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

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ABSTRACT

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

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

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

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

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

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INTRODUCTION

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

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

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

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

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

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

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

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

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

A preliminary attempt was also made to determine the nature of

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

LITERATURE REVIEW

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

A. Radiochemical methods for measuring thyroid activity,

B. Influence of temperature on fish thyroid activity,

C. Influence of stable iodide levels on radioiodine metabolism,

D. Iodoamino acids in thyroid and serum.

Where possible information has been reduced to tabular form.

A. Radiochemical Methods for Measuring Thyroid Activity

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

Different measures of thyroid activity using radioiodine have been developed.

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

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

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

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

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

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

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

B. Influence of Temperature on Fish Thyroid Activity

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

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

C. Influence of Stable Iodide Levels on Radioiodide Metabolism

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

TABLE I. Influence of Temperature on Fish Thyroid Activity

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

TABLE I (continued)

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

Fontaine, 1960).

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

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

D. Iodoamino acids in Thyroid and Serum

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

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

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

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

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

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

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

TABLE II (Continued)

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

TABLE II (Continued)

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

+ represents presence, % unknown

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

MATERIALS AND METHODS

A. Living Material

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

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

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

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

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

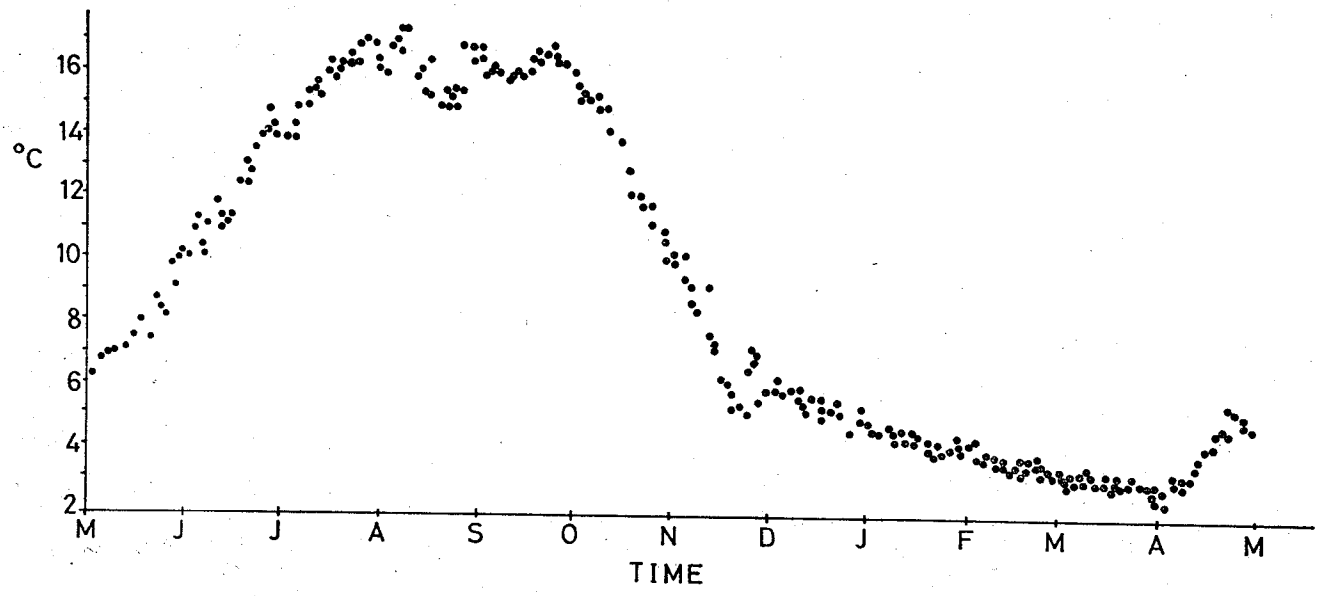
B. Injection of ^{125}I

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

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

C. Collection of Blood and Thyroid Samples

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

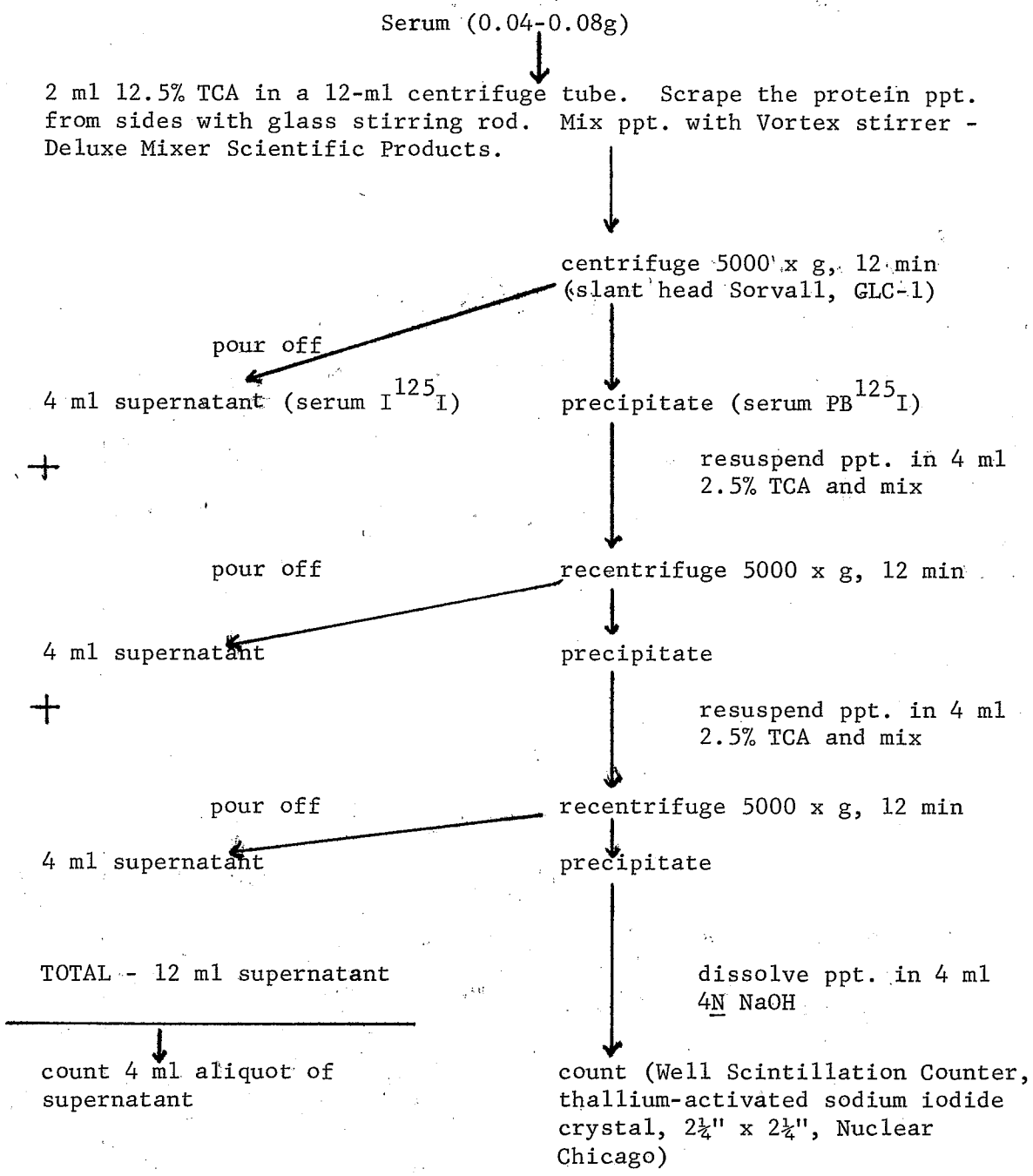
The thyroid region, between the first and third gill arches of the lower jaw, as mapped by Drury (1967), was cut out, counted and frozen at -20 C for analysis at a later time.

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

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

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

FIGURE 2. Flow chart of TCA separation of $I^{125}I$ and $PB^{125}I$ of the serum.


Serum (0.04-0.08g)
2 ml 12.5% TCA in a 12-ml centrifuge tube. Scrape the protein ppt. from sides with glass stirring rod. Mix ppt. with Vortex stirrer - Deluxe Mixer Scientific Products.
centrifuge 5000 x g, 12 min (slant head Sorvall, GLC-1)
pour off
4 ml supernatant (serum $I^{125}I$)
precipitate (serum PB ^{125}I)
resuspend ppt. in 4 ml 2.5% TCA and mix
pour off
recentrifuge 5000 x g, 12 min
4 ml supernatant
precipitate
resuspend ppt. in 4 ml 2.5% TCA and mix
pour off
recentrifuge 5000 x g, 12 min
4 ml supernatant
precipitate
TOTAL - 12 ml supernatant
dissolve ppt. in 4 ml 4N NaOH
count 4 ml aliquot of supernatant
count (Well Scintillation Counter, thallium-activated sodium iodide crystal, 2¼" x 2¼", Nuclear Chicago)

(experiment from February to April, 1969 carried out on fish held at 10 C).

Some iodide contamination of the $PB^{125}I$ fraction occurred due to imperfect washing of the precipitate. This was corrected in this study where output of radiohormone was so slow, by considering the CR on the earliest day of sampling (day 2 or 5) as the level of contamination and subtracting this from all later values. In the experiment (February to April, 1969) where the first samples were taken at 15 days, iodide contamination was evaluated by performing a separation on serum from an uninjected fish to which inorganic iodide (^{125}I) had been added.

E. Serum $I^{125}I$ and Serum $PB^{125}I$

Serum $I^{125}I$ and serum $PB^{125}I$ represent the inorganic and organic radioiodide fractions, respectively, of the serum. They are calculated in the same manner with the weight of the serum used and the body weight of the fish taken into account.

$$\frac{\%\text{ injected dose}}{\text{serum wt(g)}} \times \text{body wt(g)}$$ where % injected dose equals $$\frac{I^{125}I \text{ or } PB^{125}I \text{ fraction of TCA precipitation (cpm)}}{\text{standard (cpm)}} \times 100$$

F. Hydrolysis of Thyroid Tissue

In preliminary experiments thyroids were hydrolyzed using pancreatin (1.5%) in tris buffer (pH 8.5), but pronase (Malan, 1968) was found to provide a better hydrolysis (Fig 3). The extent of thyroglobulin digestion was checked by precipitating proteins from the

FIGURE 3. Flow chart of the hydrolysis of thyroid regions.

Thyroid region (lower jaw between first and third gill arches) cut into 4 - 5 pieces with scissors.

↓

homogenize in 2 ml 0.9% NaCl (teflon pestle in a glass tube attached to a Tri - R - Stir - R homogenizer, Model S63C, Tri - R Instrument, Inc.)

↓ pour into 10-ml test tube

add 4 ml tris buffer (pH 8.0) + 200 PUK pronase + 1 drop toluene to prevent bacterial growth.

↓ seal tube with cork

incubate 24 hr (37C) with constant shaking (200 rpm) Metabolyte Water Bath Shaker, Model G77, New Brunswick Scientific Co., Inc., New Brunswick, N.J.

thyroid hydrolysate of an injected fish with 12.5% TCA. After 12 hr of incubation 4.0% of the radioactivity remained in the precipitate, presumably as part of the thyroglobulin molecule, and at 24 hr 3.5%. After 12 hr hydrolysis was probably complete.

The possibility of deiodination of the thyroid hormones during the entire procedure was also checked by homogenizing a thyroid from an uninjected fish and incubating it with 125 I - L - thyroxine (Mallinckrodt Nuclear, St. Louis, Missouri) as above. The amount of deiodination was insignificant (radioiodide content of T_{4}-^{125}I before incubation 6.3%, after incubation 4.8%).

G. Acid-butanol Extractions of Thyroid Tissue and Serum

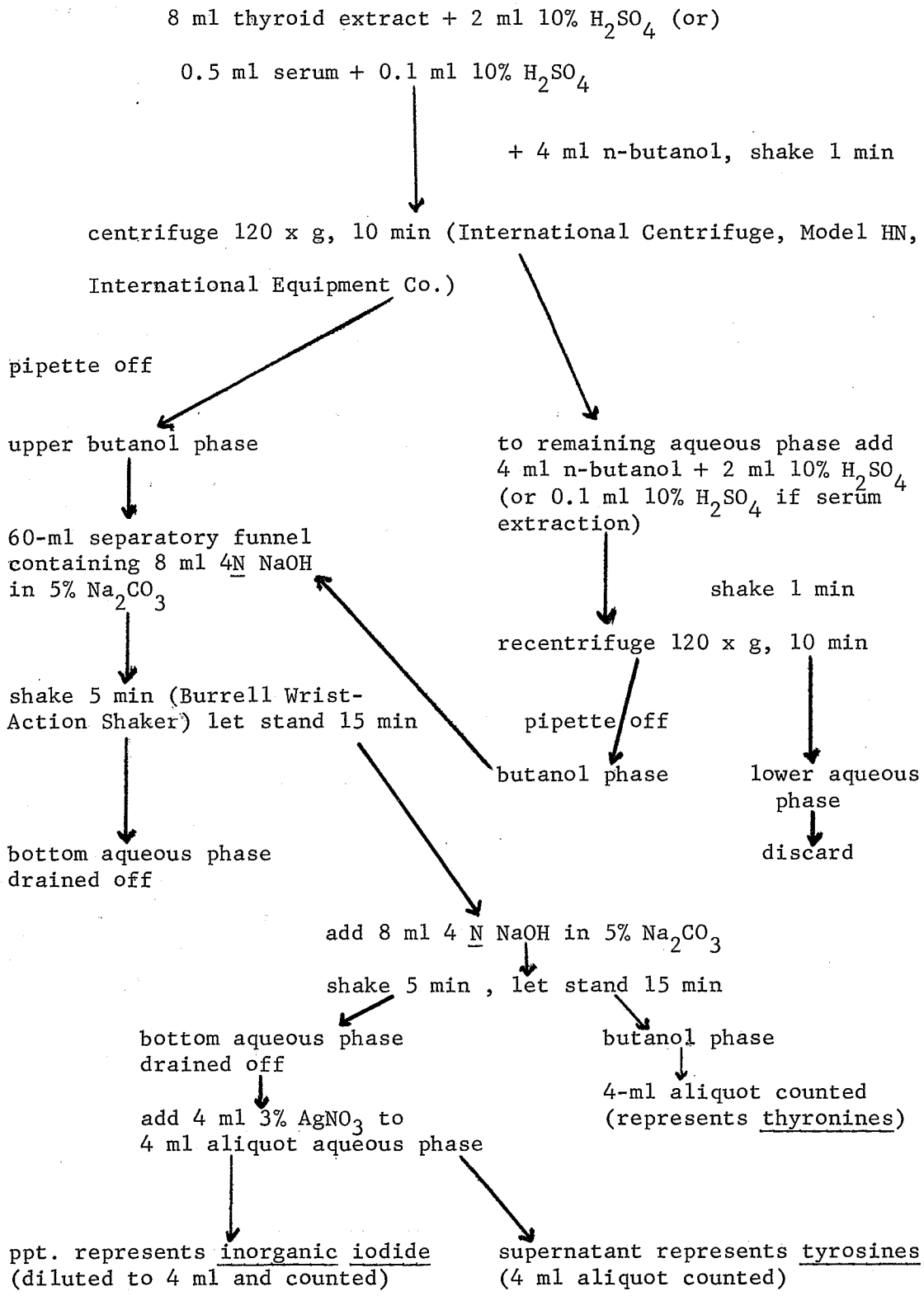
Iodocompounds of thyroid extracts and serum were separated by the method of Fisher et al. (1964) in which the iodide, iodotyrosines and iodothyronines are extracted in acid-butanol, and the iodide and iodotyrosines are then removed by alkali washes (Fig 4). Using this method iodide and tyrosine contamination of the butanol phase was found to be 4.5%.

There is a possibility that some iodotyrosines may adhere to the iodide precipitate. Precipitation of iodide from the tyrosines with silver nitrate was not checked.

H. Instant Thin Layer Chromatography (ITLC) of Serum Iodoamino acids

The instant thin layer sheets (prepared silica gel (SG), Gelman, 8" x 8") were desiccated overnight and then activated before use (110 C for 2 hr). Three hundred lambda of concentrated serum butanol extract (concentrated in vacuum oven from 8 ml to 1 ml at 37 C)

FIGURE 4. Flow chart of acid-butanol extraction of thyroid tissue and serum.


8 ml thyroid extract + 2 ml 10% H_2SO_4 (or)
0.5 ml serum + 0.1 ml 10% H_2SO_4
+ 4 ml n-butanol, shake 1 min
centrifuge 120 x g, 10 min (International Centrifuge, Model HN, International Equipment Co.)
pipette off
upper butanol phase
60-ml separatory funnel containing 8 ml 4N NaOH in 5% Na_2CO_3
shake 5 min (Burrell Wrist-Action Shaker) let stand 15 min
bottom aqueous phase drained off
to remaining aqueous phase add 4 ml n-butanol + 2 ml 10% H_2SO_4 (or 0.1 ml 10% H_2SO_4 if serum extraction)
shake 1 min
recentrifuge 120 x g, 10 min
pipette off
butanol phase
lower aqueous phase
discard
add 8 ml 4 N NaOH in 5% Na_2CO_3
shake 5 min , let stand 15 min
bottom aqueous phase drained off
add 4 ml 3% $AgNO_3$ to 4 ml aliquot aqueous phase
ppt. represents inorganic iodide (diluted to 4 ml and counted)
butanol phase
4-ml aliquot counted (represents thyronines)
supernatant represents tyrosines (4 ml aliquot counted)

were slowly applied along a 2.5-cm line using a warm air stream. Five µg MIT, DIT and the sodium salt of 3, 3', 5- triiodo L-thyronine (T_3) were spotted in a volume of 5λ and 10 µg of the sodium salt of L - T_4 and I^- (KI) were spotted in volumes of 10 lambda as standards.

After application, the sheet was desiccated for 30 min in darkness, then put directly in the Gelman thin layer chamber and equilibriated for 15 min with concentrated NH_4OH. Solvent (n-butanol) was added until it touched the bottom of the sheet. The chamber was levelled and placed in darkness. After 80 min the sheet was removed, the solvent front marked, and the sheet allowed to dry in a fume hood. Ninhydrin spray (Sigma Chemical Co.) was used to detect the standard spots (amino acids mauve, iodide yellow). The Rf values for the standards are shown below.

Substance	Rf
MIT	.43
DIT	.39
T_3	.81
T_4	.62
I^-	1,00

I. Determination of $PB^{127}I$, $BE^{127}I$, Total ^{127}I and $I^{127}I$ in Serum and Food

Total ^{127}I, $I^{127}I$, $PB^{127}I$ and $BE^{127}I$ were measured in the food using the Hycel PBI cuvette system (Fig 5). The major reaction involves the reduction of ceric ammonium sulfate by arsenious acid which is catalyzed by iodine. A colour reaction results and the

FIGURE 5. Flow chart of $PB^{127}I$, $BE^{127}I$, Total ^{127}I and $I^{127}I$ determinations in serum.

1. $\underline{PB^{127}I}$

serum (0.1 ml) diluted 5 times with distilled water

↓ + Inorganic Iodine Resin (50% by volume)

shake 1 hr (Burrell Wrist-Action Shaker)

↓ pipette 0.1 ml of diluted serum to the bottom of a cuvette (19 x 150 mm). Avoid transferring any resin particles. Also 0.1 ml of 5 known concentrations of potassium iodide.

(A.) add 2 ml perchloric acid digestion reagent, mix gently (allow reagent to flow down inner wall of cuvette.)

↓ at 10 sec intervals place cuvettes in preheated block (230±5 C), under fume hood, for 6 min

at same 10 sec intervals remove cuvettes from heating block

↓ cool to room temperature (15 min)

add 2 ml ceric reagent (mix well using Vortex mixer)

↓ place in water bath for 1 hr, 37 C

at timed 30 sec intervals, add 2 ml arsenious reagent to each cuvette, mix well (Vortex mixer)

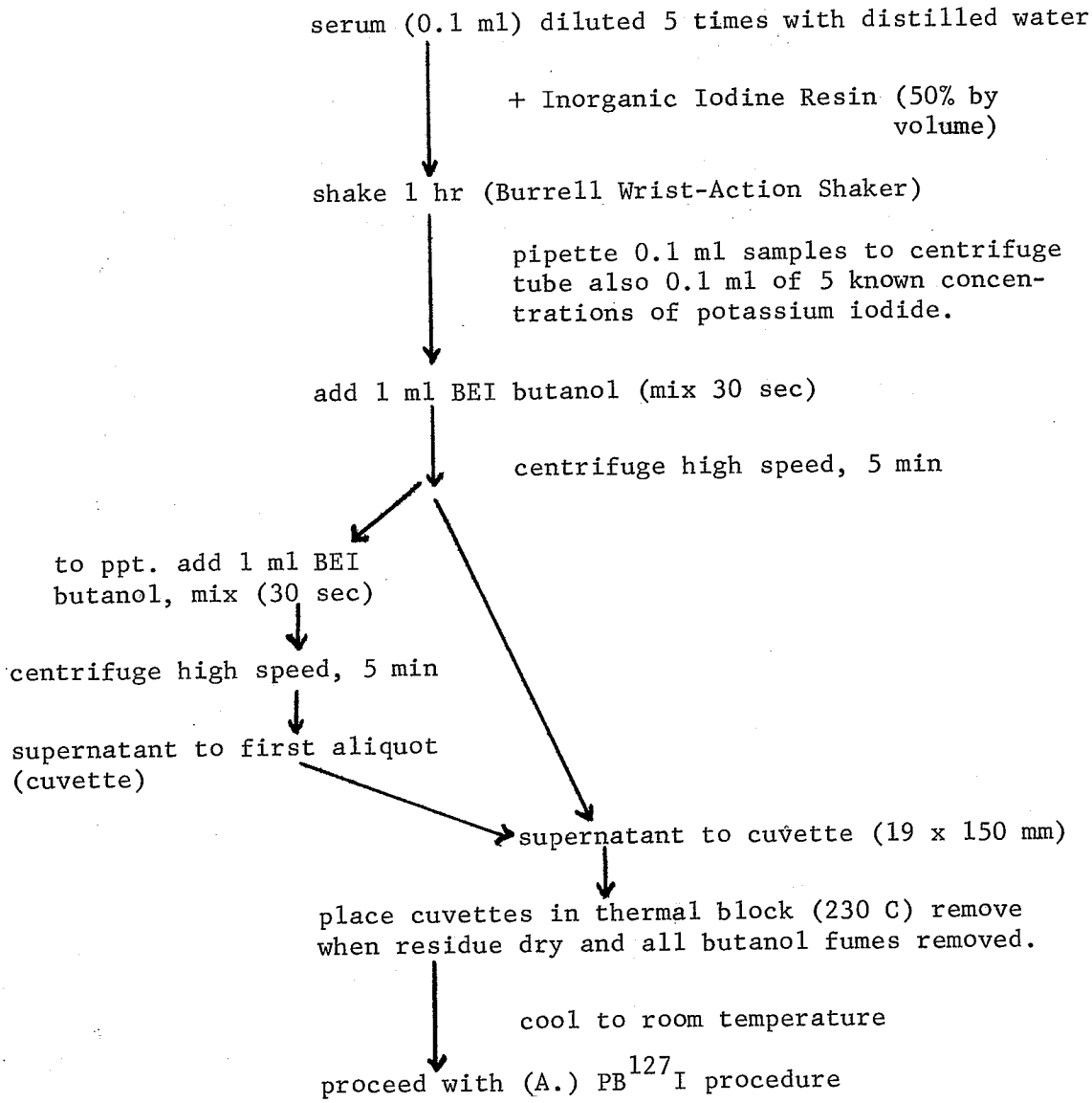
↓ place in water bath (37 C), 20 min

in same 30 sec intervals remove cuvettes from water bath, mix and read optical density against water.

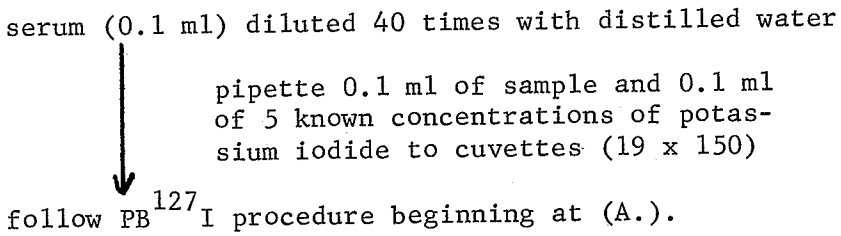
↓ plot optical density of standards against concentration.

obtain samples values

2. BE^{127}I



3. Total ^{127}I



4. $\underline{I^{127}I}$ is obtained by subtracting $BE^{127}I$ from Total ^{127}I. In the present studies this was preferable to the more usual method of subtracting $PB^{127}I$ from Total ^{127}I owing to the probable high contamination of $PB^{127}I$ with the high level of $I^{127}I$ present.

depth of colour is measured with a spectrophotometer at 420 mμ. The rate of catalysis is directly proportional to the iodine concentration at standard time and temperature.

J. Methods of Measuring Thyroid Activity

(i) Methods associated with radioiodide uptake by the thyroid

(a) Per cent of the injected dose (% thyroid)

The percent of the injected dose of radioiodide in the thyroid is considered a measure of the iodide pump. It is expressed simply as:

$$\% \text{ thyroid} = \frac{\text{thyroid (cpm)}}{\text{standard (cpm)}}$$

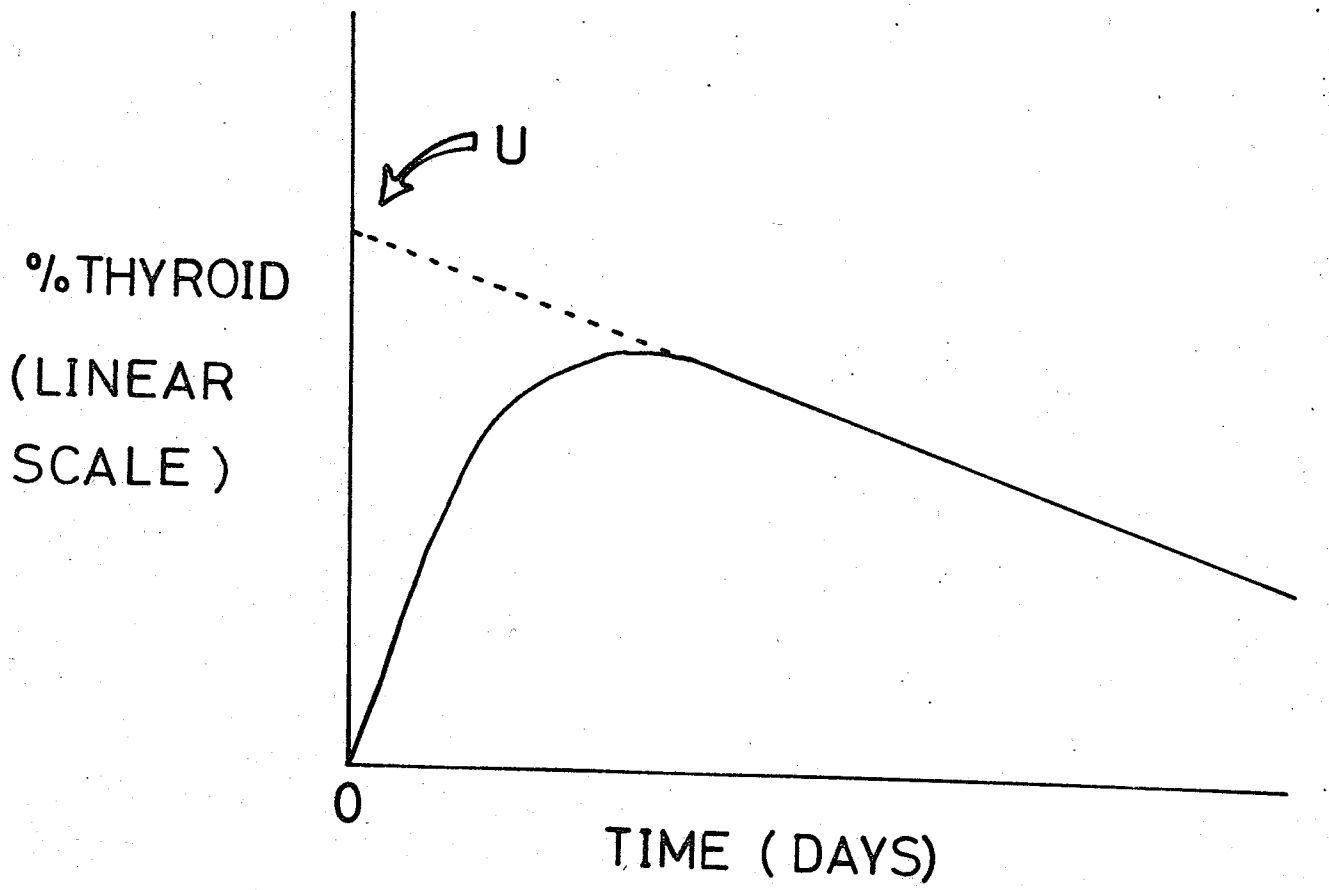
The theoretical maximum ^{125}I uptake (U) of the thyroid was determined by extrapolating the peak of the % thyroid curve to zero time (Fig 6).

(b) Thyroid:Serum (T/S) index

As used in this study T/S was measured as:

$$T/S = \frac{\% \text{ thyroid}}{\text{Serum } {}^{125}I}$$

This index has been used to provide a measure of thyroid uptake. Since it assesses the thyroid uptake of ^{125}I relative to the ^{125}I level in the serum it was considered by Eales (1964) to be superior to % thyroid. The latter measurement might be considerably influenced by changes in the serum ^{125}I pool available for thyroid uptake as a result of loss of iodide through extra-thyroidal routes. The T/S index is only considered, however, to provide an approximate correction for changes in extrathyroidal ^{125}I excretion.


U
% THYROID
(LINEAR
SCALE)
0
TIME (DAYS)

(c) Rate constant for thyroid ^{125}I uptake (K_1)

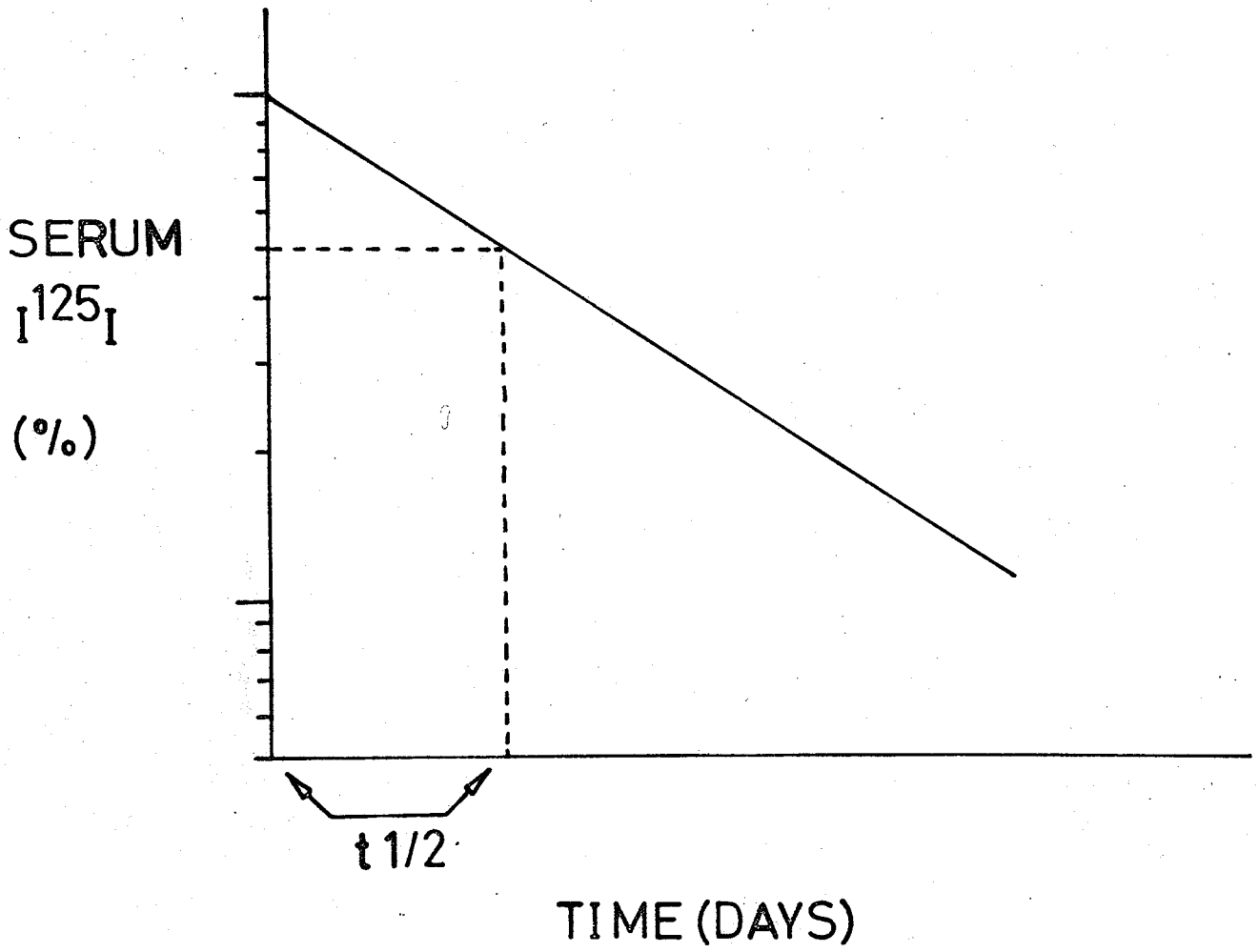
This is the preferred method of measuring thyroid ^{125}I uptake as it makes a theoretically exact allowance for alteration in thyroid uptake due to change in the excretion rate of ^{125}I from the serum. It has not been used to measure thyroid activity in fish before, being adapted from Robertson and Falconer (1961) where it is used in mammals. It does, however, require a considerable series of dterminations in time.

The rate constant for thyroid ^{125}I uptake (K_1) and the rate constant for ^{125}I excretion via all other routes (K_2) may be obtained as follows. The decrease in serum $I^{125}I$ with time is exponential and becomes a linear relationship when plotted semilogarithmically. From the slope of the exponent, the biological half life ($t_{\frac{1}{2}}$) can be calculated. This represents the time for the inorganic radioiodide in the serum to decrease by exactly 50% (Fig 7). From $t_{\frac{1}{2}}$ we can then calculate the fractional rate of loss (rate constant, K) of radioiodide from the serum according to the equation:

$$t_{\frac{1}{2}} = \frac{\ln 2}{\text{rate constant (K)}} = \frac{0.693}{\text{rate constant (K)}}$$

Since the decrease in serum inorganic ^{125}I is determined both by the rate constant for ^{125}I excretion by all extrathyroidal routes (K_2) and also by the rate constant for ^{125}I uptake by the thyroid (K_1), then, $K_1 + K_2 = K$.

$$\text{Serum } I^{125}I_{t_{\frac{1}{2}}} = \frac{0.693}{K_1 + K_2} \qquad \text{equation (1)}$$


SERUM
$I^{125}I$
(%)
t 1/2
TIME (DAYS)

However, $K_1 + K_2$ can be related in another way where

$$\frac{U}{100} = \frac{K_1}{K_1 + K_2} \qquad \text{equation (2)}$$

If the maximum % uptake by the thyroid (U) is known, $K_1 + K_2$ can be calculated simultaneously from equation (1) and (2) (Robertson and Falconer, 1961).

K_1 may be considered a useful index of thyroid activity as it assesses thyroid uptake of ^{125}I taking extrathyroidal excretion of radioiodide into account. K_2 is not an index of thyroid activity but provides a measure of extrathyroidal excretion of iodide through all other routes (i.e. gills, skin, kidney, uptake by tissues, loss to gastrointestinal tract). More detailed theory has been given by Robertson and Falconer (1961).

(ii) Methods associated with ^{125}I release from the thyroid

(a) Rate constant for thyroid ^{125}I release (K_3)

The method used to determine the rate constant for the release of ^{125}I from the thyroid, K_3, involves the measurement of the exponentially declining ^{125}I content of the thyroid. First of all an apparent rate constant (K'_3) is calculated from equation (3) (Robertson and Falconer, 1961)

$$t_{\frac{1}{2}} = \frac{0.693}{K'_3} \qquad \text{equation (3)}$$

where $t_{\frac{1}{2}}$ is the half life of the % thyroid curve, which decreases linearly with time. The true rate constant for thyroid ^{125}I release

(K_3) is calculated from equation (4) (Robertson and Falconer, 1961),

$$K_3 = \frac{K'_3}{\left(1 - \frac{U}{100}\right)} \qquad \text{equation (4)}$$

where K'_3 and U are already known. The apparent rate constant (K'_3) is less than the true rate constant (K_3) since it takes into account the reutilization of iodide resulting from the hormone (Brownell, 1951).

(b) Conversion ratio (CR)

The CR is a measure of radiohormone release. It is a useful index but not an absolute measure of thyroid activity. Hickman (1961) applied the CR to studies of thyroid activity in fish as follows:

$$CR = \frac{PB^{125}I(cpm)}{PB^{125}I(cpm) + I^{125}I\ (cpm)}$$

K. Analysis of Linear Regressions

Straight line regressions were fitted by the method of least squares. An analysis of covariance program ("Simple Covariance", #15, Computer Centre, University of Manitoba) was applied to the straight line regressions (serum $I^{125}I$) of 10 and 16 C experiments (Snedecor, 1956). The program tested the significance between adjusted group y means (slopes), and between adjusted group means (intercepts), as well as certain covariance assumptions:

1. homogeneity of residual variances--Bartlett's chi-square test.

2. homogeneity of group regression coefficients (F-test).
3. pooled within-group regression coefficient, and its t-test of significance.

If Bartlett's chi-square is significant the variances of the data do not show homogeneity. The departure from homogeneity causes a loss of efficiency and distortion of the treatment estimates (Cochran, 1947). The amount of distortion depends on the extent of error in the variances. If the error is large, the losses may be far from the true values. Therefore, if Bartlett's chi-square was significant, the test of significance values of the covariance program were not used, since the amount of distortion was not known.

RESULTS

A. Preliminary Experiments on Radioiodine Metabolism at 10 C

A preliminary experiment was conducted to determine the time-course of radioiodine metabolism in the brook trout. Knowledge of the length of time to maximum uptake of radioiodide by the thyroid, the rate of radioiodine excretion, and the time for maximum radiohormone production were needed for later studies.

From May 31 to July 10, 1968, 60 fish (range 7.1 g to 35.6 g; average 14.5 g) acclimated to 10 ± 1 C were injected with ^{125}I (0.3μCi). Eight fish were killed at each of 10, 14, 18, 22, 26, 30 and 40 days and serum $I^{125}I$, % thyroid, T/S, serum $PB^{125}I$ and CR values measured (Fig 8, 9).

Serum $I^{125}I$ decreased exponentially with time. The % thyroid reached a peak value between 10 and 15 days and began to fall after 25 days, probably due to loss of radiohormone to the serum ($t_{\frac{1}{2}}$ = 36.3 days). The T/S rose with time and did not level off, which was due to the fact that $I^{125}I$ was being continually lost from the serum, as was evident from the $I^{125}I$ curve. The CR values for the experiment were calculated.

Time days	CR $\bar{x}$ ± s.e.	No. of samples
10	9.67	8
14	0	8
18	0	8
22	25.6±11.5	8

FIGURE 8. Preliminary experiment on radioiodine metabolism at 10 C: Relationship between log serum $I^{125}I$(▲)log T/S(●)and time (days). Each point is the average of 6-8 fish. The standard error (s.e.) of the point is shown.

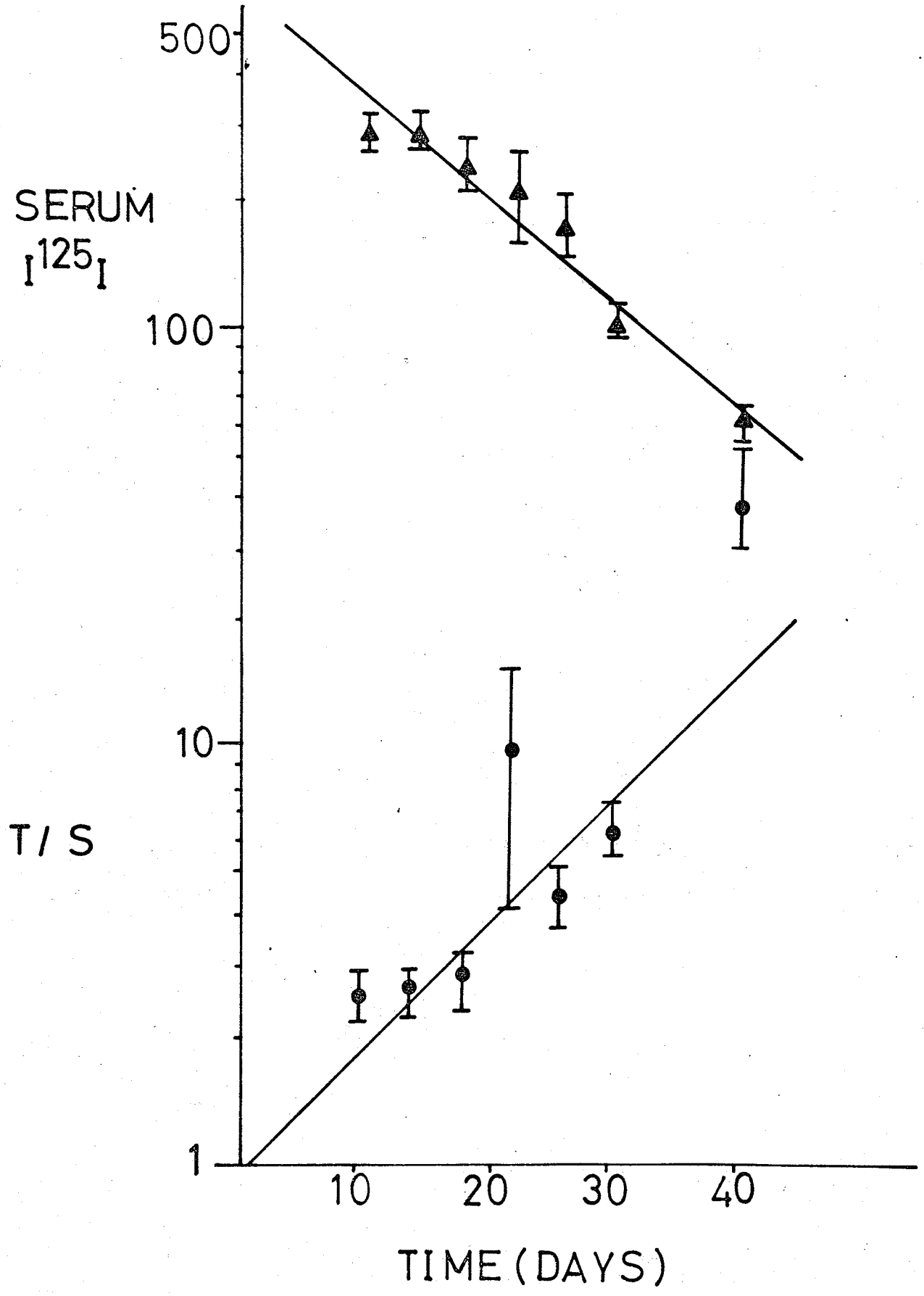
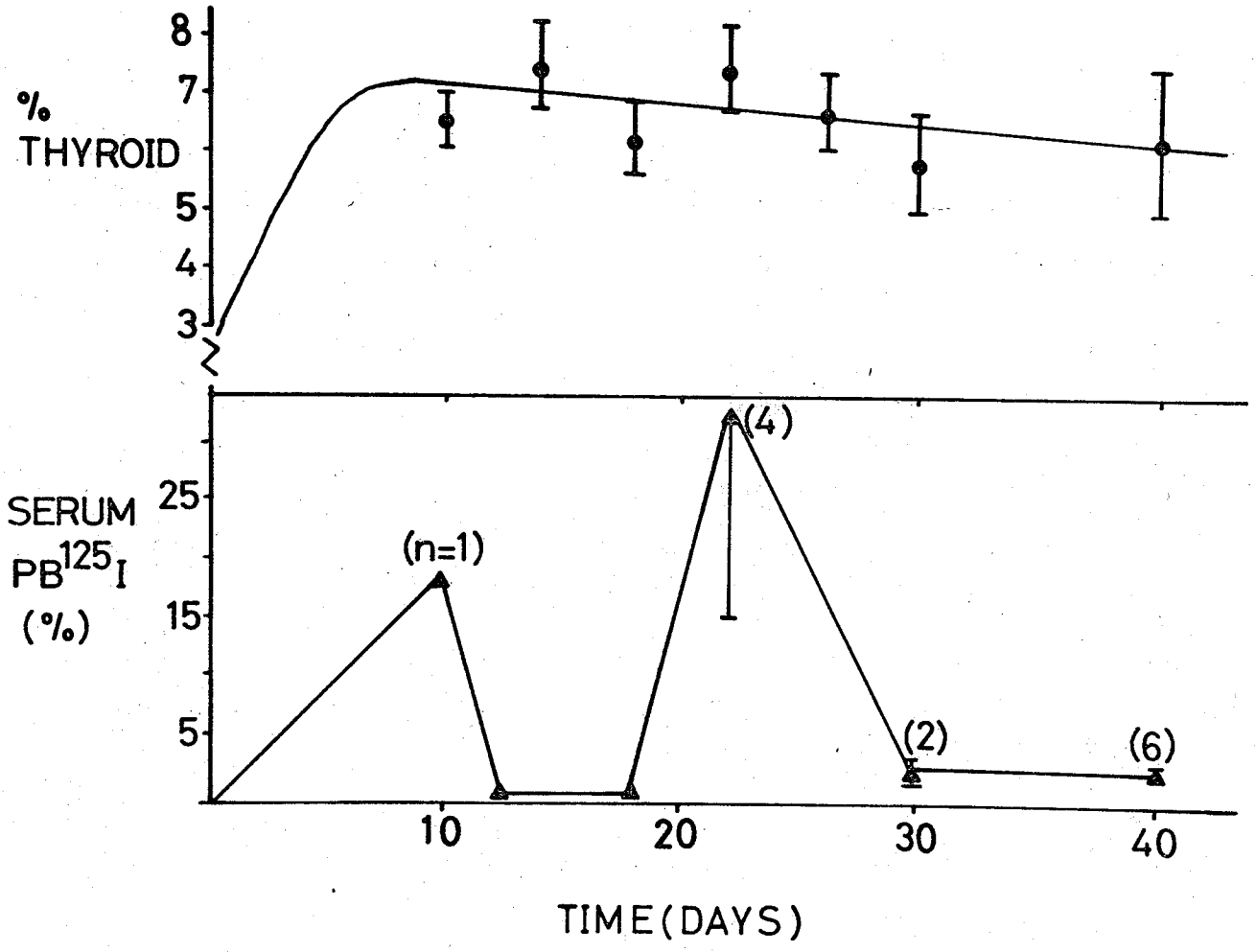

500
100
10
1
SERUM
I125I
T/S
10
20
30
40
TIME (DAYS)

FIGURE 9. Preliminary experiment on radioiodine metabolism at 10 C: Relationship between % thyroid(●), serum PB^{125}I(▲) and time (days). Each point is the average of 6-8 fish unless shown otherwise. The standard error of the point is shown.


8
7
%
THYROID
5
4
3
SERUM
PB125I
(%)
25
15
5
(n=1)
(4)
(2)
(6)
10
20
30
40
TIME(DAYS)

26	9.7±4.2	8
30	1.2±1.0	8
40	9.0±2.4	8

The value on day 22 was the highest, and indicated an output of radiohormone to the serum at this time. The serum PB^{125}I fluctuated greatly but did demonstrate output of radiohormone to the serum as the % thyroid curve decreased. There were peaks at both 10 (18.6%) and 22 days (34.2%) with zero values between the peaks. The great variability in the PB^{125}I values, as well as CR values, was probably due to iodide contamination from imperfect washing of the protein precipitate.

It can be concluded from this preliminary experiment that the brook trout used have a very low thyroid uptake of radioiodine as well as a very low output of radiohormone.

B. Relationship Between Dose of Injected ^{125}I and Various ^{125}I Parameters.

This experiment was conducted to determine if the amount of radioiodine injected into the brook trout affected the thyroid activity as measured by various ^{125}I parameters.

From June 4 to 16, 1968, 40 fish (range 6.3 g to 19.0 g; average 12.1 g) were acclimated to 10 ± 1 C in a 200-liter tank and, 4 groups of 10 fish each, injected with 0.03, 0.3, and 30.0 µCi of radioiodide, providing doses from 0.0017 to 4.29 µCi/g body wt. In another experiment, June 21 to July 2, 1968, 40 fish (range 9.2 g to 24.8 g; average 16.8 g), 4 groups of 10 fish each, were injected with

4.0, 10, 15, 20 μCi of ^{125}I, providing doses from 0.237 to 1.89 μCi/g body wt. All fish were killed 10 days postinjection and CR, T/S, % dose in thyroid and serum $I^{125}I$ measured. There was a low correlation between all of these parameters and the dose of ^{125}I injected. The behaviour of injected ^{125}I (10 C) after 10 days was independent of dose administered up to 4.29 μCi/g (Table III). In later experiments doses of ^{125}I were kept well below this level and damage to the thyroid or modification of thyroid function was assumed to be negligible.

C. Influence of Temperature on Longterm Metabolism of ^{125}I (10, 16 C).

These experiments were conducted in an attempt to trace the effect of temperature on thyroid activity and are divided into three sections:

(i) Radioiodine metabolism

(ii) Stable iodide levels of serum

(iii) Thyroid hormone biosynthesis

(i) Radioiodine metabolism

From July 10 to September 8, 1968, 80 fish acclimated to 10 ± 1 C (range 6.4 g to 56.0 g; average 19.0 g) were injected with ^{125}I (5 μCi) and 6 fish killed at 5, 10, 15, 18, 21, 24, 27, 30, 35, 40, 50 and 60 days. From July 15 to September 11, 1968, 80 fish acclimated to 16 ± 1 C (range 8.1 g to 58.3 g; average 22.7 g) were injected with a similar dose. Six fish were killed at 2, 5, 7, 10, 15, 20, 25, 30, 35, 39, 50 and 58 days and radiochemical parameters

TABLE III. Coefficients for the correlations between dose of injected ^{125}I and several ^{125}I parameters. None of 'r' values were significant (p=.05). Significance determined with a t-test in correlation analysis.

Experiment	Serum $I^{125}I$	% thyroid	T/S	CR
I	0.2615	-0.2420	-0.2223	-0.1424
II	-0.1600	-0.0584	-0.0947	0.0207

measured (Fig 10). Differences between parameters are summarized in Table IV.

The form of the thyroid uptake curves was clearly dependent on temperature. The % thyroid at 16 C (max 6.5%) was higher than at 10 C (max 4.8%). The rate constant for ^{125}I uptake (K_1) was probably the best index for thyroid uptake. K_1 for the trout at 16 C was higher than for those at 10 C. This means that the trout at 16 C collected more radioiodine. Both curves reached a plateau between 10 and 15 days and then began to decrease. The half-lives of these curves differed. At 16 C the half-life was 33.8 days whereas at 10 C it was 63.3 days, suggesting that at 16 C the thyroid produced more radiohormone than at 10 C, though loss of inorganic ^{125}I from the thyroid cannot be excluded. The rate constant for thyroid ^{125}I release (K_3) was however higher at 16 C.

Serum $PB^{125}I$ values for fish at 16 C reached a maximum (13.5%) at 10 days whereas at 10 C a maximum of 9.8% occurred at 25 days. The plots of CR and T/S by time (Fig 10) both suggest that at 16 C the thyroid turned radioiodide over faster than at 10 C. Examination of the serum $I^{125}I$ graph allowed us to conclude that trout at 16 C lose serum $I^{125}I$ faster than at 10 C.

It was concluded that trout at 16 C have (1) a greater uptake of radioiodide by the thyroid (2) a greater release of radioiodide from the thyroid and (3) a more rapid build up of radiohormone in the serum than trout at 10 C.

(ii) Stable iodide levels of serum

$PB^{127}I$, $BE^{127}I$, ^{127}I and total ^{127}I determinations were

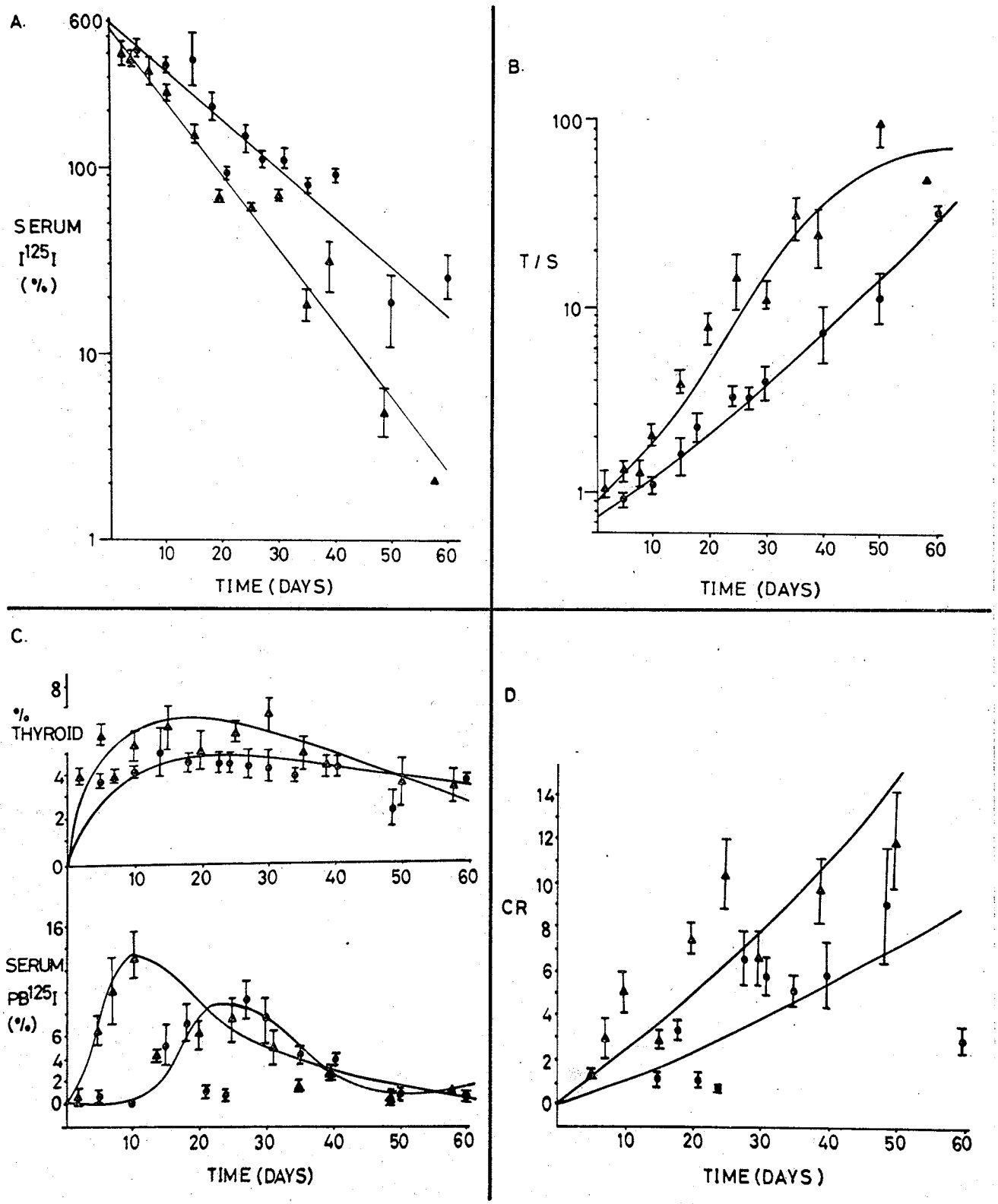

A.
600
100
10
1
SERUM
$I^{125}I$
(%)
10 20 30 40 50 60
TIME (DAYS)
B.
100
10
1
T / S
10 20 30 40 50 60
TIME (DAYS)
C.
8
%
THYROID
4
2
0
10 20 30 40 50 60
16
SERUM
$PB^{125}I$
(%)
6
4
2
0
10 20 30 40 50 60
TIME (DAYS)
D
14
12
10
8
6
4
2
0
CR
10 20 30 40 50 60
TIME (DAYS)

TABLE IV. Summary of parameters of thyroid function and radioiodine metabolism. (10, 16 C) July to September, 1968 (60 days)

Parameters	10 C	16 C	Difference	% change
Size of fish $\bar{x}$ (g)	18.95	20.84	+ 1.89	+ 9.07
Max % uptake	4.80	6.50	+ 1.70	+26.15
Max % uptake U at zero time	5.40	8.60	+ 3.20	+37.21
$t_{\frac{1}{2}}$ % thyroid (days)	63.25	33.75	-29.50	-46.64
$t_{\frac{1}{2}}$ serum $I^{125}I$ (days)	10.9	7.70	- 3.20	-29.36
T/S (10 days)	1.12	2.14	+ 1.02	+47.66
K_1	.004	.008	+ .004	+50.00
K_2	.060	.082	+ .022	+26.83
$K_1 + K_2$	.064	.090	+ .026	+28.89
K_3'	.011	.021	+ .010	+47.62
K_3	.012	.023	+ .011	+47.83
CR max (%)	9.10 @ 50 days	21.79 @ 58 days	+12.69	+58.24
Max $PB^{125}I$ (%)	8.80 @ 25 days	13.50 @ 10 days	+ 4.70	+34.81

carried out on serum samples from brook trout at 10 and 16 C (Table V). Unfortunately, the serum samples used for these determinations were not from experimental fish. Large fish ($\bar{x}$ = 102.27 g) were killed in April, 1969 and serum samples taken. The stable iodide values obtained from these serum samples were considered to be similar to those of the large fish in the 10 C experiment--February to April, 1969. Small fish ($\bar{x}$ = 22.77 g) were killed in June, 1969 and serum samples obtained. Stable iodide values obtained from these small fish were considered to be similar to those of small fish in the 1968 experiments (May to June, and July to September, 1968).

(iii) Thyroid Hormone Biosynthesis

Enzyme hydrolysis followed by butanol extraction were conducted on thyroid tissues from trout acclimated at 10 and 16 C. This procedure separated iodoamino acids into a butanol phase (thyronines or BEI) and an aqueous phase (iodide and tyrosines). Iodide was then precipitated from the aqueous phase with $AgNO_3$ leaving behind the tyrosines. However, part of the aqueous phase was discarded during extraction so that percentages of thyronines, tyrosines and iodide could not be calculated. However, thyronine:tyrosine ratio was calculated (Fig 11). It is concluded that the thyroid gland of trout at 16 C synthesizes a larger amount of radiohormone at a faster rate than the thyroid at 10 C.

D. Identification of Serum Iodoamino acids in Brook Trout at 10 C.

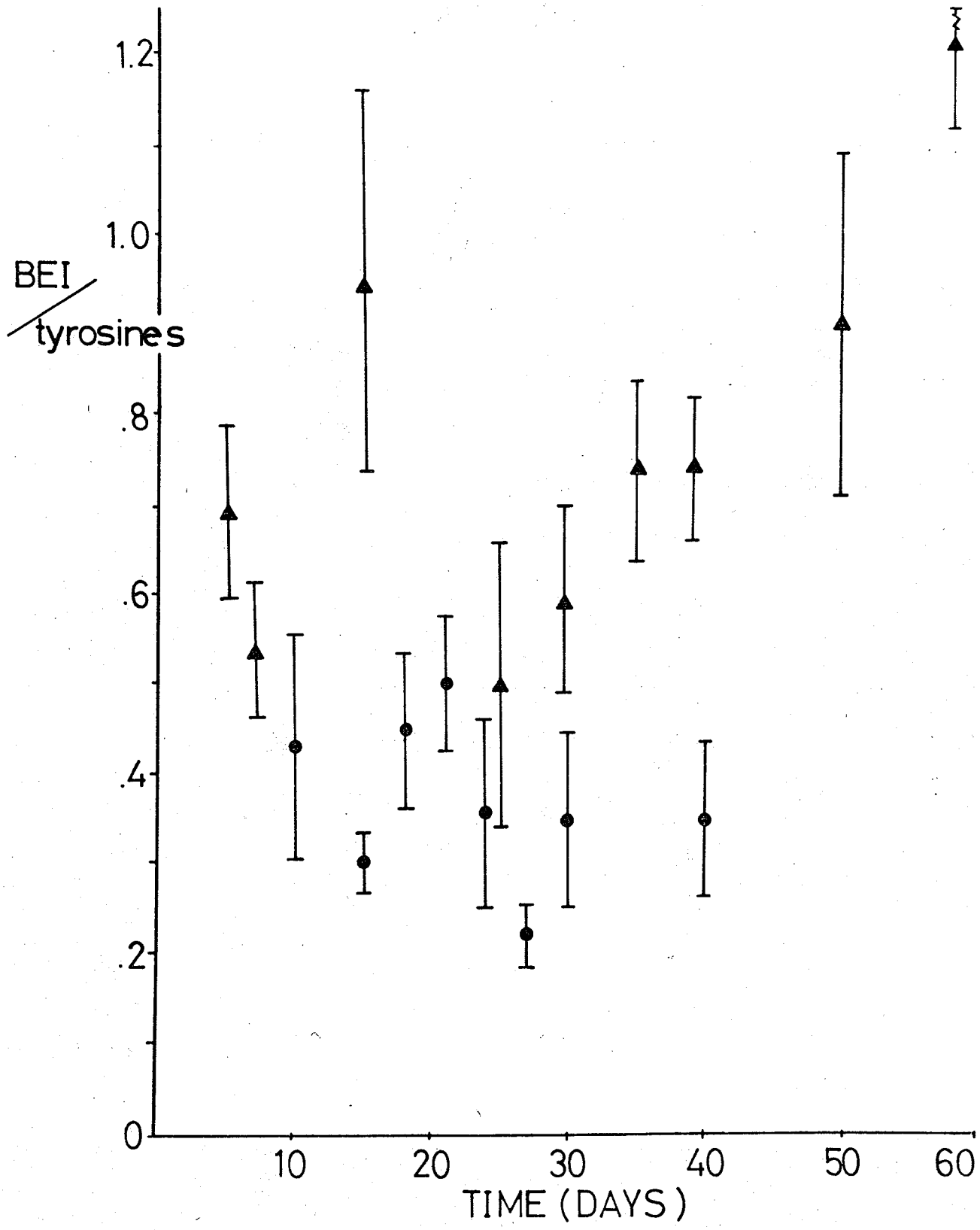
A preliminary experiment was carried out where serum samples were spotted directly on the ITLC sheets following the method of

TABLE V. Stable iodine determinations (^{127}I) of Brook Trout serum (µg/100 ml) $\bar{x}$ ± s.e.

Temp	$PB^{127}I$	$BE^{127}I$	^{127}I	Total ^{127}I	Samples
Wt = 102.27 ± 15.37 g (April, 1969)					
10 C	25.42 ± 5.89	15.28 ± 8.24	508.57 ± 45.56	523.85 ± 53.80	10
16 C	21.46 ± 0.84	20.88 ± 1.05	730.62 ± 7.68	751.50 ± 8.73	4
Wt = 22.77 ± 0.58 g (June, 1969)*					
10 C	2.14 ± 0.43	2.83 ± 0.75	84.72 ± 6.76	87.55 ± 7.51	7

* indicates data from Fung, 1969 (unpublished)

FIGURE 11. Acid-butanol extraction of 10(●), 16(▲)C thyroids of brook trout:Relationship between BEI(cpm)/tyrosines (cpm) and time (days). Points are means of up to eight samples. The standard error is shown.


1.2
1.0
BEI
tyrosines
.8
.6
.4
.2
0
10
20
30
40
50
60
TIME (DAYS)

Jacoby and Hickman (1966). Owing to the excessive ^{125}I levels and very low iodinated organic compounds in the serum it was difficult to identify the peaks of radioactivity on the chromatograms against the background created by the high level of ^{125}I.

The serum samples were then extracted with acid-butanol and chromatography carried out on the butanol phase. Also a higher level of ^{125}I was injected into the fish to increase the levels of radiohormone in the serum.

From February 28 to April 19, 1969, 40 fish (range 71.2 to 216.2 g; average 118.3 g) acclimated to 10 ± 1 C were injected with 10 μCi of ^{125}I. Eight fish were killed at 15, 25, 30 and 40 days, and ITLC carried out on the butanol phase of the extracted serum samples. The results of butanol extraction and subsequent ITLC are shown in Table VI and examples of thin-layer separations are plotted in Fig 12.

The TLC separations (Fig 12) show peaks for T_3 and T_4. They have been identified by comparison to stable standards. Radioiodotyrosine peaks are not definite and it can only be suggested that iodotyrosines occur in brook trout serum.

A very high percentage of the thyronine portion of the serum was shown still to be iodide (0.40% at 25 days which is 50.8% of the butanol phase). The thyronines (T_4 and T_3) reached a peak value of 0.16 and 0.15%, respectively, at 30 days. A similar thyronine peak was shown in the plot of serum PB^{125}I (Fig 10) at 10 C, between 20 and 30 days. A quantitative comparison of the thyronines, T_4 and T_3, was made by applying a paired t-test. The t-value was not significant

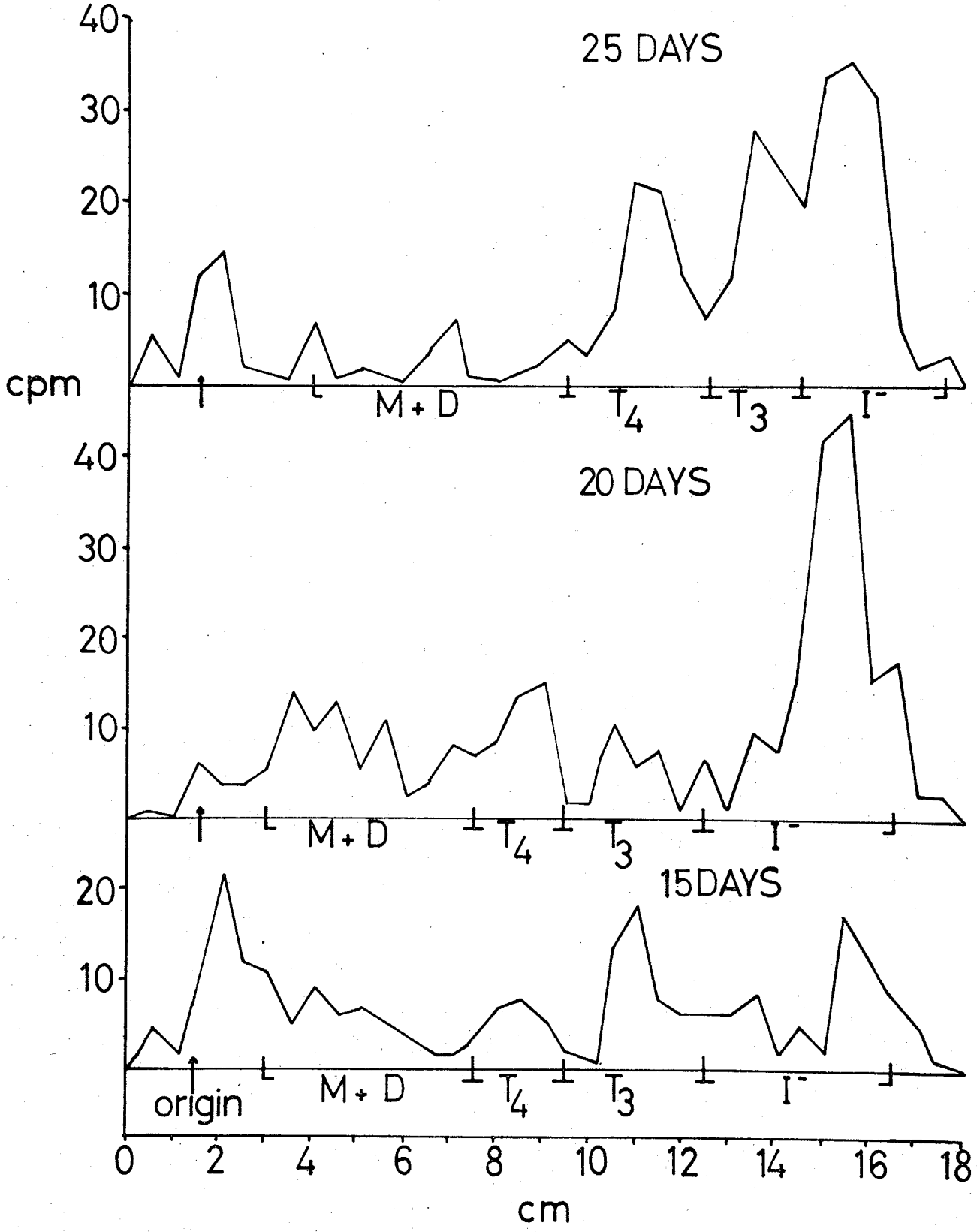
TABLE VI. Results of acid-butanol extraction of fish serum and subsequent ITLC separation of the butanol phase (February to April, 1969; 10 C)

Time (days)	TCA Precipt'n CR (PBI%)	Acid-butanol extraction (%) BEI (%)	I^- in BEI	I^- + tyrosines*	Chromatography of butanol phase (%) Origin	MIT and DIT	T_4	T_3	# of Samples
15	2.1	0.71	0.23	99.52	0.26	0.10	0.05	0.07	9
25	2.7	0.80	0.40	99.61	0.17	0.06	0.06	0.10	7
30	4.6	0.91	0.27	99.37	0.18	0.14	0.16	0.15	8
40	6.6	0.87	0.36	99.49	0.23	0.13	0.09	0.06	3
$\bar{x}$	4.0	0.82	0.32	99.50	0.21	0.11	0.09	0.10	6.8

BEI = thyronine portion

* indicates minimum value of iodide since some discarded during extraction.

N.B. All percentages are calculated in terms of the radioactivity in the acid-butanol fraction.


25 DAYS
20 DAYS
15DAYS
cpm
40
30
20
10
M + D
T_4
T_3
I⁻
origin
0
2
4
6
8
10
12
14
16
18
cm

($p = .05$), that is, the mean per cent of T_4 in the serum did not significantly differ from that of T_3 (50:50).

E. Variation in ^{125}I Metabolism at 10 C.

Certain parameters of thyroid function of 2 radioiodine metabolism experiments at 10 C (July to September, 1968; February to April, 1969) are compared in Fig 13 and summarized in Table VII. Owing to lack of homogeneity of variance, covariance analysis could not be applied.

The 2 experiments are similar in uptake of ^{125}I (K_1) and ^{125}I release (K_3). A small difference occurred in the half-life of the excretion of serum $I^{125}I$ but it was not statistically significant. Both the CR and serum $PB^{125}I$ showed considerable variation. Part of this variation was probably due to inorganic ^{125}I contamination. A different level of contamination was subtracted from each and could help to account for the different levels of the T/S and serum $PB^{125}I$ plots (Fig 13).

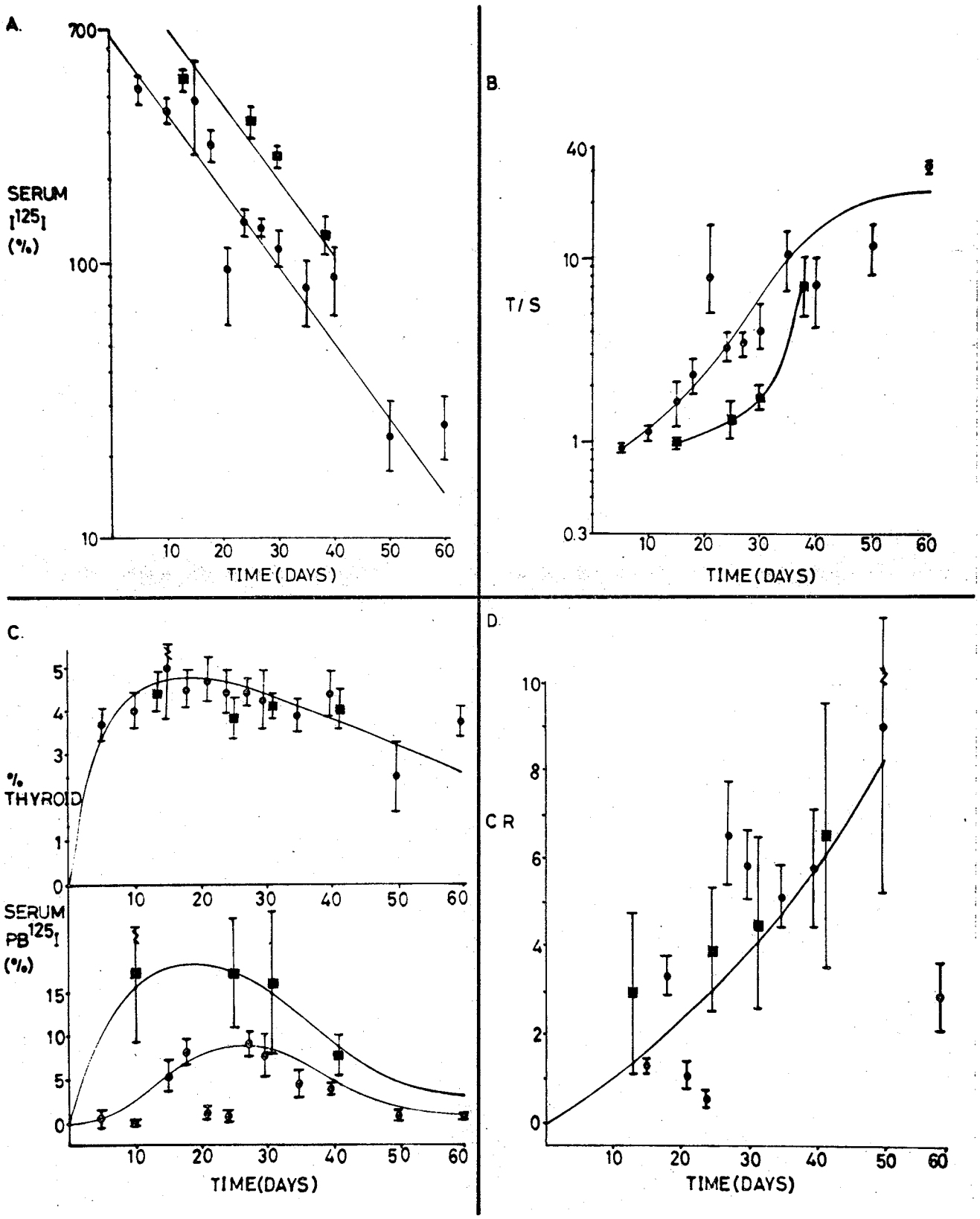

A.
700
100
10
SERUM
$I^{125}I$
(%)
10 20 30 40 50 60
TIME(DAYS)
B.
40
10
1
0.3
T/S
10 20 30 40 50 60
TIME(DAYS)
C.
5
4
3
1
0
%
THYROID
10 20 30 40 50 60
SERUM
$PB^{125}I$
(%)
15
10
5
0
10 20 30 40 50 60
TIME(DAYS)
D.
10
8
6
4
2
0
CR
10 20 30 40 50 60
TIME(DAYS)

TABLE VII. Summary of parameters of thyroid function and radioiodine metabolism at 10 C.

Parameters	July to Sept., 1968 (60 days)	Feb. to April, 1969 (40 days)	Difference	% change
Size of fish $\bar{x}$ (g)	18.95	118.31	+99.36	+524.33
Max % uptake	4.80	4.80	0	0
Max % uptake (0) U	5.80	5.80	0	0
$t_{\frac{1}{2}}$ % thyroid (days)	63.25	63.25	0	0
$t_{\frac{1}{2}}$ serum $I^{125}I$ (days)	10.90	11.00	+ 0.10	+ 0.91
T/S (15 days)	1.68	.98	- .70	- 41.67
K_1	.004	.004	0	0
K_2	.060	.059	- .001	- 1.67
$K_1 + K_2$	.064	.063	- .001	- 1.56
K_3'	.011	.011	0	0
K_3	.012	.012	0	0
CR 40 day (%)	5.77	6.58	+ 0.81	+ 12.31
Max $PB^{125}I$ (%)	8.8 @ 25 days	19.0 @ 16 days	+10.2	+ 53.68

DISCUSSION

Using increasing doses of injected radioiodine, a dose effect was not determined on the parameters of thyroid function (serum $I^{125}I$, % thyroid, T/S and CR) up to 4.29 μCi/g body wt, over a 10-day experimental period. Subsequent experiments were run for longer than 10 days and a dose effect may have developed after this time. However, the dose of ^{125}I used was kept well below 4.29 μCi/g in these experiments.

The dose of injected ^{125}I has been shown to be related to various parameters of thyroid function. Chavin and Cukrowski (1968) state that even low dose levels (0.006 μCi/g) reduced thyroid uptake by 37% after 171 days. This was in goldfish where % thyroid uptake was 10.3 ± 1.7% for control fish. They also found that thyroid epithelial height increased and abnormalities in the thyroid follicles evoked. Hoar and Eales (1963a) showed that in goldfish (20 C) and chum salmon (12 C) a correlation existed between 4-day CR and the dose of ^{131}I over a dose range of 1.1 to 8.2 μCi/g body mass. Data obtained from chub (4, 20 C), steelhead trout (4 C) and sockeye salmon (4 C) did not suggest this correlation. They concluded that CR values particularly of goldfish can be altered by dose and the effect of dose is modified by several factors including temperature and the activity of the gland itself.

Radioiodine metabolism was found to be slow in the brook trout at both 10 and 16 C. Thyroid uptake of radioiodide remained low (max uptake 4.8% at 10 C and 6.5% at 16 C, after 20 days). Up to 25 days

elapsed before any appreciable release of radiohormone occurred. The CR values were low after 20 days, 1.6 at 10 C and 5.5 at 16 C, and reached only 8.6 at 10 C and 13.7 at 16 C after 60 days (Fig 10d). This slow rate of radioiodide turnover is probably due to the high iodide (^{127}I) pool (Table V). According to Leloup and Fontaine (1960) fish having high serum levels of ^{127}I take us less radioiodide into the thyroid in proportion to fish with low levels. Hickman (1962) also notes that a change in the stable iodide level changes the amount of ^{125}I taken into the thyroid.

The high total ^{127}I in the trout serum may be accounted for by the ability of the serum proteins to bind iodide. This ability is noted among migratory fishes (Leloup and Fontaine, 1960) to which the brook trout belongs.

The total ^{127}I content of the serum at 16 C is significantly greater than at 10 C. This is contrary to the results of Leloup and Fontaine (1960) in the eel, where the stable iodide of the serum decreases significantly at higher temperatures. However, the $BE^{127}I$ is not significantly altered by temperature in the brook trout determinations.

A low level of radioiodine metabolism has also been noted in goldfish (Hoar and Robertson, 1959), where thyroid uptake reached a maximum of 2 to 3.5% at 20 C. Drury and Eales (1968) also concluded that brook trout had a slow turnover of radioiodide at 13 C (% thyroid, 7% in 8 days and had not reached a plateau). A low radiohormone output was also present occurring approximately 1-3 days after radioiodine injection. Drury and Eales concluded that this small peak at 3 days

was due to contamination. In comparison, Salmo gairdneri (Eales, 1964) have a considerably higher rate of radioiodide turnover (% thyroid, 9% in 6 days and CR reached a value of 20 in 11 days), as do the goldfish used by Hoar and Eales (1963b) which reached a CR value of 19.0 to 26.4 in 10 to 20 days at 18 C.

All measured parameters of thyroid function in the brook trout were found to be higher at the higher acclimation temperature (Table IV).

Olivereau (1955b) and Berg, Gorbman and Kobayashi (1959) determined a higher thyroid activity at lower temperatures using thyroid uptake of radioiodide as the measure of thyroid activity; whereas Leloup and Fontaine (1960), Eales (1964, 1965), Leray and Febvre (1968), and Drury and Eales (1968) notes increased thyroid activity with increasing temperature using the following radiochemical parameters--CR, T/S and thyroid uptake. The discrepancy might be accounted for by factors influencing iodide uptake. Iodide uptake into the thyroid is the result of--the concentrating ability of the gland, radioiodide excretion and ^{127}I content of the blood (Leloup and Fontaine, 1960). High temperature increases the speed of ^{125}I excretion from the serum of Salmo gairdneri (Eales, 1964) and in the brook trout of the present study. Therefore a gland may take up less iodine even though it is more active. This could account for the findings of Olivereau, and Berg, Gorbman and Kobayashi that lower temperature increases the % uptake of radioiodide.

Why may thyroid activity be considered to increase with temperature? The pituitary is the link between the receptor organs

and the thyroid gland. Environmental effects are mediated through the pituitary to the thyroid by means of the thyrotropic hormones. Seasonal changes in thyroid activity suggest that thyrotropic hormones are produced in varying amounts at different seasons. It is reasonable to issume that photoperiod and temperature act through the pituitary to control production of thyrotropin (Hoar, 1957). However Eales (1964) argues that temperature directly influences thyroid function without the mediation of the pituitary.

An increase in temperature causes an increase in the metabolic rate of the animal, which produces increased metabolic demands. The thyroid itself may be directly stimulated by some particular metabolic demand, or indirectly through the action of light and temperature on the pituitary-thyroid mechanism.

During temperature acclimation in fish certain enzyme changes have been noted to occur. The most notable being a metabolic shift during low temperature acclimation in goldfish (Hochachka, 1962)for (Hoar and Eales, 1963), so that the hexose monophosphate pathway (HMP) or pentose shunt was more favoured at low temperatures. Little doubt remains that temperature compensation in goldfish is at least partially an enzyme adjustment. Mammalian TSH was found to increase low temperature resistance of goldfish, however, the results could not be related to the role of the thyroid (Hoar and Eales, 1963).

Owing to large amounts of inorganic iodide it was difficult to determine the iodocompounds in brook trout serum. Chromatography of serum from fish at 10 C tentatively suggested that T_3 and T_4 may be present in approximately equal amounts. Iodotyrosines (MIT, DIT)

may also be present.

The presence of iodotyrosines in fish serum has been noted by Leloup (1956) in Periophthalmus koelreuteri and by Jacoby and Hickman (1966) in Salmo gairdneri. They were not, however, found by Osborn and Simpson (1959) who carried out an analysis of the circulating thyroid hormones in the serum of plaice. Osborn and Simpson indicate that the evidence obtained for iodotyrosine presence in fish serum by Leloup, and Jacoby and Hickman is only slight. They suggest that the compounds may be metabolites of thyroxine, but this does not argue against the presence of idotyrosines in fish serum.

Radioiodine metabolism experiments run at 10 C on fish of different sizes and at different seasons of the year show a great deal of similarity. The rate constants for ^{125}I uptake and release from the thyroid (K_1, K_3) are equal (Table VII). There is a difference however, in the max PB ^{125}I and 40-day CR values. Higher values are present in the larger, mature fish of the February to April experiment. The increased maturity of the larger brook trout as well as the seasonal variation could account for these differences. Swift (1960) notes an increased activity of the thyroid gland at spawning time, reflected in a great increase in the production of labelled thyroxine in Fundulus heteroclitus.

A total survey of the 2 experiments shows more similarities than differences. However, before it can be concluded that thyroid activity is not significantly altered with season when the temperature is kept constant, experiments must be carried out where fish are kept at constant temperature over all the seasons.

Further experiments are necessary to confirm the $PB^{125}I$ peaks, obtained at both 10 and 16 C, because of the presence of the large pool of contaminating iodide and very low levels of protein-bound radioiodide. The method of radioisotopic equilibrium (Jacoby and Hickman, 1966) makes it possible to increase the radioiodine content of the serum and consequently the $PB^{125}I$ can be measured more accurately. Further work could then be done to identify and quantitate the radioiodoamino acids in the serum. Also a determination of the optimal dose of radioiodine to be used as a tracer dose for experiments would be an asset, or experiments to prove conclusively that no dose effect occurred after 10 days with the amount of radioiodine used.

In conclusion, it was found that an increased temperature increased radioiodine metabolism and thyroid hormone biosynthesis in the brook trout. The slow rate of radioiodine turnover at both 10 and 16 C can be attributed to the high serum iodide (^{127}I) pool. The level of thyroid activity at different seasons of the year did not vary significantly when the temperature remained constant.

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APPENDIX

TABLE I

TESTS FOR COVARIANCE ASSUMPTIONS:

RADIOIODINE METABOLISM EXPERIMENTS (10, 16 C)

Test	Serum $I^{125}I$	T/S	CR
Bartlett's χ^2	$\chi^2_{(1)} = 14.703$	$\chi^2_{(1)} = 0.067$	$\chi^2_{(1)} = 0.177$
(homogeneity of within group variances)	$\chi^2 > \chi^2\ \alpha$	$\chi^2 < \chi\ \alpha$	$\chi^2 < \chi^2\ \alpha$
	No homogeneity	Homogeneity of variances	No homogeneity of variances
Homogeneity of within group regression coefficients	$F(1,137)=17.017$	$F(1,137)=13.5$	$F(1,138)=24.919$
	$F > F\ \alpha$	$F > F\ \alpha$	$F > F\ \alpha$
	No homogeneity	No homogeneity	No homogeneity
Significance of pooled within group regression coefficient	$t(138)=-15.436$	$t(138)=11.077$	$t(139)=8.237$
	$t > t\ \alpha$	$t > t\ \alpha$	$t > t\ \alpha$
	Significantly different from zero	Significantly different from zero	Significantly different from zero

TABLE II

TESTS FOR COVARIANCE ASSUMPTIONS:

RADIOIODINE METABOLISM EXPERIMENTS (10 C)

MAY TO JULY, 1968, JULY TO SEPT 1968, FEB TO APRIL, 1969

Test	Serum $I^{125}I$	T/S	CR
Bartlett's χ^2	$\chi^2_{(2)} = 6.981$	$\chi^2_{(2)} = 42.459$	$\chi^2_{(2)} = 58.452$
(homogeneity of within group variances)	$\chi^2 > \alpha^2\ \alpha$	$\chi^2 > \chi^2\ \alpha$	$\chi^2 > \chi^2\ \alpha$
	No homogeneity of variances	No homogeneity of variances	No homogeneity of variances
Homogeneity of within group regression coefficients	$F(2,154)=1.189$	$F(2,153)=4.883$	$F(2,154)=1.477$
	$F < F\ \alpha$	$F > F\ \alpha$	$F > F\ \alpha$
	Homogeneity	No homogeneity	Homogeneity
Significance of pooled within group regression coefficient	$t(156)=-14.798$	$t(155)=9.121$	$t(156)=3.049$
	$t > t\ \alpha$	$t > t\ \alpha$	$t > t\ \alpha$
	Significantly different from zero	Significantly different from zero	Significantly different from zero

TABLE III

EQUATIONS FOR STRAIGHT LINE

REGRESSIONS USED IN FIGURES

No. of Figure	Temp (C)	Radiochemical Parameter	Equation of the line y = a + b x (log)	Date
8	10	Serum $I^{125}I$	y = 2.928 - (0.0342)x	May to July, 1968
8	10	T/S	y = -0.050 + (0.0302)x	May to July, 1968
10	10	Serum $I^{125}I$	y = 2.760 - (0.0262)x	July to Sept., 1968
10	16	Serum $I^{125}I$	y = 2.752 - (0.0396)x	July to Sept., 1968
13	10	Serum $I^{125}I$	y = 2.760 - (0.0262)x	July to Sept., 1968
13	10	Serum $I^{125}I$	y = 3.136 - (0.0276)x	Feb. to April, 1969