. Doudoroff. 1963. The influence of oxygen concentration on the swimming performance of juvenile Pacific salmon at various temperatures. Trans. Amer. Fish. Soc. 92:111-124.

Dean, M.J., and C.J. Goodnight. 1964. A comparative study of carbohydrate metabolism in fish as affected by temperature and exercise. Physiol. Zool. 37:280-299.

DeGroot, L.J. 1966. Action of potassium iodide on thyroxine metabolism. J. Clin. Endocrinol. 26:778-779.

De Nayer, Ph., P. Malvaux, M. Ostyn, H.G. Van Den Schrieck, C. Beckers, and M. De Visscher. 1968. Serum free thyroxine and binding-proteins after muscular exercise. J. Clin. Endocrinol. 28: 714-716.

Dickson, I.W. 1968. Factors influencing respiratory metabolism of rainbow trout, Salmo gairdneri. Ph.D. Thesis, Utah State University, Logan, Utah.

Dodd, J.M., and A.J. Matty. 1964. Comparative aspects of thyroid function. In "The Thyroid Gland" (R. Pitt-Rivers and W.R. Trotter, eds.) Vol. I:303-356, Butterworths, London.

Dorey, C.D. 1970. The effect of temperature on radioiodine metabolism and thyroid hormone biosynthesis in brook trout, Salvelinus fontinalis (Mitchill). M.Sc. Thesis, University of Manitoba.

Drexler, E., and B. von Issekutz, Jr. 1935. Die Wirkung des Thyroxins auf den Stoffwechsel koltblütiger Wirbeltiere. Arch. f. Exp. Path. u. Pharmakol. 177:435-441. (Cited in Smith and Everett, 1943.).

Drury, D.E. 1967. A comparison of methods for measuring thyroid activity in the eastern brook trout (Salvelinus fontinalis Mitchell). M.Sc. Thesis, University of New Brunswick.

Drury, D.E., and J.G. Eales. 1968. The influence of temperature on histological and radiochemical measurements of thyroid activity in the eastern brook trout, Salvelinus fontinalis (Mitchell). Can. J. Zool. 46:1-9.

Eales, J.G. 1963. An analysis of the thyroid role in juvenile steelhead (Salmo gairdneri Richardson) and factors responsible for its seasonal fluctuation in activity. Ph.D. Thesis, University of British Columbia.

Eales, J.G. 1963. A comparative study of thyroid function in migrant juvenile salmon. Can. J. Zool. 41:811-824.

Eales, J.G. 1964. The influence of temperature on thyroid histology and radioiodine metabolism of yearling steelhead trout, Salmo gairdneri. Can. J. Zool. 42:829-841.

Eales, J.G. 1970. Biliary excretion of radiothyroxine by the brook trout, Salvelinus fontinalis (Mitchill). Gen. Comp. Endocrinol. 14:385-395.

Eales, J.G., L. Welsh, and Hooi Har Chan. 1970. Biliary excretion of ^{125}I-3, 5, 3 triiodo-l-thyronine by the brook trout Salvelinus fontinalis Mitchill. Gen. Comp. Endocrinol. (In press.)

Escobar del Rey, F., and G. Morreale de Escobar. 1956a. Studies on the peripheral disappearance of thyroid hormone. II. The effect of swimming for two hours on the I^{131} distribution in thyroidectomized, l-thyroxine maintained rats after the injection of I^{131}-labeled l-thyroxine. Acta Endocrinologica, 23:393-399.

Escobar del Rey, F., and G. Morreale de Escobar. 1956b. Studies on the peripheral disappearance of thyroid hormone. III. The effect of running for 12 hours on the I^{131} distribution in thyroidectomized, l-thyroxine maintained rats after the injection of I^{131}-labeled l-thyroxine. Acta Endocrinologica, 23:400-406.

Etkin, W., R.W. Root, and B.P. Mofshin. 1940. The effect of thyroid feeding on oxygen consumption of the goldfish. Physiol. Zool. 13:415-429.

Falkner, N.W. 1970. The binding of ^{125}I- 3, 5, 3′triiodo-l-thyronine and ^{125}I-l-thyroxine to the plasma proteins of the brook trout, Salvelinus fontinalis Mitchill. Ph.D. Thesis, University of Manitoba.

Farmer, G.J., and F.W.H. Beamish. 1969. Oxygen consumption of Tilapia nilotica in relation to swimming speed and salinity. J. Fish. Res. Bd. Canada, 26:2807-2821.

Featherston, W.R., R. Boehm, and J.C. Rogler. 1966. Influence of dietary iodine on I^{131} retention and distribution in the chick. Proc. Soc. Exp. Biol. and Med. 123:73-76.

Fontaine, M., and J. Leloup. 1959. Influence de la nage à contre-courant sur le métabolisme de l'iode et le fonctionnement thyroidien chez la Truite arc-en-ciel (Salmo gairdnerii Rich.). Compt. Rend. Acad. Sci. 249:343-347.

Fromm, P.O., and E.P. Reineke. 1956. Some aspects of thyroid physiology in rainbow trout. J. Cell. Comp. Physiol. 48:393-404.

Galton, V.A., and S.H. Ingbar. 1962. Observations on the effects and the metabolism of thyroid hormones in Necturus maculosus. Endocrinology, 71:369-377.

Galton, V.A., and S.H. Ingbar. 1967. Effect of large doses of iodide on the peripheral metabolism of thyroxine in rats. Endocrinology, 81:1439-1441.

Galton, V.A., and B.C. Nisula. 1969. Thyroxine metabolism and thyroid function in the cold-adapted rat. Endocrinology, 85:79-86.

Gibson, E.S., and F.E.J. Fry. 1954. The performance of the lake trout Salvelinus namaycush, at various levels of temperature and oxygen pressure. Can. J. Zool. 32:252-260.

Glass, N.R. 1968. The effect of time of food deprivation on the routine oxygen consumption of large-mouth black bass (Micropterus salmoides). Ecology, 49:340-343.

Gorbman, A., and O. Berg. 1955. Thyroidal function in the fishes Fundulus heteroclitus, F. majalis, and F. diaphanus. Endocrinology, 56:86-92.

Gorbman, A., and H.A. Bern. 1962. A textbook of comparative endocrinology. John Wiley and Sons, New York.

Gorbman, A. 1969. Thyroid Function and its control in fishes. In Fish Physiology, "The Endocrine System" (W.S. Hoar and D.J. Randall, eds.), Vol. 2, Academic Press.

Gorman, C.A., E.V. Flock, C.A. Owen, Jr., and J. Paris. 1966. Factors affecting exchange of thyroid hormones between liver and blood. Endocrinology, 79:391-405.

Graham, J.M. 1949. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, Salvelinus fontinalis. Canadian J. Res., Sect. D. 27:270-288.

Green, W.L. 1966. Further studies of the effects of inorganic iodide on thyroidal intermediary metabolism in vitro. Endocrinology, 79:1-9.

Greer, M.A., Y. Grimm, and H. Studer. 1968. Qualitative changes in the secretion of thyroid hormones induced by iodine deficiency. Endocrinology, 83:1193-1198.

Haarmann, W. 1936. Über den Einfluss von Thyroxin auf den Sauerstoff= ver-brauch über-lebender Gewebe. Arch. exp. Path. Pharmak. 180:167-182. (Cited in Pickford and Atz, 1957.)

Hammond, B.R., and C.P. Hickman, Jr. 1966. The effect of physical conditioning on the metabolism of lactate, phosphate, and glucose in rainbow trout, Salmo gairdneri. J. Fish. Res. Bd. Canada, 23:65-83.

Hasler, A.D., and R.K. Meyer. 1942. Respiratory responses of normal and castrated goldfish to teleost and mammalian hormones. J. Exp. Zool. 91:391-404.

Heath, A.G., and A.W. Pritchard. 1962. Changes in the metabolic rate and blood lactic acid of bluegill sunfish, Lepomis macrochirus, Raf. following severe muscular activity. Physiol. Zool. 35: 323-329.

Heinemann, F., and R. Weber. 1966. The oxygen consumption in the regressive tail tissue of Xenopus larvae during spontaneous metamorphosis and regressive metamorphosis caused by thyroxine "in vitro." Helv. Physiol. Pharmacol. Acta, 24:124-138.

Henschel, H., and M. Steuber. 1931. 'Über die Bedeutung der Schilddrüse fürden Stoffwechsel der Amphibien.' Arch. exp. Path. Pharmakol 160:401-427. (Cited by Dodd and Matty, 1964.)

Hickman, C.P., Jr. 1959. The osmoregulatory role of the thyroid gland in the starry flounder, Platichthys stellatus. Can. J. Zool. 37:997-1060.

Hickman, C.P., Jr. 1961. The conversion ratio as a discriminatory test for thyroid activity in fish. Nature, 189:1012-1013.

Hickman, C.P., Jr. 1962. Influence of environment on the metabolism of iodine in fish. Gen. Comp. Endocrinol. Suppl. 1:48-62.

Hoar, W.S. 1958. Effects of synthetic thyroxine and gonadal steroids on the metabolism of goldfish. Can. J. Zool. 36:113-121.

Hoar, W.S., and G.M. Bell. 1950. The thyroid gland in relation to the seaward migration of Pacific salmon. Can. J. Res. 28:126-136.

Hoar, W.S., and J.G. Eales. 1963. The thyroid gland and low-temperature resistance of goldfish. Can. J. Zool. 41:653-669.

Hoar, W.S., M.H.A. Keenleyside, and R.G. Goodall. 1955. The effects of thyroxine and gonadal steroids on the activity of salmon and goldfish. Can. J. Zool. 33:428-439.

Hochachka, P.W. 1961. The effect of physical training on oxygen debt and glycogen reserves in trout. Can. J. Zool. 39:767-776.

Hochachka, P.W. 1962. Thyroidal effects on pathways for carbohydrate metabolism in a teleost. Gen. Comp. Endocrinol. 2:499-505.

Hoffert, J.R., and P.O. Fromm. 1959. Estimation of thyroid secretion rate of rainbow trout using radioactive iodine. J. Cell. Comp. Physiol. 54:163-169.

Hopper, A.F. 1959. The effect of mammalian thyroid powder on oxygen consumption in Lebistes reticulatus. Growth, 23:13-19.

Hopping, A. 1931. Action of thyroxin on tissue metabolism. Proc. Soc. Exp. Biol. (N.Y.) 28:726-728.

Huang, C.T., and C.P. Hickman, Jr. 1968. Binding of inorganic iodide to the plasma proteins of teleost fishes. J. Fish. Res. Bd. Canada, 25:1651-1666.

Intoccia, A., and L. Van Middlesworth. 1959. Thyroxine excretion increase by cold exposure. Endocrinology, 64:462-464.

Irvine, C.H.G. 1967. Thyroxine secretion rate in the horse in various physiological states. J. Endocrinol. 39:313-320.

Irvine, C.H.G. 1968. Effect of exercise on thyroxine degradation in athletes and non-athletes. J. Clin. Endocrinol. 28:942-948.

Job, S.V. 1955. The oxygen consumption of Salvelinus fontinalis. Publ. Ontario Fish. Res. Lab., No. 73, 39p.

Kaciuba-Uscilko, H., K.F. Legge, and L.E. Mount. 1970. The development of the metabolic response to thyroxine in the new-born pig. J. Physiol. 206:229-241.

Kamra, S.K. 1966. Effect of starvation and refeeding on some liver and blood constituents of Atlantic cod (Gadus morhua L.). J. Fish. Res. Bd. Canada, 23:975-982.

Karmarkar, M.G., N. Kochupillai, M.G. Deo, and V. Ramalingaswami. 1969. Adaptation of thyroid gland to iodine deficiency. Life Sciences, 8:1135-1141.

Kassenaar, A., L.D.F. Lameyer, and A. Querido. 1959. Studies on the peripheral disappearance of thyroid hormone. Acta Endocrinologica 32:575-578.

Kutty, M.N. 1968. Respiratory quotients in goldfish and rainbow trout. J. Fish. Res. Bd. Canada, 25:1689-1728.

La Roche, G., C.L. Johnson, and A.N. Woodall. 1965. Thyroid function in the rainbow trout (Salmo gairdneri, Rich.). I. Biochemical and histological evidence of radiothyrioidectomy. Gen. Comp. Endocrinol. 5:145-159.

Lashof, J.C., P.K. Bondy, K. Sterling, and E.B. Man. 1954. Effect of muscular exercise on circulating thyroid hormone. Proc. Soc. Expl. Biol. Med. 86:233-235.

Leloup, J. 1965. Métabolisme de la thyroxine chez L'Anguille normale et hypophysectomisée en fonction de la température. Gen Comp. Endocrinol. 5:Abstract.

Leloup, J., and M. Fontaine. 1960. Iodine metabolism in lower vertebrates. Ann. N.Y. Acad. Sci. 86:316-353.

Lewis, E.J.C., and E. Frieden. 1959. Biochemistry of amphibian metamorphosis: Effect of triiodothyronine, thyroxin, and dinitrophenol on the respiration of the tadpole. Endocrinology, 65: 273-282.

Lowenstein, J.M., and R.C. Stebbins. 1969. Iodine metabolism and thyroid activity in parietalectomized tropical lizards. Copeia 4:664-669.

Lynn, W.G., and H.E. Wachowski. 1951. The thyroid gland and its functions in cold-blooded vertebrates. Quart. Rev. Biol. 26:123-168.

MacCrimmon, H.R., and J.S. Campbell. 1969. World distribution of brook trout, Salvelinus fontinalis. J. Fish. Res. Bd. Canada, 26: 1699-1725.

Madanmohanrao, G. 1961. Effect of thyroid feeding on the rate of oxygen consumption of Tilapia mossambica (Peters). Proc. Natl. Inst. Sci. India, Part B. Biol. Sci. 27:343-346.

Maher, M.J. 1961. The effect of environmental temperature on metabolic response to thyroxine in the lizard, Lacerta muralis. American Zoologist 1:461.

Maher, M.J. 1964. Metabolic response of isolated lizard tissues to thyroxine administered in vivo. Endocrinology, 74:994-995.

Maher, M.J. 1965. The role of the thyroid gland in the oxygen consumption of lizards. Gen. Comp. Endocrinol. 5:320-325.

Maher, M.J., and B.H. Levedahl. 1959. The effect of the thyroid gland on the oxidative metabolism of the lizard, Anolis carolinensis. J. Exp. Zool. 140:169-189.

Marine, D. 1914. The rapidity of the involution of active thyroid hyperplasias of brook trout following the use of fresh sea fish as a food. J. Exp. Med. 19:376-382.

Marine, D., and C.H. Lenhart. 1910. Observations and experiments on the so-called thyroid carcinoma of brook trout (Salvelinus fontinalis) and its relation to ordinary goitre. J. Exp. Med. 12:311-337.

Massey, B.D., and C.L. Smith. 1968. The action of thyroxine on mitochondrial respiration and phosphorylation in the trout (Salmo trutta Fario L.). Comp. Biochem. Physiol. 25:241-255.

Matthews, S.A., and D.C. Smith. 1947. The effect of thiourea on the oxygen consumption of Fundulus. Physiol. Zool. 20:161-164.

Matty, A.J. 1954. Thyroidectomy of the dogfish, Scyllium canicula (L.), and the effect dogfish thyroid upon the oxygen consumption of rats. J. Mar. Biol. Ass. U.K. 33:689-697.

Matty, A.J. 1957. Thyroidectomy and its effect upon oxygen consumption of a teleost fish, Pseudoscarus guacamaia. J. Endocrinol. 15:1-8.

McNabb, R.A. 1969. The effects of thyroxine on glycogen stores and oxygen consumption in the leopard frog, Rana pipiens. Gen. Comp. Endocrinol. 12:276-281.

Mohsen, T., and R. Godet. 1960. Action of thyroxine on the rate of oxygen consumption of the lung-fish (Protopterus). Nature, 185:108.

Myant, N.B. 1957. Faecal clearance rate of endogenous thyroid hormone in rats. J. Physiol. 136:198-202.

Myant, N.B., and C. Osorio. 1964. Biliary excretion of thyroxine in monkeys. J. Physiol. 177:75-76.

Müller, J. 1953. Über die Wirkung von Thyroxin und thyreotropem Hormon auf den Stoffwechsel und die Färbung des Goldfisches. Z. vergl. Physiol. 35:1-12.

Ochi, Y., and L.J. DeGroot. 1969. TSH-or LATS-stimulated thyroid hormone release is inhibited by iodide. Endocrinology, 84: 1305-1309.

Osborn, P.E. 1951. Some experiments on the use of thiouracil as an aid in holding and transporting fish. Progressive Fish Culturist, 13:75-78.

Osorio, C., and N.B. Myant. 1965. Relation between the biliary excretion of thyroxine and its binding by the serum proteins in monkeys. Endocrinology, 76:938-948.

Parvatheswararao, V. 1965. Effects of short term starvation on the metabolism of a fresh-water fish, Puntius sophore (Hamilton). Current Science, 34:563-564.

Parvatheswararao, V. 1967. Effects of prolonged starvation on the metabolism of fresh-water fishes, Etroplus maculatus and Cirrhina reba. Life Sciences, 6:163-172.

Pickford, G.E., and J.W. Atz. 1957. The Physiology of the Pituitary Gland of Fishes. New York Zoological Society, New York.

Picos, C.A., D. Schmidt, and E. Popovici. 1969. Der Einflub des Thioharnstoffs auf den Sauerstoffverbrauch der Fische in hypothermischen Bedingungen. Z. vergl. Physiologie 63: 146-150.

Pitt-Rivers, R., and W.R. Trotter. 1964. The Thyroid Gland. Butterworths, London, 1:442p.

Pritchard, A.W., and A. Gorbman. 1960. Thyroid hormone treatment and oxygen consumption in embryos of the spiny dogfish. Biol. Bull. 119:109-119.

Radulescu, I., D.G. Vasiliu, E. Ilie and S.F. Snieszko. 1968. Thyroid hyperplasia of the eastern brook trout, Salvelinus fontinalis, in Romania. Trans. Amer. Fish. Soc. 97:486-488.

Ramsden, D., F.H. Passant, C.O. Peabody, and R.G. Speight. 1967. Radioiodine uptakes in the thyroid. Studies of the blocking and subsequent recovery of the gland following the administration of stable iodine. Health Physics, 13:633-646.

Rao, G.M.M. 1968. Oxygen consumption of rainbow trout (Salmo gairdneri) in relation to activity and salinity. Can. J. Zool. 46:781-786.

Rhodes, B.A. 1967. Effect of exercise on the thyroid gland. Nature, 216:917-918.

Robertson, O.H., and A.L. Chaney. 1953. Thyroid hyperplasia and tissue iodine content in spawning rainbow trout: A comparative study of Lake Michigan and California sea-run trout. Physiol. Zool. 26:328-340.

Robertson, H.A., and I.R. Falconer. 1961. The estimation of thyroid activity: An evaluation of certain parameters. J. Endocrinol. 21:411-420.

Root, R.W., and W. Etkin. 1937. Effect of thyroxine on oxygen consumption of the toadfish. Proc. Soc. Exp. Biol. Med. 37:174-175.

Rosenberg, L.L., M. Goldman, G. La Roche, and M.K. Dimick. 1964. Thyroid function in rats and chickens. Equilibration of injected iodide with existing thyroidal iodine in Long-Evans rats and White Leghorn chickens. Endocrinology, 74:212-225.

Ruhland, M.L. 1969. Relation entre l'activité de la glande thyroide et la consommation d'oxygène chez les Téléostéens, Cichlidés. Experientia 25:944-945.

Sage, M. 1965. The effects of thyroxine and thiourea on the respiration and activity of the teleost Lebistes reticulatus Peters. Gen. Comp. Endocrinol. 5:706-707.

Sage, M. 1968. Respiratory and behavioral responses of Poecilia to treatment with thyroxine and thiourea. Gen. Comp. Endocrinol. 10:304-309.

Smit, H. 1965. Some experiments on the oxygen consumption of goldfish (Carassius auratus L.) in relation to swimming speed. Can. J. Zool. 43:623-633.

Smith, D.C., and G.M. Everett. 1943. The effect of thyroid hormone on growth rate, time of sexual differentiation and oxygen consumption in the fish, Lebistes reticulatus. J. Exp. Zool. 94:229-240.

Smith, D.C., and S.A. Matthews. 1948. Parrot fish thyroid extract and its effect upon oxygen consumption in the fish, Bathystoma. Amer. J. Physiol. 153:215-221.

Srivastava, P.N. 1960. Utilization of radioiodine by the thyroid gland of goldfish, Carassius auratus L. Physiol. Zool. 33:277-280.

Srivastava, L.S., and C.W. Turner. 1967. Comparison of thyroxine secretion rate of docile and flighty lines of fowls. Proc. Soc. Exp. Biol. Med. 124:325-327.

Sterling, K. 1964. Thyroxine in blood. Mayo Clinic Proceedings, 39: 586-608.

Stevens, E.D., and E.C. Black. 1966. The effect of intermittent exercise on carbohydrate metabolism in rainbow trout, Salmo gairdneri. J. Fish. Res. Bd. Canada, 23:471-485.

Subrahmanyam, S., and M.F. Lockett. 1965. The effect of hypophysectomy on the action of thyroxine and adrenaline on the metabolic rate of mice. J. Endocrinol. 32:77-82.

Svedmyr, N. 1966. Studies on the relationships between some metabolic effects of thyroid hormones and catecholamines in animals and man. Acta Physiol. Scand. 68:Suppl. 274, 46p.

Tanche, M., and A. Therminarias. 1969. Thyroxine and catecholamines during cold exposure in dogs. Federation Proceedings, 28: 1257-1261.

Tata, J.R. 1964. Distribution and metabolism of thyroid hormones. In "The Thyroid Gland" (R. Pitt-Rivers and W.R. Trotter, eds.) pp 163-186, Butterworths, London.

Tata, J.R. 1964a. Basal metabolic rate and thyroid hormones. Advan. Metab. Disorders, 1:153-189.

Tata, J.R. 1964b. Biological Action of thyroid hormones at the cellular and molecular levels. In "Actions of Hormones on Molecular Processes" (G. Litwack, and D. Kritchevsky, eds.) pp 58-130, Wiley, New York.

Tata, J.R. 1967. The action of thyroid hormones. J. Clin. Path. 20: 323-326.

Taylor, A. 1936. Athyroidism in the salamander Triturus torosus, Rathke. J. Exp. Zool. 73:153-181. (Cited in Lynn and Wachowski, 1951.)

Taylor, R.E. Jr., and S. B. Barber. 1967. Absence of an in vitro thyroxine effect on oxygen consumption and sodium or water transport by anuran skin and bladder. Gen. Comp. Endocrinol. 9:129-134.

Thornburn, C.C., and A.J. Matty. 1963. The effect of thyroxine on some aspects of nitrogen metabolism in the goldfish (Carassius auratus) and the trout (Salmo trutta). Comp. Biochem. Physiol. 8:1-12.

Thornburn, C.C., and A.J. Matty. 1964. The effect of thyroid hormones in vitro on the oxygen consumption of toad tissues. J. Endocrinol. 28:213-217.

Thornburn, C.C., and A.J. Matty. 1966. Effects of long-term treatment and of low concentrations of thyroid hormones on the oxygen uptake of toad tissues. J. Endocrinol. 36:221-229.

Tirri, R., M. Pantio, and H. Tarkkonen. 1968. Metabolic effects of thyroxine in infant rats. Experientia 24:365-367.

Tonoue, T., and K. Yamamoto. 1967. The disappearance rate of thyroxine from the blood and the metabolic rate in thyroidectomized rabbits. Jap. J. Physiol. 17:405-414.

Van Middlesworth, L. 1957. Thyroxine excretion, a possible cause of goiter. Endocrinology, 61:570-573.

Warren, M.R. 1940. Studies on the effect of experimental hyper-thyroidism on the adult frog, Rana pipiens, Schreber. J. Exp. Zool. 83:127-159. (Cited in Pickford and Atz, 1957.)

Wendt, C. 1965. Liver and Muscle glycogen and blood lactate in hatchery-reared Salmo salar L. following exercise in winter and summer. Rep. Inst. Freshw. Res. Drottningholm, 46:167-184.

Wiggs, A.J. 1963. Notes on the use of the conversion ratio as an index of thyroid activity. Can. J. Zool. 41:1176-1177.

Wilhoft, D.C. 1966. The metabolic response to thyroxine of lizards maintained in a thermal gradient. Gen. Comp. Endocrinol. 7:445-451.

Woodhead, A.D. 1959. Variations in the activity of the thyroid gland of the cod, Gadus callarias L., in relation to its migrations in the Barents sea. II. The 'dummy run' of the immature fish. J. Mar. Biol. Ass. U.K. 38:417-422.

Yamamoto, K. 1964. Changes in thyroxine deiodinase of the frog, Xenopus laevis Daudin, during metamorphosis and growth. Gen. Comp. Endocrinol. 4:360-369.

Zaks, M.G., and M.A. Zamkova. 1952. On the influence of thiourea on the gaseous exchanges of the larvae of salmon and sevriuga. Dokl. Akad. Nauk, USSR, 84:1101-1103. (Cited in Pickford and Atz, 1957.)