The University of Manitoba

THE INFLUENCE OF TESTOSTERONE PROPIONATE ON THYROID FUNCTION IN IMMATURE RAINBOW TROUT, SALMO GAIRDNERI, RICHARDSON

BY

David William Carey Hunt

A THESIS

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ABSTRACT

The effects of intramuscularly injected testosterone propionate (TP) on thyroid function were determined in fed immature rainbow trout held at 11.0 - 12.2°C.

Three-week treatment with a graded series of TP doses elevated plasma 3,5,3'-triiodo-L-thyronine (T_3) levels while plasma L-thyroxine (T_4) levels showed no consistent response. TP increased plasma binding of T_3 and T_4, apparently by elevating plasma protein concentrations. Plasma T_4 levels were elevated significantly 24 hr following a single 30-µg TP injection. No alterations in plasma T_3 levels or plasma binding of T_4 or T_3 occurred in these fish.

Thyroid epithelial cell height increased significantly in fish receiving a high TP dose although all TP-injected trout showed increased colloid reabsorption.

Radiothyroxine studies indicated that, relative to sham-injected control trout, TP-injected trout demonstrated an increased T_4 degradation rate, increased T_4 deiodination rate and conversion to T_3. TP altered biliary excretion of ^{125}I-radioactivity.

TP-injected fish had an increased % thyroid $^{131}I^-$ uptake and an increased plasma level of labeled T_3 following radioiodine injection. Plasma inorganic iodide levels increased significantly in TP-injected fish.

The data indicate that TP has caused a widespread stimulation of the trout thyroidal system. TP may exert its effects on the thyroid indirectly by increasing the general metabolism of the fish and/or directly by acting upon the hypothalamo-pituitary-thyroid axis.

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INTRODUCTION

Several lines of evidence point to a strong relationship between thyroidal and reproductive functions in teleost fish (Sage 1973). Alterations in thyroid activity occur in sexually maturing teleosts (Barrington and Matty, 1954; Dodd and Matty, 1964; Fontaine and Leloup, 1962). Administration of various sex steroids may produce similar alterations (Higgs et al., 1977; Matty et al., 1958; Sage and Bromage, 1970; Singh, 1969; van Overbeeke and McBride, 1971).

Changes in teleost thyroid status induced by sex steroids have been evaluated in terms of thyroid histology and thyroid radioiodide uptake. These methods are sometimes poorly correlated, and, at best, give only a general index of thyroid activity, without regard to thyroid hormone production and subsequent metabolism. Measurement of plasma L-thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) by radioimmunoassay combined with T_4 degradation studies supplements the classical methods to provide a fuller understanding of thyroid function and its alterations.

The objective of this study was to observe the effect of testosterone propionate (TP) administration on thyroid function of immature rainbow trout. Thyroid function was evaluated in

several different ways. These included the classical indices based on the histological appearance of the thyroid, thyroid uptake of inorganic radioiodide and release from the thyroid of labeled hormones. In addition the plasma T_4 and T_3 levels, the degradation rate of T_4, the extent of T_4 loss via enterohepatic and deiodination pathways, and conversion of T_4 to T_3 have been studied. To this end, four experiments were conducted. In the first three, the effects of TP on plasma T_4, T_3, plasma protein-binding of T_4 and T_3, and thyroid histological appearance were studied. In the final experiment, effects of TP on radioiodide and radiothyroxine kinetics were monitored using a double isotope approach.

TP was administered to immature rainbow trout and thyroidal responses measured. This steroid was chosen since it is an ester of the sex hormone testosterone, which is found in the plasma of sexually maturing salmonids (Idler et al., 1971). Testosterone is cleared rapidly from the plasma of immature rainbow trout (Schreck, 1972). Administration of esters potentiates the action of the steroid (Kruskemper, 1968).

Rainbow trout were chosen because they were readily available and have been used frequently in thyroidal studies in our laboratory and elsewhere.

LITERATURE SURVEY

A. The Trout Thyroid System

The thyroid gland of the trout is dispersed throughout the basibranchial area of the first three gill arches of the ventral aorta. The major units of the thyroid, the follicles, concentrate inorganic iodide which is used in the synthesis of thyroid hormone. Iodide may be exchanged between the water and the gills (Leloup, 1970) or obtained from the diet via the gut (Gregory and Eales, 1975). In trout plasma, a large percentage of the inorganic iodide is protein bound (Huang and Hickman, 1968).

Iodide is incorporated into the iodoprotein thyroglobulin within the thyroid follicle. Proteolysis of thyroglobulin is followed by the release of the thyroid hormone. Thyroxine (T_4) appears to be the major iodothyronine released by the trout thyroid (Chan and Eales, 1975). T_4 may be converted to 3,5,3'-triiodo-L-thyronine (T_3) in peripheral tissues by the removal of a single phenolic iodine atom (Eales, 1977b). It has been suggested for mammals that many thyroid hormone actions may rely upon the extrathyroidal conversion (deiodination) of T_4 to T_3 (Surks and Oppenheimer, 1977).

Thyroid function appears to be under the control of thyroid-stimulating hormone (TSH) produced by

the pituitary gland. Removal of the pituitary greatly reduces thyroid activity (Ball and Baker, 1969). TSH release may in turn be regulated by a thyroid inhibitory hormone (TIH) produced by the hypothalamus (Peter, 1973). Environmental and internal stimuli may influence the activity of the hypothalamo-pituitary-thyroid axis. T_4 appears to regulate the activity of this axis via a negative feedback mechanism (Sage and Bromage, 1970; Peter, 1973). It is unknown whether T_3 also acts in this fashion in fish.

T_4 and T_3 have rapid turnover rates in the trout (Higgs and Eales, 1977; Eales, 1977a). The majority of plasma T_4 and T_3 is bound to protein while a small proportion exists in the free form (Falkner and Eales, 1973). T_4 and T_3 are lost from the circulation by biliary excretion (Sinclair and Eales, 1972). The hormones enter the gall gladder from the liver unaltered or in a glucuronide conjugate form (Sinclair and Eales, 1972). Feeding causes the discharge of bile into the intestine (Eales and Sinclair, 1974).

Thyroid activity may be altered by water temperature (Eales, 1964; Smith and Eales, 1977), nutritional status (Higgs and Eales, 1977, 1978), stress (Brown, 1977), and seasonal changes (Eales, 1965).

The thyroid appears to participate in fish growth (Higgs et al., 1976, 1977), osmoregulation (Bonnet, 1970), ionic regulation (Pang, 1973), protein metabolism (Narayansingh and Eales, 1975a), purine metabolism (Premdas and Eales, 1976), lipid metabolism (Narayansingh and Eales, 1975b), carbohydrate metabolism (Hochachka, 1962), colour (La Roche et al., 1966), migration (Baggerman, 1960), activity (Hoar et al., 1955), nervous and sensory systems (La Roche et al., 1966), metamorphosis (Eales, 1965) and reproduction (Fontaine and Leloup, 1962; White and Henderson, 1977).

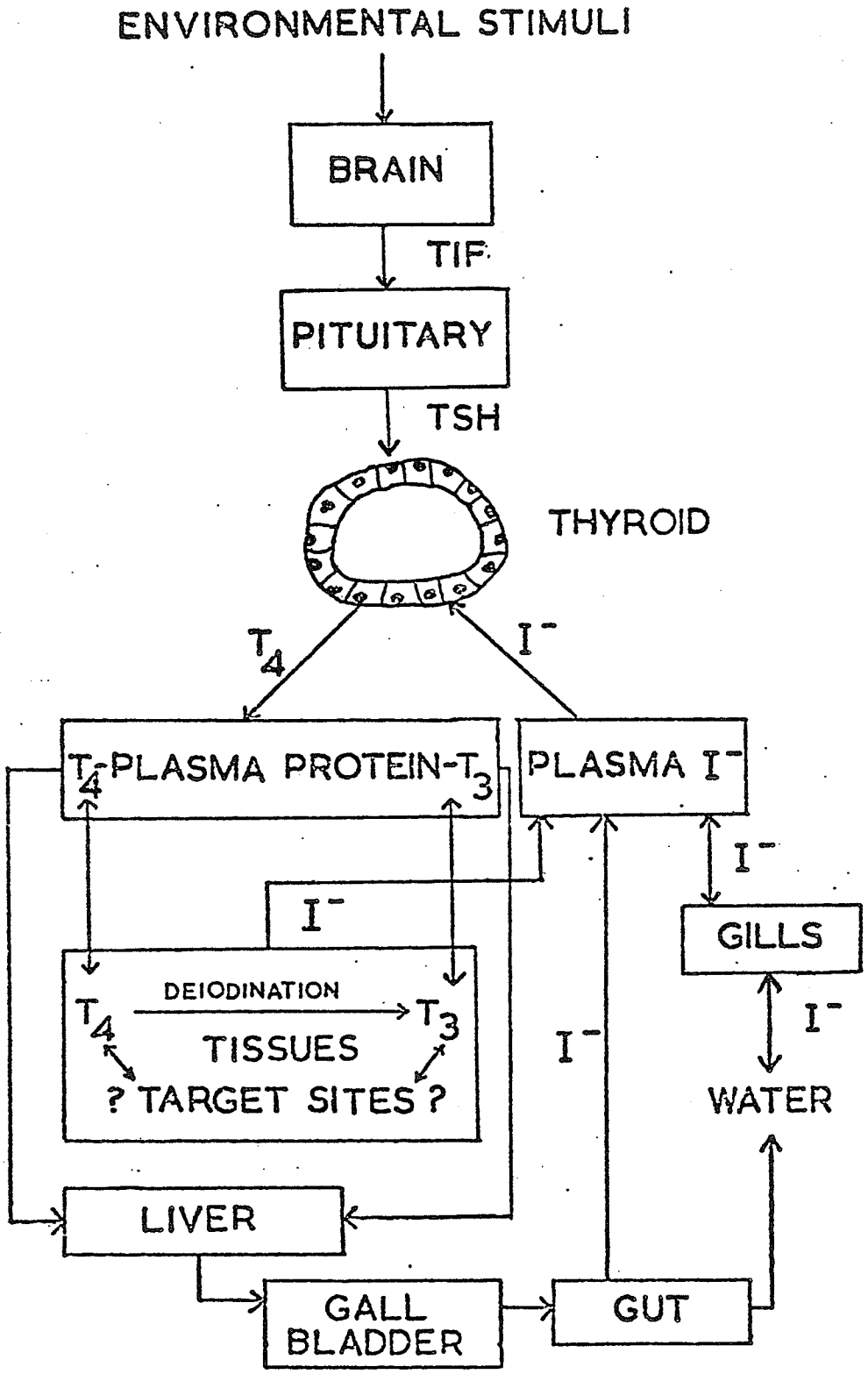
Thyroid hormones may exert their biological effects by interacting with specific 'receptor sites' within the cells of target tissues. However the mode of action of thyroid hormones in fish is not understood. Saturable T_3-binding sites have recently been demonstrated for rainbow trout liver nuclei (Van Der Kraak, personal communication).

A flow diagram describing the trout thyroid system is presented in Figure 1.

B. Influence of Androgens on Teleost Thyroid Function

The influence of male sex steroid hormones (androgens) on thyroid function has received some consideration in teleosts. Matty (1960) showed that the thyroid of Sparisoma squalidum responded

Figure 1. Diagrammatic summary of thyroid hormone and inorganic iodide (I^-) metabolism in the rainbow trout. Arrows represent directional movement of hormone or iodide.


ENVIRONMENTAL STIMULI
BRAIN
TIF
PITUITARY
TSH
THYROID
T_4
I^-
T_4-PLASMA PROTEIN-T_3
PLASMA I^-
I^-
I^-
GILLS
DEIODINATION
T_4
T_3
TISSUES
? TARGET SITES ?
I^-
I^-
WATER
LIVER
GALL BLADDER
GUT

histologically to a single injection of 500 ug testosterone phenylacetate or tri-weekly injections of 1 mg methyltestosterone. Within 14 days following the start of treatment the thyroid appeared completely hypertrophied. Matty showed that this was a direct effect of androgen upon the thyroid, and not a result of stimulation of thyroid stimulating hormone (TSH)-producing cells of the pituitary.

Singh (1968) demonstrated that large doses of methyltestosterone (150-200 ug/fish) increased thyroid $^{131}I^-$ uptake in the freshwater catfish, Mystus vittatus without increasing the thyroid-stimulating potency of the pituitary gland.

Thyroidal radioiodine uptake was significantly elevated 24 hr following $Na^{131}I$ injection in hypophysectomized Mystus vittatus treated for 3 weeks with testosterone propionate (Singh, 1969). Sage and Bromage (1970) showed that a decrease in dry weight per unit area of thyroid colloid occurred in Poecilia reticulata following 7, 14, or 21 days of methyltestosterone treatment. This indicated a stimulation of the thyroid. No alterations in pituitary TSH cells were noted in fish treated for 7 days with the androgen.

van Overbeeke and McBride (1971) described a pronounced histological activation of the small thyroid follicles in gonadectomized sockeye salmon

(Oncorhynchus nerka) injected with 2.5 mg 11 - ketotestosterone or methyltestosterone twice weekly for 4 or 7 weeks.

Higgs et al. (1977) found increased epithelial cell heights in the thyroids of yearling coho salmon (Oncorhynchus kisutch) which had received methyltestosterone in the diet for 59 days. This effect was enhanced when the fish received a weekly injection of bovine growth hormone in addition to methyltestosterone.

Matty et al. (1958) demonstrated that an exophthalmic condition was produced in Sparisoma squalidum and Scarus croicensis after treatment with methyltestosterone or testosterone phenylacetate. Exophthalmia is classically associated with hyperthyroidism in mammals. This condition can be created in some fish by administration of T_4 or T_3 (Langford, 1957). It is suggested that if a thyroidal product elicits exophthalmia in fish the androgen effect described above may be due to thyroid stimulation.

Since androgen-induced alterations in thyroid activity have been shown to exist in teleost fish, it was felt that a more detailed study of this effect would be of some value. It is of particular interest to observe changes in the peripheral metabolism of T_4 and its possible conversion to T_3, aspects not previously considered. The formation of T_3 may be especially

important should it prove to be the active form of thyroid hormone at the cellular level.

MATERIALS AND METHODS

A. Fish Maintenance

One and two-year old sexually immature rainbow trout, Salmo gairdneri Richardson, obtained from the Federal Fish Hatchery, Balmoral, Manitoba and from the Provincial Trout Hatchery, West Hawk Lake, Manitoba were held in 2.3-k litre fiberglass tanks with flowing, aerated, dechlorinated Winnipeg City water containing 1.85 ug I/litre. Stock fish were fed a diet of one to 2.0% (percentage wet bodyweight per day) Ewos trout pellets (Astra Chemicals Ltd., Mississauga, Ontario). Iodine content of the food was 0.52 ug I/g dry weight.

Experimental fish were held in 125-litre fiberglass tanks in a controlled environment room. Tanks were covered with translucent plastic and the photoperiod was adjusted to 12 hours light and 12 hours darkness (light 0800-2000).

Aeration and water flow were standardized between tanks. Water temperature during experiments varied from 11.0 - 12.2^{o}C. Holding and feeding regimes

will be described in the protocol for individual experiments.

Immature fish were used in all experiments. Schreck et al. (1972) showed that immature rainbow trout have low plasma androgen levels. Plasma androgen levels appear to be positively correlated with gonadal development (Schreck et al., 1972). Fish with ripening gonads were omitted from analysis.

B. Injections

Fish were anaesthetized for approximately two minutes by immersion in tricaine methane sulfonate (MS 222, Kent Laboratories, Vancouver; 0.075 g/litre) before all injections. Testosterone propionate (Sigma) was dissolved in peanut oil and a volume of 20 ul was injected intramuscularly from a 1-ml tuberculin syringe (needle size 25 G, 1") adapted to a repeating dispenser (PB-600-1, Hamilton Company, Reno, Nevada). Control fish received 20 ul of peanut oil. Injection was into the dorsal musculature with the needle pointed anteriorally, parallel to the lateral line, midway below the dorsal fin. To minimize leakage the needle was slowly removed and the injection site gently massaged. Injections alternated between the left and right side of the fish in long-term experiments.

Radiothyroxine (^{125}I-T_4, Industrial Nuclear Co., St. Louis; Specific Activity 750 mCi/mg) dissolved in 20 ul propylene glycol was injected into the heart of anaesthetized trout from a 1-ml tuberculin syringe (needle size 30 G, 1/2") adapted to a repeating dispenser. Injection was at a point opposite the origin of the pectoral fins along the midventral line, with the needle pointed straight downward. $^{125}I^-$ contamination of ^{125}I-T_4 was found to be 2.2% at the time of injection.

Carrier free $Na^{131}I$ obtained from the Atomic Energy Commission, Commercial Products, Ottawa was diluted with distilled water and 20 ul was injected intraperitoneally from a 1-ml tuberculin repeating syringe (needle size 30 G, 1/2"). Site of injection was just anterior to the pelvic fins.

Standards were made by ejecting 20 ul of the ^{125}I-T_4 or $Na^{131}I$ injection solutions in quadruplicate into volumetric flasks which were then made up to 100 ml with 0.1 N NaOH. One ml was made up to the standard counting volume with NaOH. Standards were included in each ^{131}I or ^{125}I isotope counting run. The injected dose was obtained by multiplying standard cpm by 100.

c. Plasma, Tissue, and Organ Sampling

Fish were netted three or four at a time from their tanks and placed into isothermal, aerated,

20-litre covered tanks of water. Individual fish were anaesthetized by immersion in MS 222, blotted dry with a paper towel, and weighed to the nearest 0.1 g. Blood was taken from the caudal vessels with a preheparinzed 3-ml tuberculin syringe (20 G, 1" needle). Samples were expelled into 1.5-ml plastic centrifuge tubes and held on ice until centrifuged at 15000 g for three minutes (International Centrifuge model MB). The plasma was removed with a Pasteur pipet and refrigerated at $-20^{o}C$ in 2-ml plastic beakers covered with Parafilm. Plasma samples were analyzed within two weeks of sampling. Fish were killed by concussion, placed in individual plastic bags and frozen for future analysis.

The liver, gall bladder, and intestine including pyloric caecae and contents were removed from the frozen carcass and placed into individual counting tubes. Large organs were subdivided between one or more tubes. Livers were weighed to the nearest 0.001 g before counting. The volume of these tubes was adjusted to 4 ml with 0.1 N NaOH. Tissues were allowed to partly digest before determining total ^{125}I-radioactivity. At the time of analysis, ^{131}I was undetectable in these organs. Organ radioactivity was expressed as a percentage of the injected dose ^{125}I-T_4.

A piece of muscle was removed from the carcass and the skin peeled away. Muscle was weighed to the nearest 0.001 g, placed in a counting tube and the volume made up to 4 ml with 0.1 N NaOH. Samples were allowed to partly digest and then counted for total ^{125}I-radioactivity. Radioactivity was calculated as percent of the injected dose ^{125}I-T_4/g muscle/100 g body weight. The lower jaw was removed from the carcass and the first three ventral branchial arches containing the thyroid tissue were dissected out. Thyroids were placed in counting tubes and the volume was made up to 4 ml with 0.1 N NaOH. Thyroid uptake was expressed as percent of the injected dose (^{125}I-T_4 or $Na^{131}I$).

D. Plasma Protein Analysis

Total plasma protein was measured by the Biuret method. Standards were made by dissolving one gram bovine serum albumin in 100 ml 0.7% NaCl. From this a series of 0.1 to 1.0 ml was taken and made up to 1 ml with 0.3 N KOH in plastic tubes. Plasma proteins of samples were denatured by adding 0.9 ml 0.3 N KOH to 0.1 ml plasma. Three ml of Biuret reagent was added to all tubes and allowed to incubate at room temperature. After 30 minutes the optical density was measured at 540 nm using a Bausch and Lomb 'Spectronic 20' and protein concentrations of samples

were determined from the standard curve and expressed in grams per 100 ml.

E. T_4 and T_3 Analysis

Plasma T_4 and T_3 were measured by radioimmunoassay (RIA) (Brown and Eales, 1977). Standard stock solutions of T_4 and T_3 were made by dissolving the anhydrous sodium salt of T_4 (Eltroxin, sodium L-thyroxine pentahydrate) or T_3 (Sigma) in 0.1 N NaOH. Working standards of 0-800 ng % were prepared by further dilution with 0.1 N NaOH.

Lyophylized rabbit serum antiserum to T_3 human serum albumin (T_3 antibody) or to T_4 human serum albumin (T_4 antibody) were obtained from K & T Biological Services., Edmonton, Alberta. The suppliers specify that the T_4 and T_3 antibodies exhibit low cross reactivity with each other and other iodothyronines.

^{125}I-T_3 (T_3*) and ^{125}I-T_4 (T_4*) phenolically labeled and with initial specific activities of approximately 500 and 750 mCi/mg respectively were purchased from Industrial Nuclear, St. Louis, Missouri, in 50% propylene glycol. Stock solutions were made by diluting the T_3* or T_4* with 0.1 N NaOH such that 0.1 ml generated 3500-5000 cpm in a gamma well detector of about 50% efficiency.

The assay was carried out on miniature columns (5-ml syringe barrel) prepared from 0.45 g of G-25 (fine) Sephadex.

Barbital buffer (pH 8.6; 75 mmol/l) was prepared by dissolving 15.6 g sodium barbital in 900 ml distilled, deionized water. The pH was then adjusted to 8.6 with 6 N HCl (2.0-2.5 ml) and diluted to 1 litre (Brown, 1977).

Columns and reagents were allowed to come to room temperature before use. The columns were drained and the bottoms capped, before addition of 0.1 ml T_4 or T_3 standard or 0.1 ml of plasma. Plasma samples were run at least in duplicate. Then 0.1 ml of T_3* or T_4* was added to each column. Also 0.1 ml of T_4* or T_3* was pipetted in duplicate into counting tubes. Barbital buffer (3.9 ml, T_4 RIA or 2.9 ml, T_3 RIA) was added to each tube. These were capped and set aside as the total counts reference (TCR).

Each column was swirled and allowed to drain to waste. When plasma is applied to Sephadex columns, equilibrated with 0.1 N NaOH, T_4 and T_3 are released from their binding proteins and are absorbed into the Sephadex (Brown, 1977). After tracer and standards or plasma have completely entered each column, 3 ml (T_3 RIA) or 4 ml (T_4 RIA) barbital buffer was added to each column and the eluate allowed to run to waste. Collection of the eluate from any two columns which make up the standard curve will permit determination of the radioiodide contamination of the T_4* or T_3* used. Subtraction of cpm iodide from cpm TCR will

give the actual amount of hormonal radioactivity introduced into the column.

Once the buffer had drained through the column, each column was positioned over an empty counting tube and 1 ml of T_4- or T_3-antibody reagent was added in sequence to each column. The raw (undiluted) T_4 antiserum was diluted 1:7000 and the raw T_3 antiserum was diluted 1:22000 with barbital buffer. The columns were covered and allowed to equilibrate. After 90 minutes 3 ml (T_4 RIA) or 2 ml (T_3 RIA) of barbital buffer was added to each column in the same sequence as the antibody addition. All bound radioactivity is removed with the first 2 ml of buffer (Brown and Eales, 1977). T_4 and T_3 standard curves were formed by plotting antibody-bound cpm y axis versus hormone concentration x axis. Plasma hormone concentrations were determined by interpolation.

Columns were regenerated by washing with 5 ml distilled,deionized water followed by 1 ml of human plasma diluted 1:20 with barbital buffer. An additional 5 ml of deionized water was added before equilibrating columns with 8 ml of 0.1 N NaOH.

F. Separation of Plasma Radio-labeled Materials

Plasma samples were analyzed using a modification of a method developed by Eales (1977a). Separation of plasma radioactive materials was performed on miniature G-25 Sephadex columns previously equilibrated

at pH 13 with 0.1 N NaOH. Column preparation was identical to RIA method described previously.

Plasma (0.2 or 0.3 ml) was added to 0.1 ml 0.1 N NaOH on the top of the column with the bottom capped. Plasma and NaOH were mixed by gentle swirling and allowed to drain into the column. The eluate was collected in counting tubes. At pH 13 T_4 and T_3 dissociate from plasma proteins and bind to the Sephadex (Brown and Eales, 1977). The column was eluted with 2.8 ml barbital buffer (pH 8.6). T_3* (^{131}I-T_3 and ^{125}I-T_3) and T_4* (^{131}I-T_4 and ^{125}I-T_4 remain bound to the Sephadex but proteins and iodide are removed. One ml of buffer was added to each counting tube to make the volume up to a standard counting volume of 4 ml. This fraction contained inorganic iodide ($^{125}I^-$ and $^{131}I^-$). Tubes were capped and put aside for counting.

A second empty counting tube was positioned under each column. A volume of 0.5 ml of buffer containing rabbit antibody to T_3-human serum albumin was added onto the column and allowed to equilibrate for 30 minutes before elution with 3.5 ml of buffer. Each 0.5 ml of antibody-buffer solution represented 1×10^{-3} the quantity present in one ml of rabbit serum containing antibodies to T_3-human serum albumin.

The radioactivity still bound to the Sephadex was almost entirely T_4*.

This was eluted from the column by addition of 4 ml of buffer containing human plasma (1:10 dilution). The eluate was caught in counting tubes positioned under each column. The columns were regenerated by washing with 4 ml distilled deionized water followed by 12 ml 0.1 N NaOH.

To check the recovery of T_3*, extraction efficiencies were determined for a series of stable T_3 concentrations (0-800 ng %) to which ^{125}I-T_3 had been added. Duplicates were run for each hormone concentration.

The extraction efficiency of T_3-antibody at the dilution used was 97.2 ($\pm$ 0.8) % over a range of 0-800 ng % added T_3 (Appendix Table 1). A one half-hour incubation period was required for the removal of the majority of ^{125}I-T_3 from the column.

G. Plasma Protein Binding of T_4 and T_3

Relative T_4 and T_3 binding capacities of various plasma samples were determined by the ability of trout plasma proteins to displace a known amount of ^{125}I-labeled hormone from an equilibriated Sephadex column. This procedure was carried out on the same columns used for RIA.

Phosphate buffer (pH 7.4, 0.5 M) was made by dissolving 68.04 g KH_2PO_4 in one liter of distilled deionized water (Solution A) and 70.99 g Na_2HPO_4 in a separate liter of water (Solution B). The buffer

was obtained by combining 17.6 parts of Solution A with 60.8 parts of Solution B.

Columns were equilibriated with 0.1 N NaOH (pH 13), drained and the bottom capped. ^{125}I-T_4 (T_4^*) or ^{125}I-T_3 (T_3^*) ($\sim$ 10000 cpm) diluted with 0.1 ml 0.1 N NaOH was pipetted on to the column. Also 0.1 ml of T_4^* or T_3^* in duplicate was pipetted into counting tubes and 3.9 ml (T_4-binding) or 2.9 ml (T_3-binding) of phosphate buffer (pH 7.4) was added to each tube. These were capped and set aside as the TCR. The column was placed over a counting tube and allowed to drain. After 10 minutes 4 ml (T_4-binding) or 3 ml (T_3-binding) buffer was added to each column and the eluate collected in the counting tubes. The buffer removes inorganic $^{125}I^-$ present in the tracer from the column. Tubes were capped and set aside for counting after the columns had drained. The amount of radioactivity in the iodide fraction is subtracted from the TCR to obtain the actual quantity of T_4^* or T_3^* (cpm) added to each column.

The columns were again capped and 0.1 ml plasma diluted with 0.9 ml phosphate buffer was added. Columns were swirled and allowed to drain into an empty counting tube. After 15 minutes an additional 3 ml (T_4-binding) or 2 ml (T_3-binding) buffer was

added on to the column. The eluate was collected in the counting tubes. Radioactivity in these tubes constituted T_4^* of T_3^* bound by plasma protein and was subsequently counted. The unbound or free portion (T_4^* or T_3^* remaining on the column) was determined by subtracting the bound fraction from the corrected TCR. An index of relative plasma protein binding of T_4^* or T_3^* was obtained by dividing bound by free radioactivity.

$$\text{Plasma Protein Binding} = \frac{\text{Bound Hormone}}{\text{Free Hormone}} = B/F = \frac{\text{Radioactivity Bound (cpm)}}{\text{Radioactivity Retained on Column (cpm)}}$$

H. Iodide Determinations

The Hycel Cuvette Protein Bound Iodine method (Hycel Inc., Houston, Texas) was used to determine total plasma iodide concentrations. Iodide content of food and water was also measured.

Briefly, 0.1 ml of standard or 0.1 ml of plasma samples were pipetted into individual cuvettes. The standards represented concentrations of 0, 5, 10, 15 and 20 ug I/100 ml, prepared as Kl in water. Food was dried to a constant weight (105°C for 24 hours), ground into a powder using a mortar and pestle and a weighed amount added to a cuvette (Gregory and

Eales, 1975). For water analysis, one liter of water was boiled to 15 to 20 ml and this was centrifuged (Sorval GLG-1) for 20 minutes at 3000 rpm, (Gregory and Eales, 1975). One tenth of a ml of the supernatant was analyzed.

Two ml of digestive reagent (0.025% vanadic acid in 72% perchloric acid) were added to each of the cuvettes. Cuvettes were shaken using a Vortex Mixer and then placed at 10 second intervals in the Hycel Heating Block (230^{o}C) under a fume hood. They were left for 12 minutes, removed at 10 second intervals and left 15 to 20 minutes to cool.

Two ml of ceric reagent (0.6% ceric ammonium sulphate in 27% sulphuric acid) were added to each of the cuvettes after cooling. They were immediately vortexed and then allowed to stand for 15 to 20 min at room temperature.

After cooling, 2 ml of arsenious reagent (0.9% arsenic trioxide in 8.2% sulphuric acid) were added to the cuvettes at 30 second intervals. Cuvettes were vortexed and placed in a water bath (37^{o}C) for 20 min. At 30 second intervals they were removed from the water bath, the external surface dried and the optical density at 420 nm measured using a Bausch and Lomb 'Spectronic 20' spectrophotometer. Iodide concentration x axis was plotted against

optical density y axis. Unknown values were determined from the standard curve by interpolation.

I. Thyroid Histology

The trout thyroid is dispersed throughout the region of the ventral aorta and dissection of a thyroid "gland" is impossible. To prepare thyroid tissues for histological examination, the lower jaw was removed and the first three gill arches along the ventral aorta were dissected out. These were rinsed with fish saline (Huang and Hickman, 1968) and then placed in a glutaraldehyde fixative (Karnovsky, 1968) for 4 hr with shaking. After two separate 2 hr immersions in cacodylate buffer the tissue was decalcified in a nitric acid-ethanol solution for 24 hr. Tissues were then treated in a 4% $NaSO_4$ solution for 3 hr before rinsing overnight in running tap water. Tissues were then routinely processed and embedded in Paraplast (mp 56°C). A transverse series of 6 µM sections was prepared and stained with haematoxylin and eosin (Humason, 1972).

An index of thyroid activity was obtained for each fish by measuring the epithelial cell height at four separate locations in six randomly selected follicles.

J. Iodide Excretion Rate (IER)

To determine iodide excretion rates, estimates of the $^{131}I^-$ distribution space (DS), $^{131}I^-$ fractional

turnover (kl), and plasma $^{127}I^-$ concentration must be obtained for each group. DS is the space in which $^{131}I^-$ would be found if it was distributed at a concentration equal to that of plasma. DS (ml/100g bodyweight) was calculated as

$$\frac{100}{\text{plasma } ^{131}I \text{ concentration extrapolated to zero time.}}$$

The $^{131}I^-$ fractional turnover rate (kl) is the fraction/hr of iodide within the DS which is excreted and replaced per unit time. It is calculated as the slope of the regression relating the logarithm of plasma $^{131}I^-$ loss with time (hr). Plasma $^{127}I^-$ (ug/ml) concentrations were measured by the Hycel Curvette Protein Bound Iodine Method (Materials and Methods, Section H). IER (ug I/day/100g) is the product of DS, kl, and $^{127}I^-$. This is the amount of iodide excreted per unit time for a body weight of 100g.

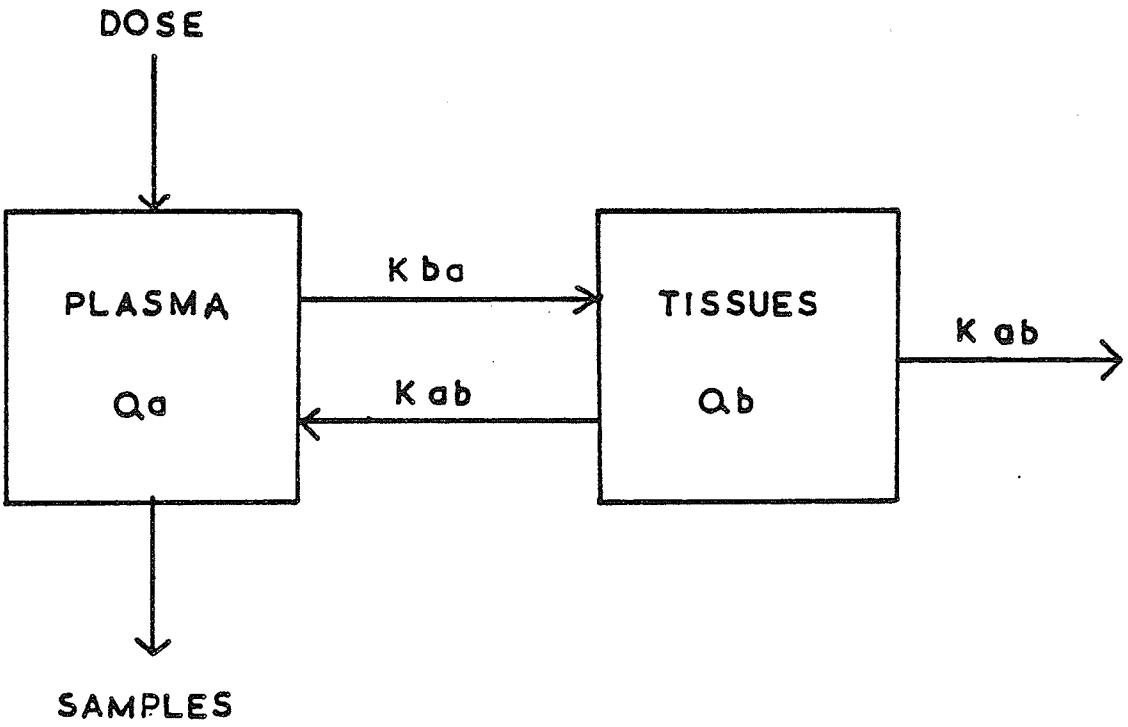
K. Kinetic Analysis of ^{125}I-T_4

Plasma ^{125}I-T_4 was expressed as % injected dose/ml plasma/100 g body weight. Geometric means and 95% confidence intervals were obtained for each sampling time and plotted on semi-logarithmic paper. The multiexponential curve describing ^{125}I-T_4 concentration with time was subjected to compartmental analysis, as

previously described for trout by Eales (1977a). The multiexponential curve for ^{125}I-T_4 loss from plasma consisted of a "fast" and a "slow" component and was used as the basis of a two-compartmental model (Shipley and Clark, 1972). The loss of ^{125}I-T_4 from 48 - 96 hr pi appeared linear and comprised the "slow" component. The equation of the line through these points was determined and extrapolated to time 0. The 0.5, 2, 6, 12, and 24 hr theoretical values were calculated from this line and subtracted from the corresponding individual points on the initial portion of the curve. The obtained "peeled" values were plotted and the equation of the new line calculated. This line represented the "fast" component. The two components are represented in the following equation: $C = Ae^{-\alpha t} + Be^{-\beta t}$, where C represents the percentage of the injected ^{125}I-T_4 dose in 1 ml plasma at time t. A and B represent the y intercepts of the "fast" and "slow" components, and α and β represent, respectively, the slopes of the "fast" and "slow" components.

On the basis of the linear appearance of the "fast" and "slow" components, the data were assumed to conform to a two-compartment model. Compartment "a" includes the plasma into which the ^{125}I-T_4 was injected and from which the samples were drawn. Compartment "b" is the extravascular T_4 pool. The model assumes there is exchange of T_4 between α and b and that T_4 is eventually lost from compartment b via

Figure 2. Two-compartment model proposed for $^{125}I\text{-}T_4$ kinetics in rainbow trout. Compartment *Qa* represents the plasma compartment into which $^{125}I\text{-}T_4$ was injected, and from which samples were withdrawn for analysis. Compartment *Qb* represents the extravascular (tissue) compartment from which $^{125}I\text{-}T_4$ was lost by degradation and excretion. *Kba* and *Kab* represent fractional transfer rates between *Qa* and *Qb*. *Kob* represents the fractional transfer rate for the irreversible loss of $^{125}I\text{-}T_4$ from *Qb*.


DOSE
PLASMA
Qa
K ba
K ab
TISSUES
Qb
K ab
SAMPLES

degradation and excretion pathways (Figure 1). Fractional transfer rates (*Kba*, *Kab*), pool sizes (*Qa*, *Qb*), flow rates (*Fba*, *Fab*) and T_4 degradation rates were calculated, as below, according to the procedure of Shipley and Clark (1972).

The normalized equation describing ^{125}I-T_4 loss with time was

$$\frac{qa}{qo} = H_1 e^{-g1t} + H_2 e^{-g2t}$$

where qa/qo = fraction of the injected dose in compartment a at time t; H_1 = A/A + B; H_2 = B/A + B; $g_1 = \alpha$; $g_2 = \beta$.

The following relationships then hold,

$$Kaa = H_1 g_1 + H_2 g_2$$
$$Kaa + Kbb = g_1 + g_2$$
$$Kaa \times Kbb - Kab \cdot Kba = g_1 g_2$$
$$Kob = Kbb - Kab$$

where (1) *Kaa* and *Kbb* are the overall turnover rates for pools a and b, respectively, and (2) *Kba*= *Kaa* since *Kba* is the sole exit route from compartment a (Shipley and Clark, 1972). *Kaa*, *Kbb*, *Kab*, *Kba*, and *Kob* were calculated as fractional change per hour from the above equations.

The volume of distribution of T_4 in compartment a (Va) was determined as 100/A + B. The T_4 pool in compartment a (Qa) was equal to Va x h ng, where h = plasma T_4 concentration (ng/ml). The pool of T_4 in compartment b was determined from the following equation:

$$Qb = \frac{Qa \times Kba}{Kbb}$$

Flow rates Fba (Qa x Kba), Fab (Qb x Kab), and Fob (Qb x Kob) were then calculated. Flow rates were expressed as nanograms of T_4 per hour for a 100-g fish.

L. T_4 Deiodination

Deiodination rates were estimated by comparing $^{131}I^-$ loss from plasma with the plasma appearance and subsequent loss of $^{125}I^-$ produced from ^{125}I-T_4 (Eales, 1977b). $^{131}I^-$ and $^{125}I^-$ concentrations were expressed as percent of the injected dose of $Na^{131}I$ or ^{125}I-T_4/ml plasma/100 g body weight. Geometric means and 95% confidence intervals were obtained and plotted on semi-logarthmic paper. Plasma $^{125}I^-$ levels were corrected for $^{125}I^-$ contamination of the injected ^{125}I-T_4 dose.

Plasma radioiodide levels decrease in an exponential manner (k1) following radioiodine injection, implying single compartment kinetics (Higgs and Eales, 1977). Plasma $^{125}I^-$ levels increase until 12 hr and then

decrease (k2) exponentially following intracardiac ^{125}I-T_4 injection in rainbow trout (Eales, 1977b). Regression lines were fitted for both isotopes between 12 and 96 hr pi. Lines were extrapolated to the *y* axis and the intercepts (Y125) and (Y131) were obtained.

The intercept Y125 depends on the extent of $^{125}I^-$ addition to plasma due to ^{125}I-T_4 monodeiodination (Eales, 1977b). This intercept must undergo correction to allow for plasma $^{125}I^-$uptake. Values for 0.5, 2, and 6 hr were taken from the extrapolated portion of the $^{125}I^-$ disappearance curve. The mean ($\bar{x}$) values of the corresponding points on the uptake portion of the curve are subtracted. A rate constant (k3) representing $^{125}I^-$ addition to plasma is obtained. The Y125 intercept is calculated as the following:

$$Y125 = \frac{k3 - k2}{k3} \times Y125 \text{ (from regression)}$$

The fraction of the Y131 intercept that is represented by the Y125 intercept indicates the fraction of the $^{125}I^-$ present in the injected ^{125}I-T_4 dose that has been removed by deiodination. This relationship gives an index of deiodination.

At the specific activity of the ^{125}I-T_4 employed,

a given ^{125}I-T_4 molecule will have only one of its two phenolic ^{127}I atoms replaced by ^{125}I (Braverman et al., 1970). Two labeled T_4 molecules must be deiodinated to produce one labeled iodide ion and one labeled T_3 molecule. Thus T_4 to T_3 conversion is underestimated by 50% by radioiodide kinetics. Doubling the index of deiodination gives the actual proportion of ^{125}I-T_4 undergoing monodeiodination. Multiplying the index of deiodination by T_4DR gives the T_4 deiodination rate in ng/hr.

Analysis of covariance was used to compare plasma $^{131}I^-$ and $^{125}I^-$ loss in control and TP-injected trout.

M. Enterohepatic Organs

Loss of ^{125}I-radioactivity from the intestine and the enterohepatic organs (intestine plus gall bladder) approximated a linear relationship between 12 and 96 hr pi. Analysis of covariance was used to compare rates of radioactive loss (kl) from these organs between groups. The regression lines were extrapolated to the y axis. The obtained intercepts underwent correction, to allow for the influence of radioactive uptake on radioactive loss. This was determined by obtaining 0.5, 2, and 6 hr values from the extrapolated regression lines and subtracting the mean ($\bar{x}$) percentage dose values of the corresponding points on the uptake portion of the

curve. A line (k2) representing the rate of radioactive uptake was obtained. The percentage of the injected dose of ^{125}I-T_4 in the intestine or in the enterohepatic organs, that would be present at time zero was then calculated as follows:

$$\% \text{ Dose } {}^{125}I\text{-}T_4 \text{ at time } 0 = \frac{k_2 - k_1}{k_2} \times \text{Y intercept of k1.}$$

N. Liver and Muscle

The amount of ^{125}I-radioactivity taken up by the liver at 0.5 hr pi was compared. No analysis of the rate ^{125}I-radioactive loss was made for this organ.

Loss of ^{125}I-labeled radioactivity from muscle was compared by analysis of covariance. Covariance analysis was also used to compare the rate of ^{125}I-radioactive loss from muscle 24 - 96 hr pi with the disappearance of $^{125}I^-$ from plasma. Since the majority of ^{125}I-labeled hormone (T_3 and T_4) is removed from plasma 24 hr following ^{125}I-T_4 injection, it was felt that the remaining ^{125}I-label in muscle 24 - 96 hr pi was inorganic iodide.

O. Thyroid $^{125}I^-$ and $^{131}I^-$ Uptake

Rates of thyroid $^{131}I^-$ and $^{125}I^-$ uptake were obtained by plotting percent of the injected dose versus time.

This approximated a linear relationship to which regression analysis could be applied. Rates of thyroid $^{125}I^-$ uptake were determined from 24 - 96 hr pi. Analysis of covariance was used to compare the slopes of the regressions for thyroid $^{125}I^-$ and $^{131}I^-$ uptake within and between groups.

$^{125}I^-$ derived from monodeiodination of ^{125}I-T_4 is underestimated by 50% since two ^{125}I-T_4 molecules must be deiodinated to create one ^{125}I ion and one ^{127}I ion. Percent dose values for thyroid $^{125}I^-$ uptake were doubled to account for the uptake of unlabeled iodide derived from ^{125}I-T_4. The ratio of the percent of the injected doses of $^{125}I^-$ and $^{131}I^-$ in the thyroid between 24 and 96 hr pi was used as an index of ^{125}I-T_4 deiodination. The thyroid $^{125}I/^{131}I$ ratio was determined for all trout sampled between 24 and 96 hr pi.

P. Radiation Counting

Plasma, organ, and tissue samples were counted twice for 10 minutes or 10000 cpm in a Nuclear Chicago Automatic Gamma System containing a 2 in (DS 202) NaI crystal. Discriminator settings were optimized for ^{125}I or ^{131}I. When samples contained ^{125}I- and ^{131}I- radioactivity, appropriate corrections were made for "crosstalk" between channels.

Q. Statistical Analysis

Wherever two means were compared by *t*-tests, two-tailed F-tests were used to test for homogeneity of variances. If more than two means were considered, Bartlett's X^2 test was used to test homogeneity of variance. One-way analysis of variance (ANOVA) was used to show if means differed between groups. If the *F*-test from the ANOVA method proved significant, Student-Newman-Keuls test was used to locate those means which differed.

Analysis of covariance was used to compare rates of radioactive uptake or loss from plasma, tissue, and organs. These statistical techniques are outlined in Snedecor and Cochran (1971) and Sokal and Rohlf (1969).

EXPERIMENTAL PROTOCOL

1. Effect of different TP doses on plasma T_3, T_4 and thyroid histology

Rainbow trout (Idaho stock; initial mean weight 54.2 g, SEM 1.11 g) were randomly divided into four groups of 12 fish. The trout were acclimated to their new tanks for 2 weeks before the start of injections. On the first day of the experiment, fish were injected with 3, 30, or 300 ug TP. Control fish received the peanut oil vehicle only. The fish were injected once every fourth day for a total of 5 injections. Fish were fed a 2% ration throughout

the acclimation and experimental periods. On the fourth day following the last injection the fish were killed, bled and the thyroid tissue removed.

2. Effect of different TP doses on plasma T_3, T_4, T_3 (B/F), T_4 (B/F), and protein

Rainbow trout (Montana stock; initial mean weight 93.5 g, SEM 1.6 g) were randomly divided into four groups of 10 - 11 fish. The experimental procedure followed was identical to that of experiment 1, except that plasma T_3 (B/F), T_4 (B/F), and protein were measured. Thyroid tissue was not examined in this experiment.

3. 24-hr effect of a single TP injection (30 ug) on plasma T_3, T_4, T_3 (B/F), T_4 (B/F), and protein

Twenty-nine trout (Montana stock; initial mean weight 105.4 g, SEM 1.7 g) were randomly divided into two groups. After a two-week acclimation period, one group received a single injection of 30 ug TP. The other group received the peanut oil vehicle only. Fish were fed a 2% ration throughout the acclimation period and once again four hr after injection. Twenty-four hr after injection, fish were bled and the plasma obtained for T_4, T_3, T_4 (B/F), T_3 (B/F), and protein analysis.

4. Effect of 30 ug TP on radiothyroxine and radioiodide metabolism

Rainbow trout (Montana stock; initial mean weight 115.3 g, SEM 1.9 g) were randomly divided into two groups of 92 fish. Fish were fed a 1% ration throughout the acclimation and experimental periods. Three weeks were allowed for acclimation before injections began.

On the first day of the experiment the trout received an injection of 30 ug TP or the peanut oil vehicle only. Injections were given every 4 days for a total of 5 injections. Four days following the final injection, fish were injected with 1 *uCi* ^{125}I-T_4 and 0.05 *uCi* $Na^{131}I$.

Sub-groups of 8 - 10 trout were anaesthetized at 0.5, 2, 6, 12, 24, 48, 72 and 96 hr post-injection (pi), bled and the plasma and carcass set aside for later analysis. Fourteen control and fourteen TP-injected trout held under identical conditions as those injected with ^{125}I-T_4 and $Na^{131}I$ were bled at times corresponding to one-half hr before and 7 hr following radioisotope injections.

RESULTS

1. Effect of different TP doses on plasma T_3, T_4, and thyroid histology

Figure 3 shows plasma T_3 and T_4 levels and thyroid

epithelial cell heights in control and TP-injected trout. No difference could be shown in plasma T_4 concentrations between control fish and fish injected with 30 ug TP, but T_4 was elevated significantly above control values in the fish injected with 3 and 300 ug TP. Plasma T_3 levels were significantly elevated in the fish injected with 300 ug TP. One-way ANOVA (Appendix Table 3) showed that the initial and final mean body weights did not differ significantly. One fish died during the experiment but all remaining fish gained weight and appeared healthy.

Results of one-way ANOVA for body weights, thyroid epithelial cell heights and plasma T_4 and T_3 are presented in Appendix Table 3.

Thyroid epithelial cell heights were significantly ($p < 0.01$) greater in trout injected with 300 ug TP. Although epithelial cell heights were not shown to differ, the thyroid colloid of trout injected with 3 and 30 ug TP contained more resorption vacuoles than that of control trout, indicating greater thyroid activity in these fish (Figures 4a, b, c). The colloid of thyroid follicles in trout injected with 300 ug TP appeared well vacuolated (Figure 4d). The major effects of TP appeared to be localized in the smaller thyroid follicles.

Figure 3. Thyroid epithelial cell heights and plasma T_4 and T_3 levels in control trout and trout injected with 3, 30, and 300 ug TP. Ranked means ($\bar{x}$) with 95% confidence intervals for 9-11 fish are presented. Horizontal bars join similar ($p < 0.05$) means determined from Student-Newman-Keuls test. Original and final fish body weights are given in Appendix Table 2.

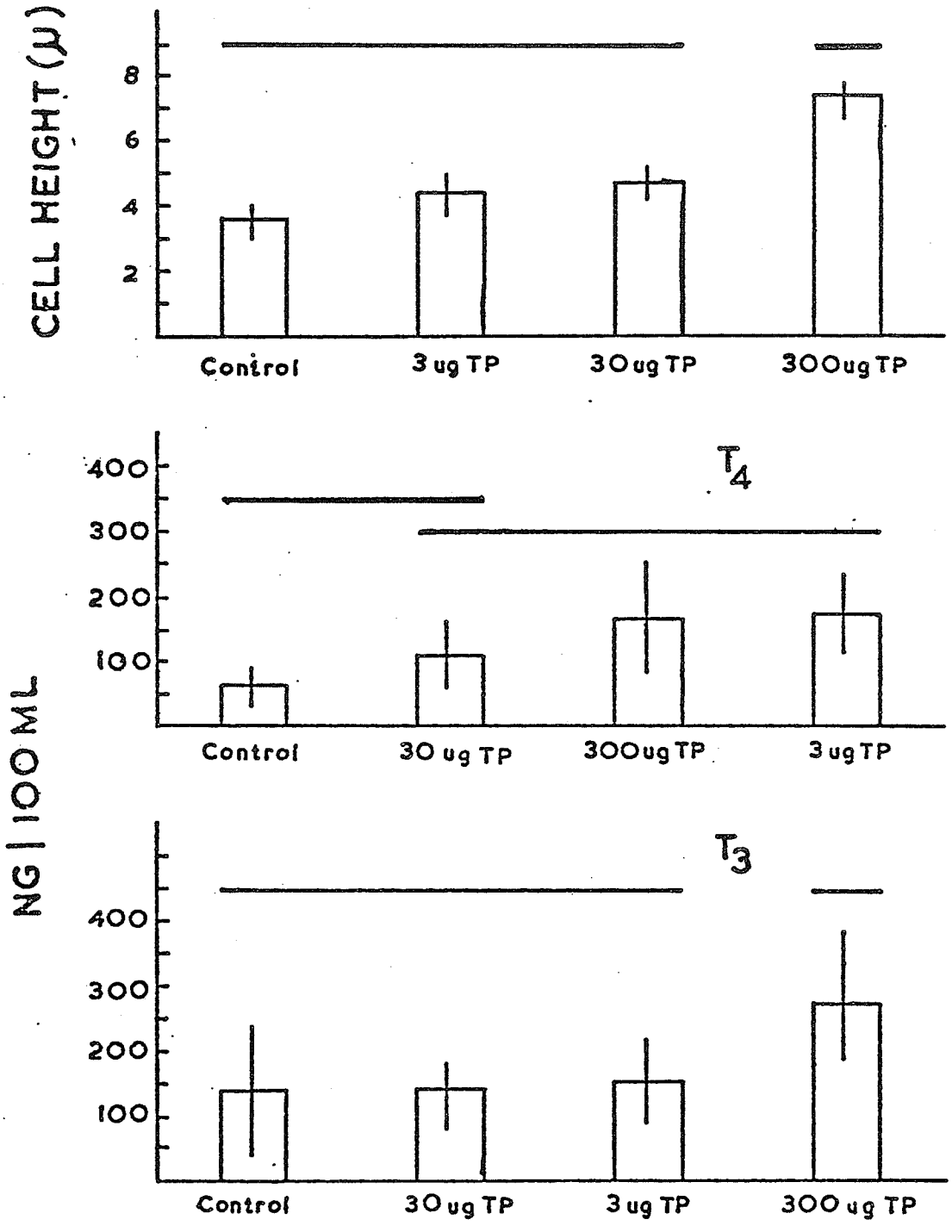
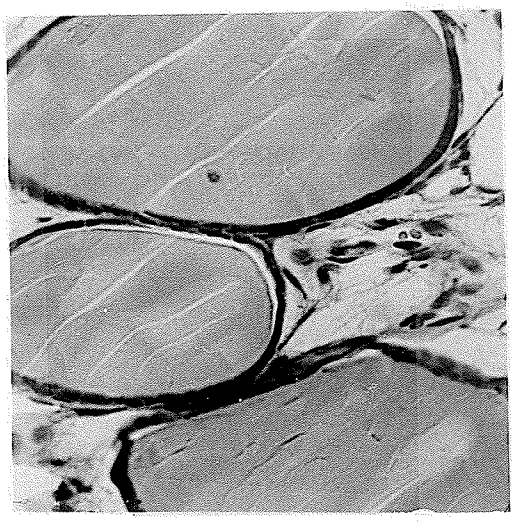
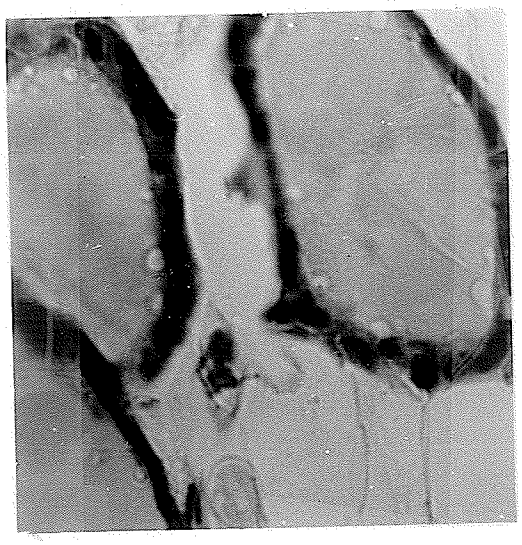

CELL HEIGHT (μ)
8
6
4
2
Control
3 ug TP
30 ug TP
300 ug TP
NG | 100 ML
T4
400
300
200
100
Control
30 ug TP
300 ug TP
3 ug TP
T3
400
300
200
100
Control
30 ug TP
3 ug TP
300 ug TP

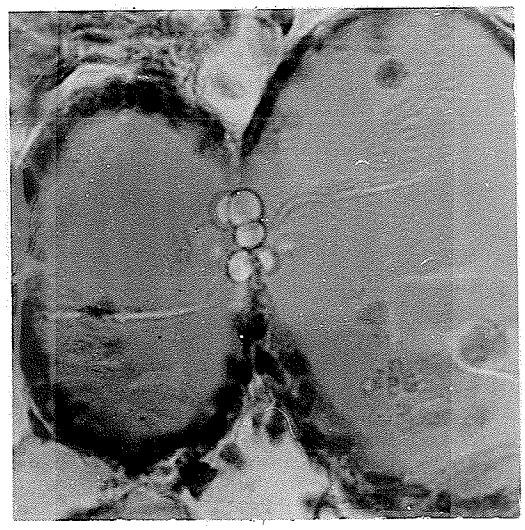
Figure 4. Photomicrographs (x 500) of thyroid tissue in a) control fish, and fish injected with b) 3 ug TP, c) 30 ug TP, d) 300 ug TP.



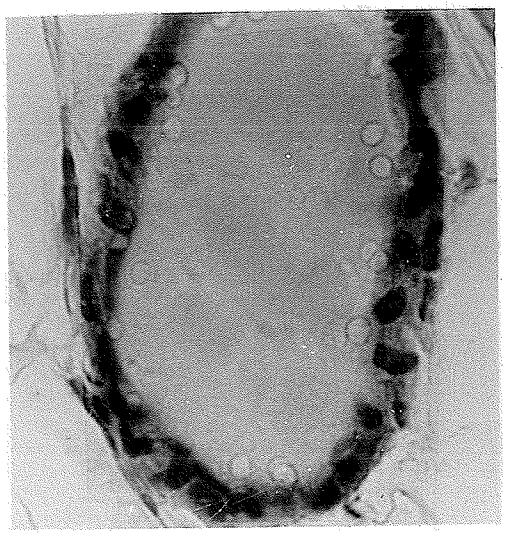
a.



b.



c.



d.

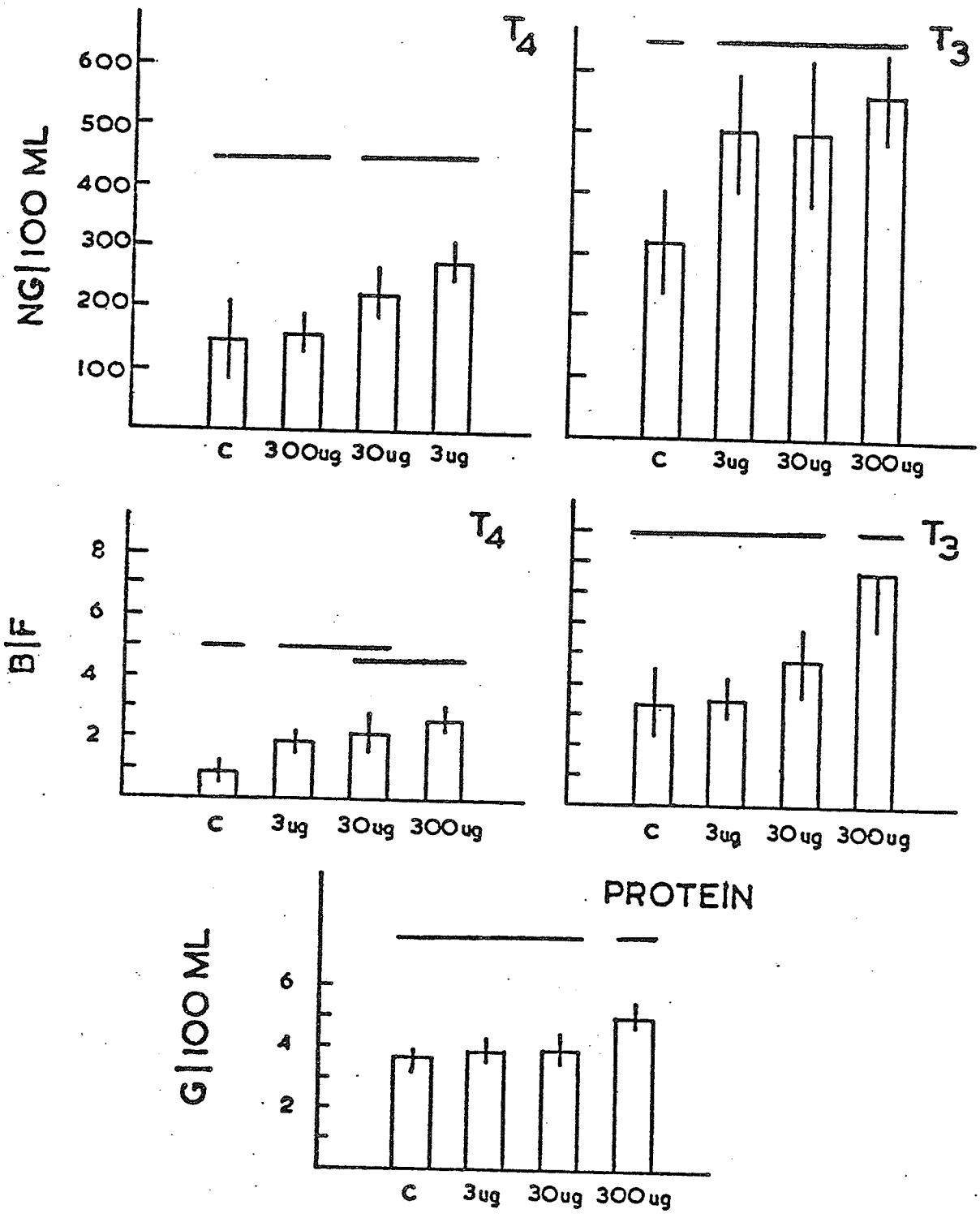
2. Effects of different TP doses on plasma T_3, T_4, T_3 (B/F), T_4 (B/F) and protein

Figure 5 shows plasma T_3, T_4, T_3 (B/F), T_4 (B/F), and protein in control and TP-injected fish. Plasma T_4 was elevated significantly in trout injected with 3 and 30 ug TP. Plasma T_3 was increases significantly above control values in all trout receiving TP. There was no significant difference in plasma T_3 levels among groups of TP-injected fish. Plasma protein concentration was significantly elevated in trout injected with 300 ug TP. All trout injected with TP showed significant elevations in plasma T_4 (B/F). One-way ANOVA showed that the initial and final mean body weights for each group did not differ. There were four mortalities during the experiment. All other fish appeared healthy. The results of one-way ANOVA for body weights, plasma T_4, T_3, T_4 (B/F), T_3 (B/F), and protein are presented in Appendix Table 5.

3. 24-hr effect of a single TP injection (30 ug) on plasma T_3, T_4, T_3 (B/F), T_4 (B/F) and protein

Plasma T_4 was increased significantly ($p < 0.05$) in fish injected with 30 ug TP 24 hr previously (Table 1). There was no statistical difference in plasma T_3, T_4 (B/F), T_3 (B/F), protein and bodyweight between the groups.

Figure 5. Plasma T_4, T_3, T_4 (B/F), T_3 (B/F), and protein in control (C) fish and fish injected with 3, 30, and 300 ug TP. Ranked means ($\bar{x}$) with 95% confidence intervals for 8-10 fish are presented. Horizontal bars join similar ($p < 0.05$) means determined from Student-Newman-Keuls test. Original and final fish body weights are presented in Appendix Table 4.


NG/100 ML
600
500
400
300
200
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T4
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300ug
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T3
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3ug
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300ug
B/F
8
6
4
2
T4
C
3ug
30ug
300ug
T3
C
3ug
30ug
300ug
PROTEIN
G/100 ML
6
4
2
C
3ug
30ug
300ug

Table 1. Mean values ($\bar{x}$) and 95% confidence intervals for plasma T_4, T_3, protein, T_4 (B/F) and T_3 (B/F) for 14 control and 14 TP-injected trout. An F-test showed that all variances were homogeneous.

	Control		TP-injected			
	$\bar{x}$	95%	$\bar{x}$	95%	t-value	significance
T_4 (ng%)	188	137-238	367	256-478	3.36	*
T_3 (ng%)	389	256-478	342	288-397	0.87	NS
Protein (g%)	4.24	3.89-4.60	4.34	4.01-4.68	0.41	NS
T_4 (B/F)	4.51	3.36-5.66	6.22	4.11-8.33	1.62	NS
T_3 (B/F)	8.88	6.40-11.37	9.97	7.92-12.01	0.73	NS

* Level of significance $p < 0.05$

4. Effect of 30 ug TP on radiothyroxine and radioiodide metabolism

a. General observations

Two fish died during the injection period. All other fish appeared healthy and gained weight during the experiment. Control fish gained an average of 6.2 g (5.25% of original weight) and TP-injected fish gained an average of 9.2 g (7.69% of original weight) during this time. There was no statistical difference between the initial and final mean bodyweights of the groups (Table 2).

Trout which had received TP injections were darker than the control fish. A few precociously mature male fish were found in both groups and were omitted from analysis.

b. Plasma T_3, T_4, protein, and inorganic iodide

Plasma T_3, T_4, protein, and inorganic iodide levels are given in Table 3 with test statistics. Plasma T_3 was elevated significantly ($p < 0.005$) in trout injected with 30 ug TP. There was no significant difference in plasma T_4 levels in these fish. Total plasma protein and inorganic iodide ($^{127}I^-$) concentrations were elevated significantly ($p < 0.01$) in trout injected with TP.

Table 2. Mean values ($\bar{x}$) with standard error of the mean (SEM) for body weights and hepatosomatic index (HSI) in control and TP-injected trout. An F-test showed all variances to be homogeneous.

	Control			TP-injected				
	n	$\bar{x}$	SEM	n	$\bar{x}$	SEM	t-value	significance
Original weight (g)	94	118.0	2.35	90	120.0	2.36	0.61	NS
Final weight (g)	92	124.2	2.53	86	129.2	2.55	1.43	NS
HSI	92	1.08	0.31	86	0.98	0.24	2.38	**

** Level of significance $p < 0.01$

Table 3. Mean values ($\bar{x}$) with 95% confidence intervals for plasma T_4, T_3, inorganic iodide ($^{127}I^-$) and protein in control and TP-injected trout. An F-test showed that all variances were homogeneous.

	Control			TP-injected			t-value	significance
	n	$\bar{x}$	95%	n	$\bar{x}$	95%		
T_4 (ng%)	14	121	74-169	14	124	82-167	0.10	NS
T_3 (ng%)	14	192	137-247	14	346	252-439	2.98	***
$^{127}I^-$ (ug%)	24	82	60-105	23	113	98-127	2.34	**
Protein (g%)	23	3.11	2.74-3.49	26	3.81	3.57-4.05	2.38	**

** Level of significance $p \leq 0.01$

*** Level of significance $p \leq 0.0005$

c. Iodide metabolism

Plasma $^{131}I^-$ decreased in an exponential fashion in both groups following $Na^{131}I$ injection (Figure 6). Control fish had a mean plasma $^{127}I^-$ level of 0.82 ($\pm$ 0.23) ug/ml. The fractional turnover rate/hr, distribution space for $^{131}I^-$ and IER were 0.0137, 13.1 ml, and 148 ng/hr/100 g bodyweight, respectively. TP-injected fish had a mean plasma $^{127}I^-$ concentration of 1.13 ($\pm$ 0.14) ug/ml, a $^{131}I^-$ distribution space of 11.3 ml, and a fractional turnover rate/hr of 0.0125. IER for these fish was 159 ng/hr/100 g bodyweight. Data are presented in Table 4.

$^{131}I^-$ accumulated in the thyroid region of fish in both groups following $Na^{131}I$ injection (Figure 7). The thyroid of TP-injected trout appeared to accumulate a smaller percentage of the injected dose at the early sampling periods (0.5 - 12 hr pi). At 24 hr pi there was no statistical difference between the percentage of $^{131}I^-$ in the thyroid gland of each group (Table 5). After 24 hr pi mean ($\bar{x}$) thyroid uptake values were higher in the TP-injected trout. The slopes of the regressions for thyroid $^{131}I^-$ uptake were 0.0194 (control) and 0.0453 (TP-injected). Analysis of covariance revealed that the slope for thyroid $^{131}I^-$ uptake was greater in the TP-injected fish (Appendix Table 6).

Figure 6. Plasma $^{131}I^-$ and $^{125}I^-$ levels in control (●) and TP-injected (o) trout at various times following simultaneous intraperitoneal $Na^{131}I$ and cardiac $^{125}I\text{-}T_4$ injection. Lower graph shows $^{125}I^-$ addition to plasma of control (— — —) and TP-injected (-----) fish. Lines were derived by "curve-peeling". Vertical bars indicate 95% confidence intervals of the geometric mean for 7 - 10 fish.

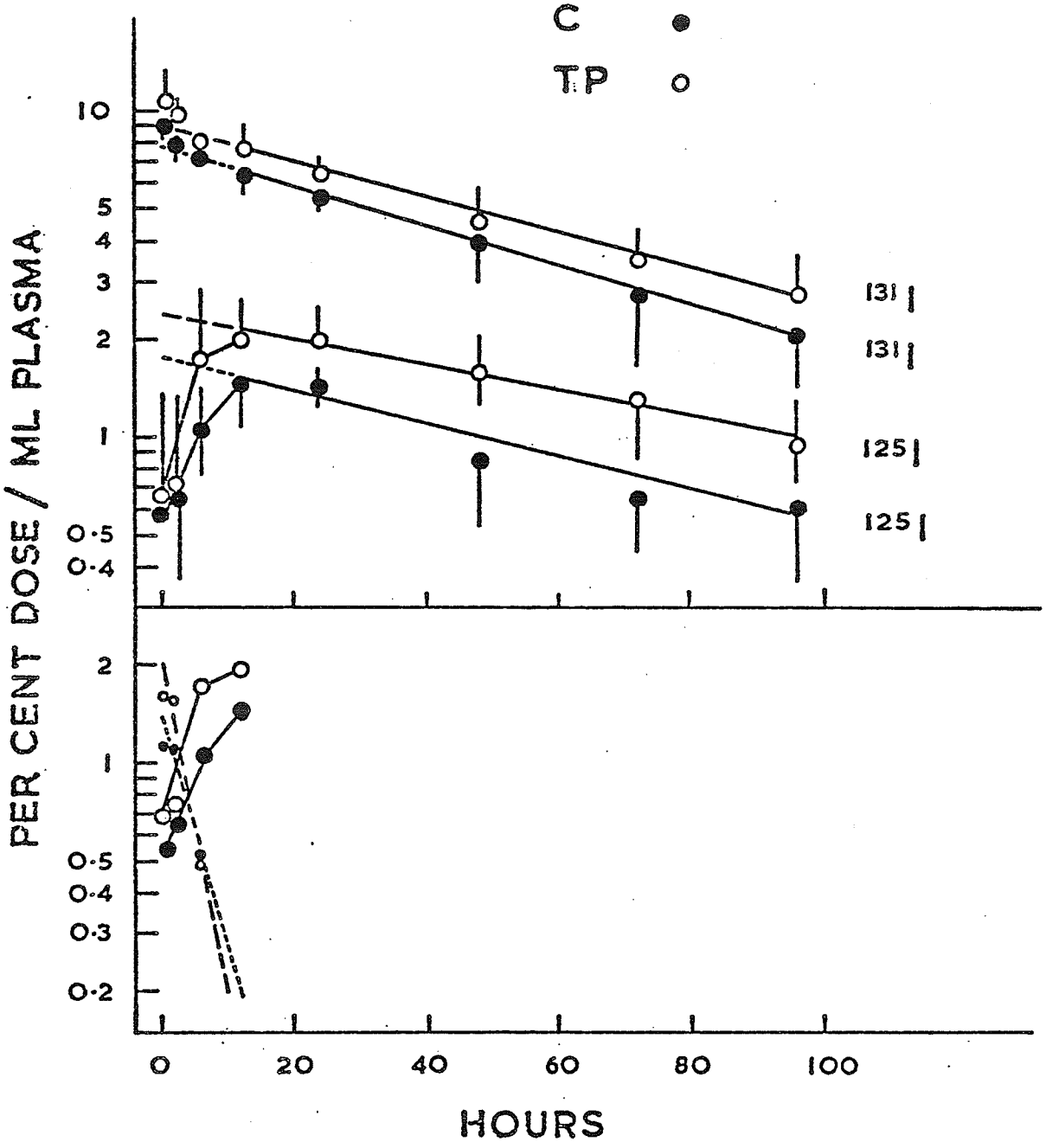

C
TP
10
5
4
3
2
1
0·5
0·4
131 I
131 I
125 I
125 I
2
1
0·5
0·4
0·3
0·2
PER CENT DOSE / ML PLASMA
0
20
40
60
80
100
HOURS

Table 4. Various parameters of iodide metabolism in control and TP-injected trout.

	Control	TP-injected
Plasma $^{127}I^-$ (ug/ml)	0.82 ($\pm$ 0.23)[1]	1.13 ($\pm$ 0.14)
$^{131}I^-$ distribution space (ml)	13.13	11.30
Iodide pool size (ug)	10.81	12.71
$^{131}I^-$ fractional turnover/hr	0.0137	0.0125
Iodide excretion rate (ng/hr/100 g body weight)	148	159
Hormonal iodine degradation rate (ng/hr/100 g body weight)	6.29	8.21

1. 95% confidence intervals of the mean

Following ^{125}I-T_4 injection, ^{125}I-radioactivity was concentrated in the thyroid region (Figure 7). High levels of ^{125}I-radioactivity from 0.5 to 12 hr pi may represent 'contamination' of surrounding gill and blood vessels with ^{125}I-T_4 and ^{125}I-T_3. The slopes of the regressions for thyroid $^{125}I^-$ uptake were determined from 24 - 96 hr pi, since the majority of ^{125}I-labeled hormone had been cleared from the plasma by this time. The slopes for the regressions/hr for thyroid $^{125}I^-$ uptake were 0.0053 (control) and 0.0198 (TP-injected). Analysis of covariance revealed that the slope of thyroid $^{125}I^-$ uptake between 24 and 96 hr pi was greater in the TP-injected fish (Appendix Table 7).

When percent dose values were doubled to account for thyroidal uptake of unlabeled iodide derived from ^{125}I-T_4, slopes of 0.0106 (control) and 0.0395 (TP-injected) were obtained. No comparison of the slopes of the regressions for thyroid $^{131}I^-$ and $^{125}I^-$ uptake in control fish was possible since variances were found to be heterogeneous (Appendix Table 8). The variances and slopes of the regressions for thyroid $^{125}I^-$ and $^{131}I^-$ uptake in the TP-injected trout were shown to be statistically similar (Appendix Table 9).

Table 5. Mean values ($\bar{x}$) with 95% confidence intervals for 24 hr pi thyroid $^{131}I^-$ (% of the injected dose), thyroid $^{125}I/^{131}I$ ratio (24-96 hr pi), 6 hr pi gall bladder ^{125}I-radioactivity (% of the injected dose), and 0.5 hr pi liver ^{125}I-radioactivity (% of the injected dose) in control and TP-injected fish. An F-test showed all variances to be homogeneous.

	Control			TP-injected				
	n	$\bar{x}$	95%	n	$\bar{x}$	95%	t-value	significance
Thyroid $^{131}I^-$ (% 24 hr pi)	10	1.03	0.74-1.32	10	0.95	0.74-1.16	0.12	NS
Thyroid $^{125}I/^{131}I$ (24-96 hr pi)	39	0.43	0.39-0.47	31	0.64	0.58-0.69	6.21	**
Gall bladder (% 6 hr pi)	10	4.20	2.25-7.86	9	1.45	0.58-3.65	2.19	*
Liver (% 0.5 hr pi)	8	18.9	10.5-34.1	10	8.2	4.7-14.2	2.44	**

* Level of significance $p < 0.05$

** Level of significance $p < 0.01$

Significant amounts of ^{131}I-labeled T_4 and T_3 were detected in the plasma of control and TP-injected trout shortly after the injection of $Na^{131}I$ (Figure 8). ^{131}I-T_3 predominated in the plasma of each group. Plasma ^{131}I-T_3 levels were higher in the TP-injected trout at all sample times. Plasma ^{131}I-T_4 tended to be slightly higher in the control fish at most sampling times.

d. T_4 kinetics

Plasma ^{125}I-T_4 decreased in a curvilinear manner in both groups (Figure 9). Disappearance curves were comprised of a "fast" and a "slow" component and this was used as the basis of a compartmental analysis. Results are present in Table 6. The equations for plasma ^{125}I-T_4 loss with time were, $C = 2.02\ e^{-0.1704} + 0.035\ e^{-0.0183}$ (control) and $C = 1.42e^{-0.18} + 0.14e^{-0.0285}$ (TP-injected). T_4DR was greater in the TP-injected fish (12.63 ng/hr) than in control fish (9.67 ng/hr). Figure 10 shows dissection of components by "curve-peeling" for both groups.

e. ^{125}I-T_3 generation from ^{125}I-T_4

The antibody separation revealed a significant production of ^{125}I-T_3 following ^{125}I-T_4 injection in both groups (Figure 11). Plasma concentrations of ^{125}I-T_3 increased from 0.5 - 6 hr pi in both groups, then declined in a curvilinear fashion. At 6 hr pi 0.99% (TP-injected) and 0.60% (control) of the injected ^{125}I-T_4 dose was ^{125}I-T_3 in the plasma.

Figure 7. ^{131}I- and ^{125}I-radioactivity in the thyroid region of control (●) and TP-injected (o) trout at various times following simultaneous intraperitoneal $Na^{131}I$ and intracardiac ^{125}I-T_4 injection. Points indicate means with 95% confidence intervals of 7-10 trout. The lower graph shows mean thyroid $^{125}I/^{131}I$ ratios 24-96 hr following isotope injections.

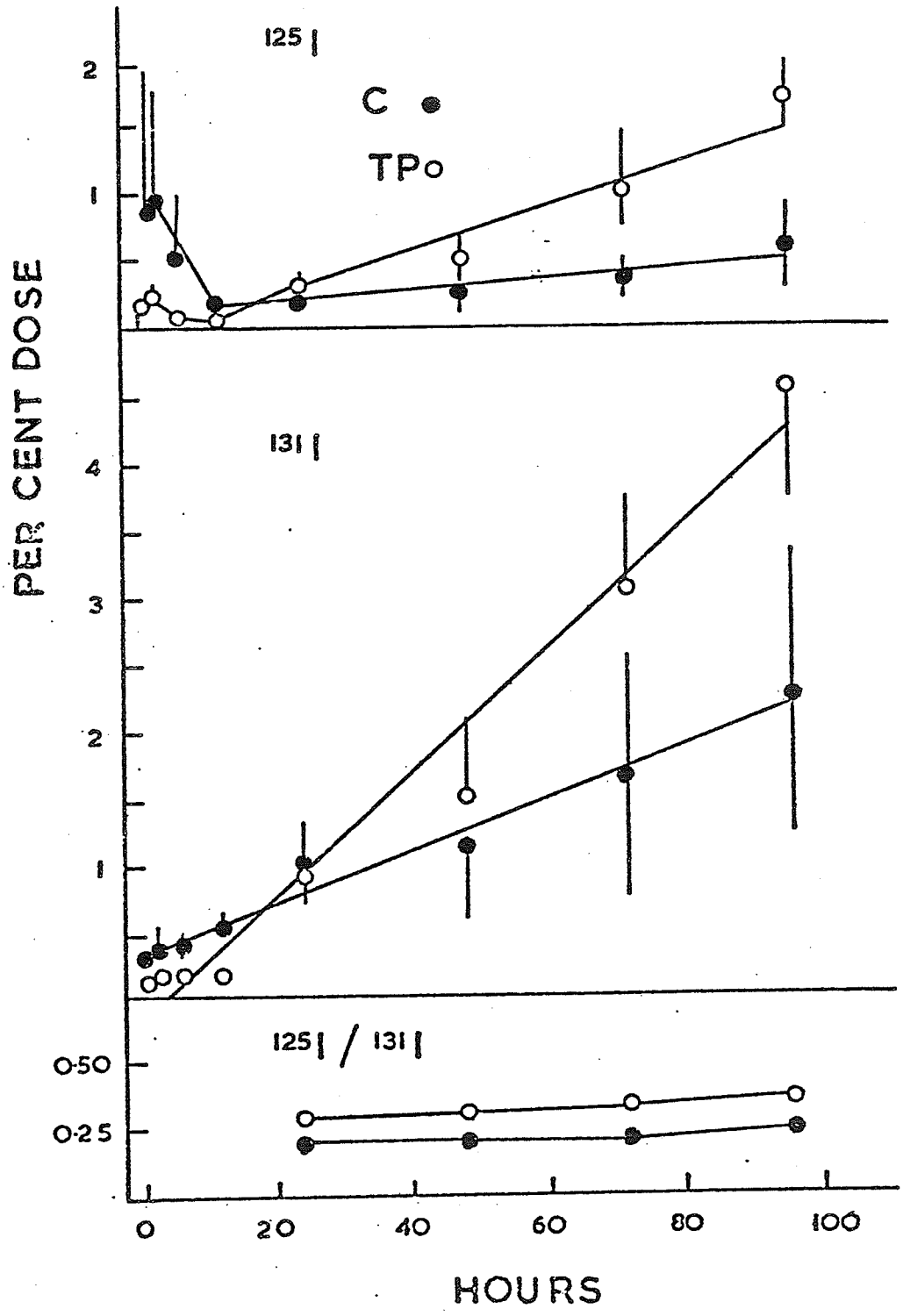

125 I
C
TPo
131 I
125 I / 131 I
PER CENT DOSE
HOURS
2
1
4
3
2
1
0·50
0·25
0
20
40
60
80
100

Figure 8. Plasma ^{131}I-T_3 and ^{131}I-T_4 levels in control (●) and TP-injected (o) rainbow trout at various times following $Na^{131}I$ injection. Points indicate geometric means with 95% confidence intervals for 7-10 fish.

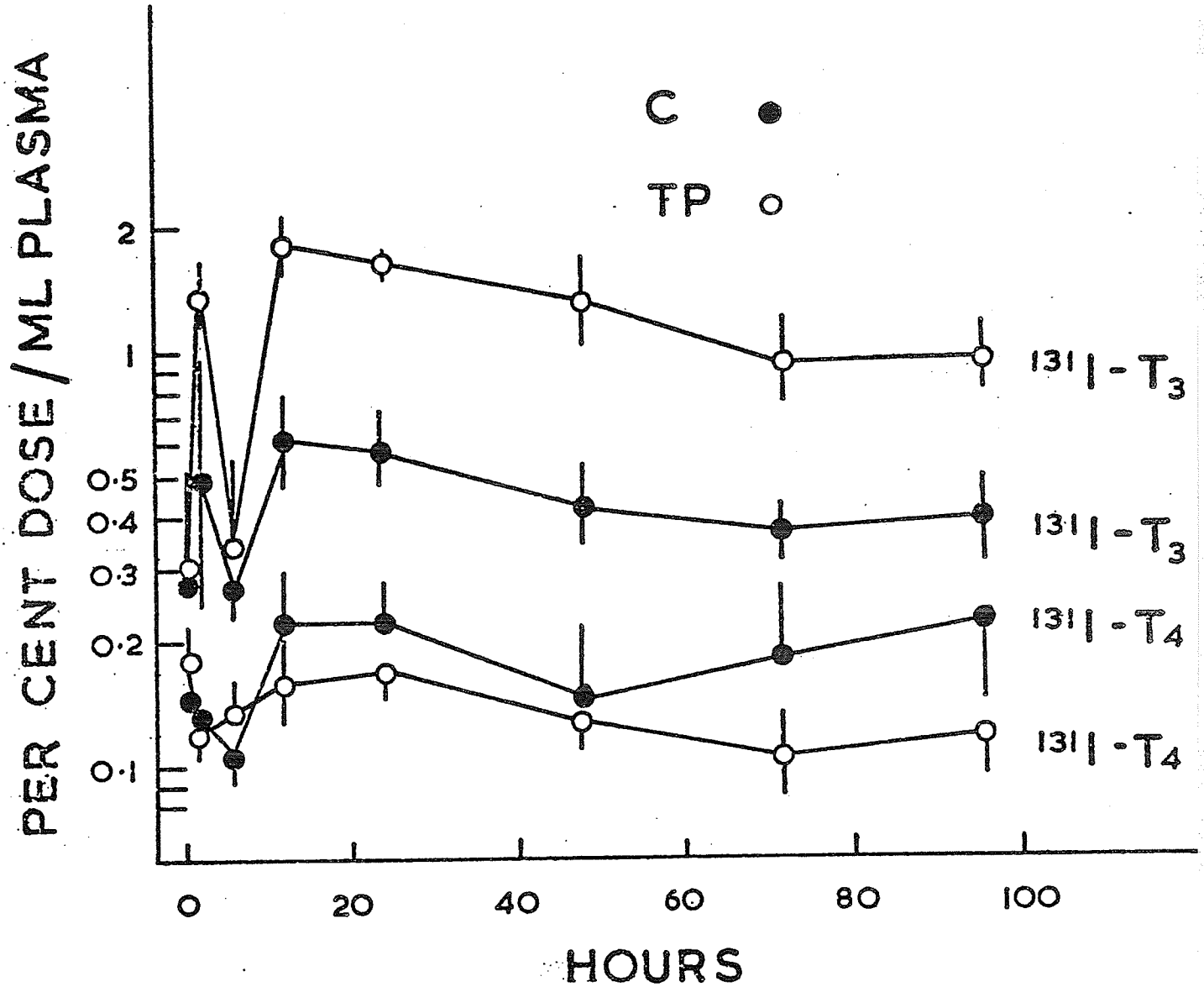

PER CENT DOSE / ML PLASMA
C
TP
2
1
0·5
0·4
0·3
0·2
0·1
^{131}I - T_3
^{131}I - T_3
^{131}I - T_4
^{131}I - T_4
0
20
40
60
80
100
HOURS

Figure 9. Plasma ^{125}I-T_4 levels in control (●) and TP-injected (o) rainbow trout at various times following cardiac ^{125}I-T_4 injection. Points indicate geometric means with 95% confidence intervals for 8-10 fish.

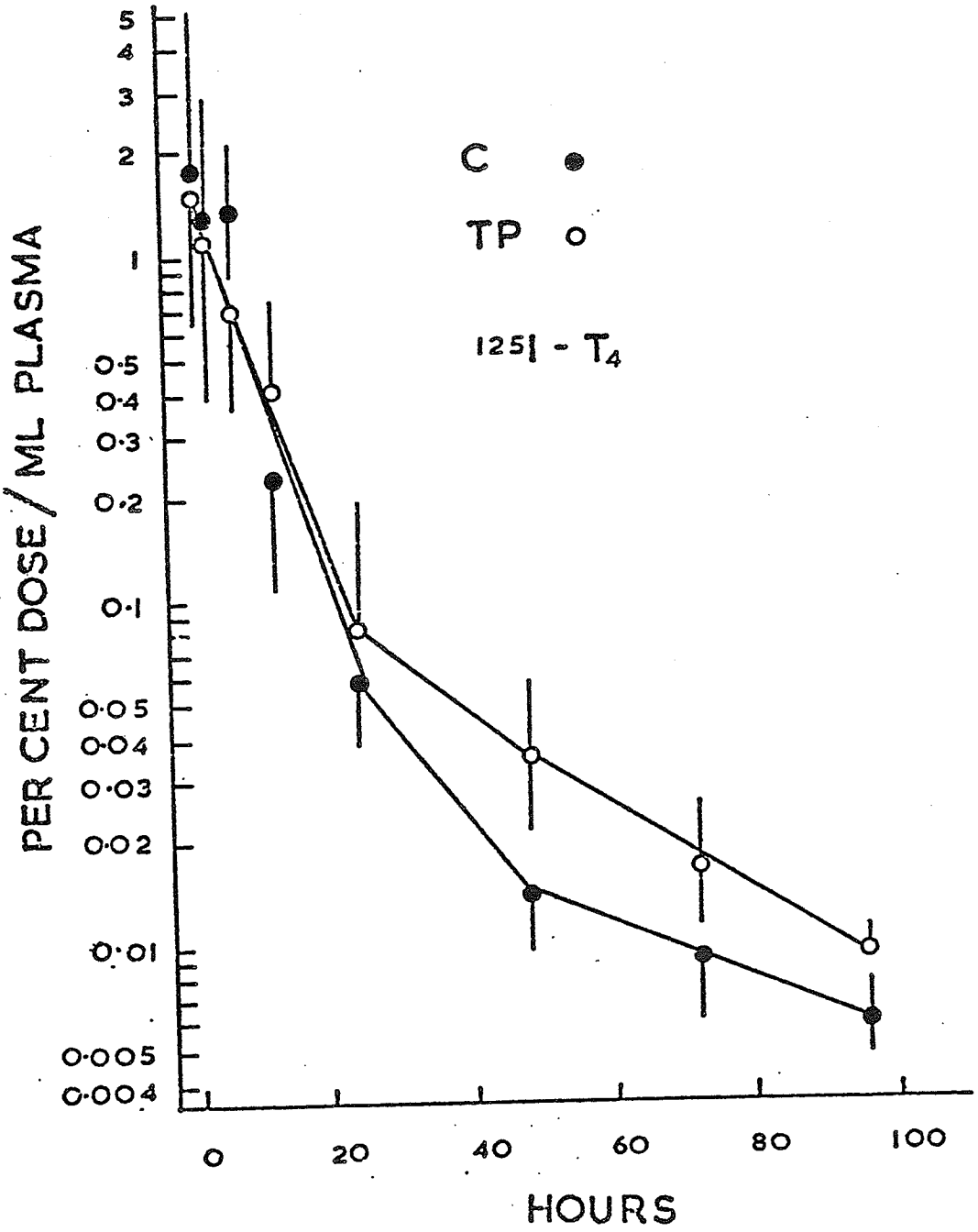
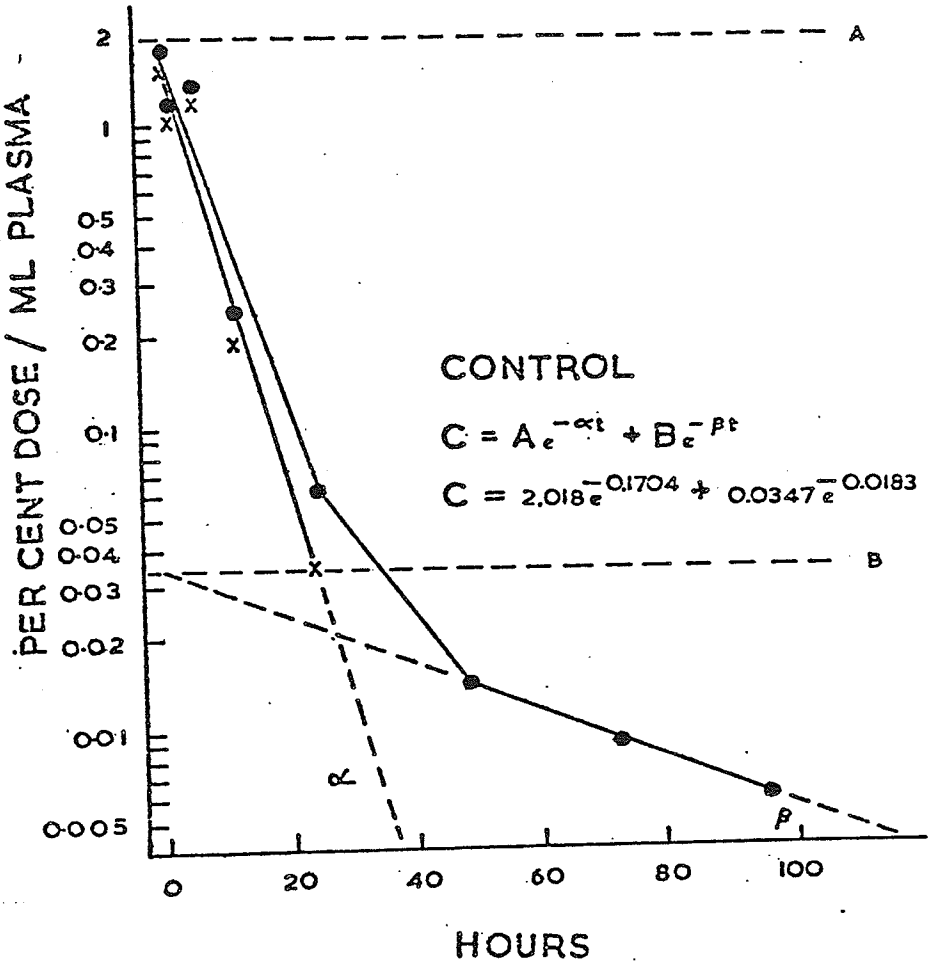

PER CENT DOSE / ML PLASMA
5
4
3
2
1
0·5
0·4
0·3
0·2
0·1
0·05
0·04
0·03
0·02
0·01
0·005
0·004
C
TP
125I - T4
0
20
40
60
80
100
HOURS

Figure 10. Dissection of the curve describing $^{125}I\text{-}T_4$ loss with time in control (●) and TP-injected (o) trout into "fast" and "slow" components. 'Peeled' values are indicated by x.


PER CENT DOSE / ML PLASMA
2
1
0·5
0·4
0·3
0·2
0·1
0·05
0·04
0·03
0·02
0·01
0·005
A
B
CONTROL
C = A e^-αt + B e^-βt
C = 2.018 e^-0.1704 + 0.0347 e^-0.0183
α
β
0
20
40
60
80
100
HOURS

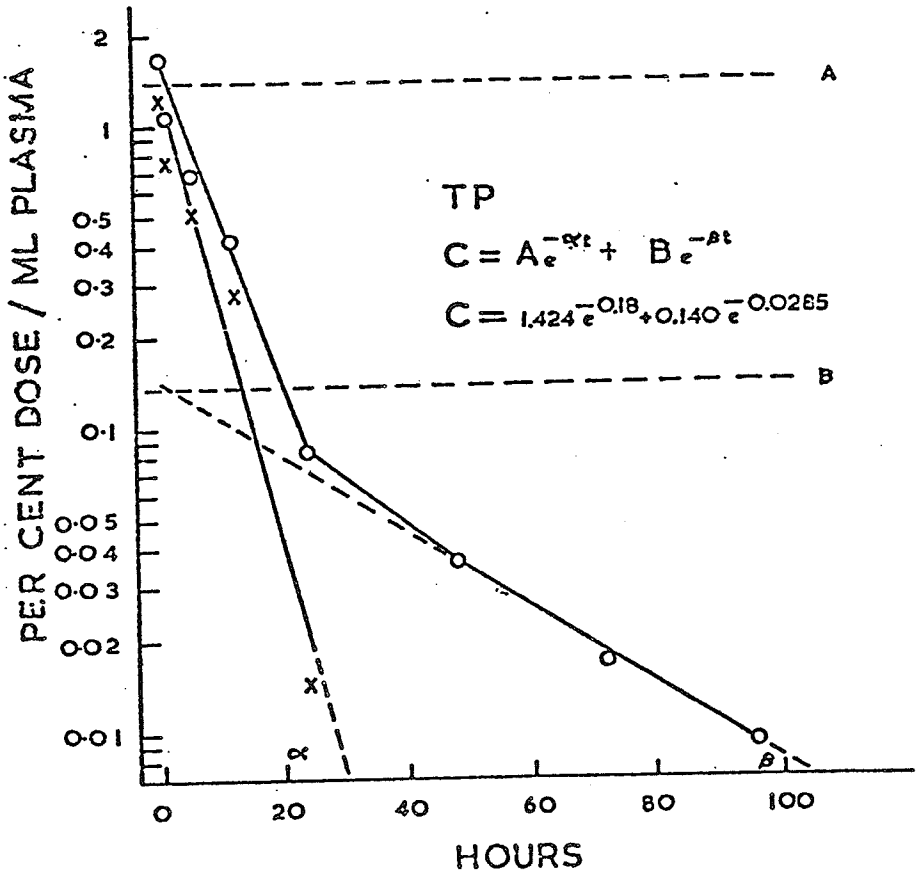

PER CENT DOSE / ML PLASMA
2
1
0·5
0·4
0·3
0·2
0·1
0·05
0·04
0·03
0·02
0·01
A
B
TP
C = A e^-αt + B e^-βt
C = 1.424 e^-0.18 + 0.140 e^-0.0285
α
β
0
20
40
60
80
100
HOURS

Table 6. T_4 kinetic parameters in control and TP-injected trout

	Control	TP-injected
Kba	0.1678	0.1664
Kab	0.0004	0.0019
Kob	0.0205	0.0402
Va (ml)	48.7	64
T_4 (ng/ml)	1.21 ($\pm$ 0.5)[1]	1.24 ($\pm$ 0.4)
Qa (ng)	59.0	79.4
Qb (ng)	471	314
Fba (ng/hr)	9.9	13.2
Fab (ng/hr)	0.2	0.6
Fob (ng/hr)	9.7	12.6

[1] 95% confidence intervals of the mean

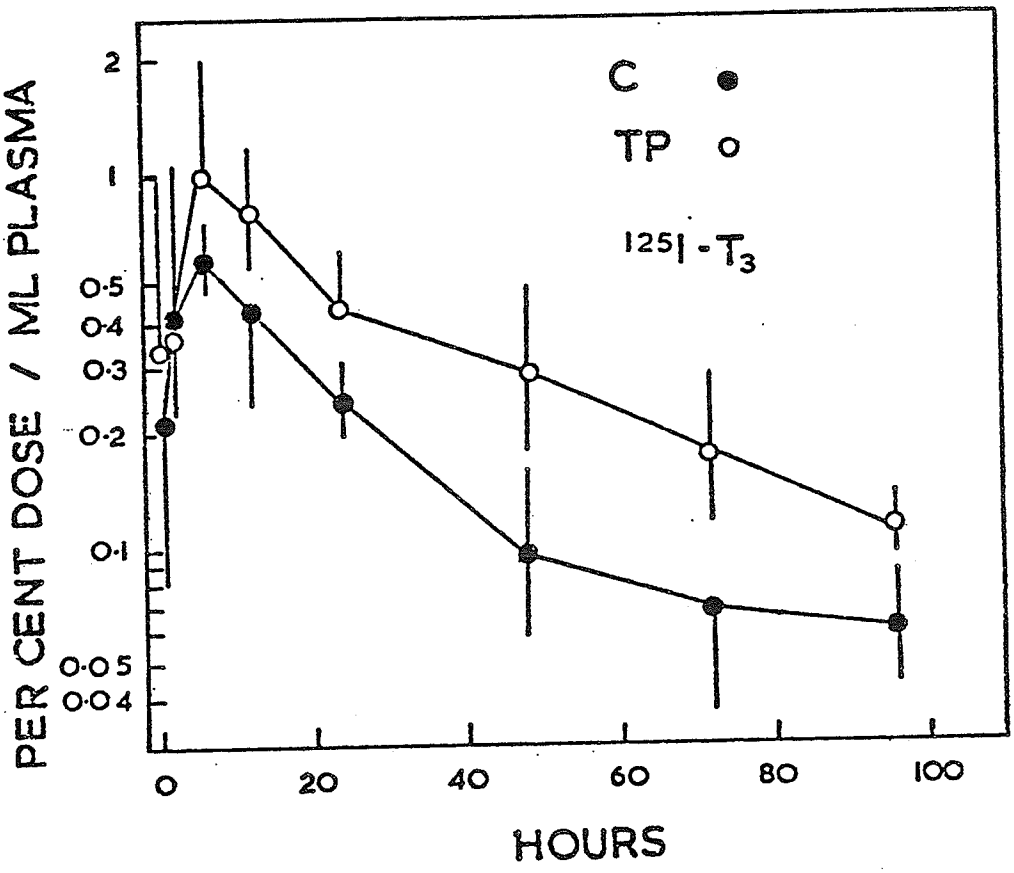
f. Deiodination

Fractional rates of $^{131}I^-$ loss/hr (k1) from plasma were 0.0137 and 0.0125 between 12 and 96 hr pi for control and TP-injected fish, respectively. The *y* intercepts (Y131) of the regressions for plasma $^{131}I^-$ loss were 7.61% for control and 8.85% for TP-injected trout. This indicated the percentage of the injected dose that would have been present in one ml of plasma at zero time for a trout weight of 100 g.

Following ^{125}I-T_4 injection there was a significant production of $^{125}I^-$ in control and TP-injected fish. Plasma $^{125}I^-$ concentrations increased until 12 hr pi, then decreased in an exponential manner for the remainder of the sampling period (Figure 5). Fractional rates of $^{125}I^-$ loss/hr (k2) from plasma were 0.0115 for control fish and 0.0091 for TP-injected fish. The *y* intercepts (Y125) of the regressions for plasma $^{125}I^-$ loss were 1.72% for control and 2.37% for TP-injected trout.

Analysis of covariance revealed no significant difference between the variances or slopes of the regressions between 12 and 96 hr pi for plasma $^{125}I^-$ and $^{131}I^-$ loss in either group (Appendix Tables 10 and 11). No comparison of plasma $^{125}I^-$ or $^{131}I^-$ kinetics between control and TP-injected trout was possible since the variances of the regressions were

Figure 11. Plasma ^{125}I-T_3 in control (●) and TP-injected trout at various times following cardiac ^{125}I-T_4 injection. Vertical bars represent 95% confidence intervals of the geometric mean for 7-10 trout.


C
TP
125I - T3
PER CENT DOSE / ML PLASMA
2
1
0·5
0·4
0·3
0·2
0·1
0·05
0·04
0
20
40
60
80
100
HOURS

found to be heterogeneous (Appendix Tables 12 and 13).

Fractional rates/hr (k3) for $^{125}I^-$ addition to plasma were 0.1571 for control fish and 0.2358 for TP-injected fish (Figure 6). Corrected Y125 intercepts were 1.59% and 2.24% for control and TP-injected fish, respectively. The fraction of the Y131 intercept that is represented by the Y125 intercept is 1.59/716. = 0.21 for control fish and 2.24/8.85 = 0.25 for TP-injected fish. These fractions represent indices of T_4 deiodination. Doubling these fractions corrects for the random ^{125}I-labeling of the phenolic ring of ^{125}I-T_4. Thus, 42% and 51% of the injected dose of ^{125}I-T_4 underwent monodeiodination, in the control and TP-injected trout, respectively.

On the basis of the T_4 degradation rates calculated previously, estimated of the rate of T_3 generation from T_4 were 0.42 x 9.67 = 4.04ng (5.21 pM)/hr/100 g body weight for control fish and 0.51 x 12.63 = 6.40 ng (8.26 pM)/hr/100 g body weight for TP-injected fish.

Estimates of T_4 deiodination rates were also obtained by comparing the relative proportions of $^{125}I^-$ and $^{131}I^-$ in the thyroid between 24 and 96 hr pi (Figure 6). This relationship appeared stable although the $^{125}I/^{131}I$ ratio tended to increase slightly at later sampling times. The $^{125}I/^{131}I$ thyroid radio between 24 and 96 hr pi was found to be elevated

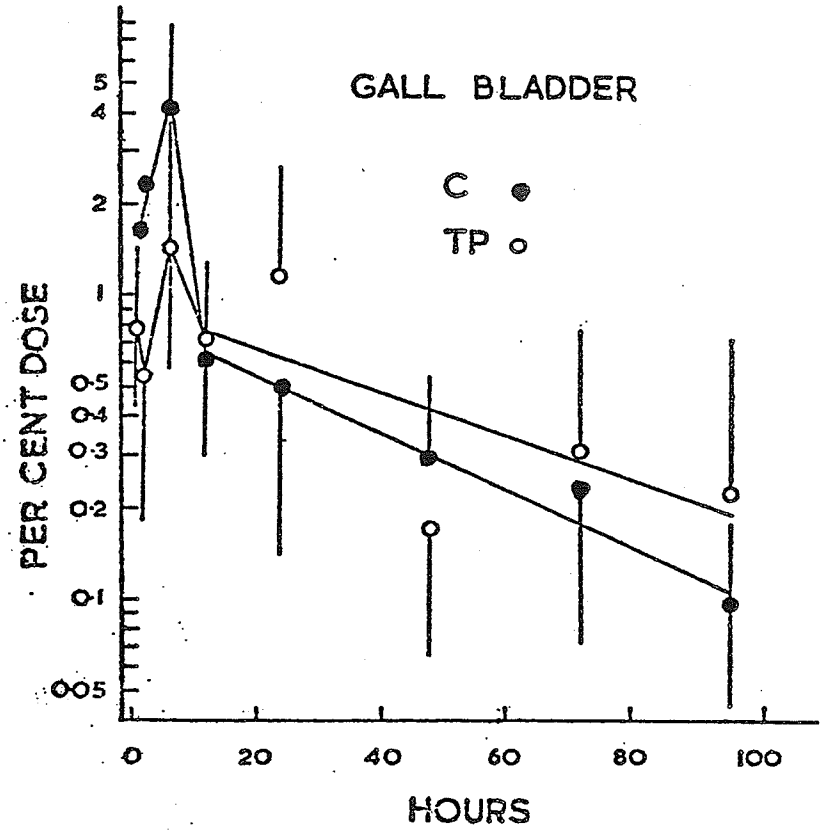
significantly ($p < 0.01$) in the TP-injected trout (Table 5). Rates of T_4 to T_3 conversion were obtained by multiplying the thyroidal $^{125}I/^{131}I$ ratios by T_4DR. T_4 deiodination rates of 0.43 x 9.67 = 4.12 ng/hr (control) and 0.64 x 12.63 = 8.03 ng/hr (TP-injected) were obtained.

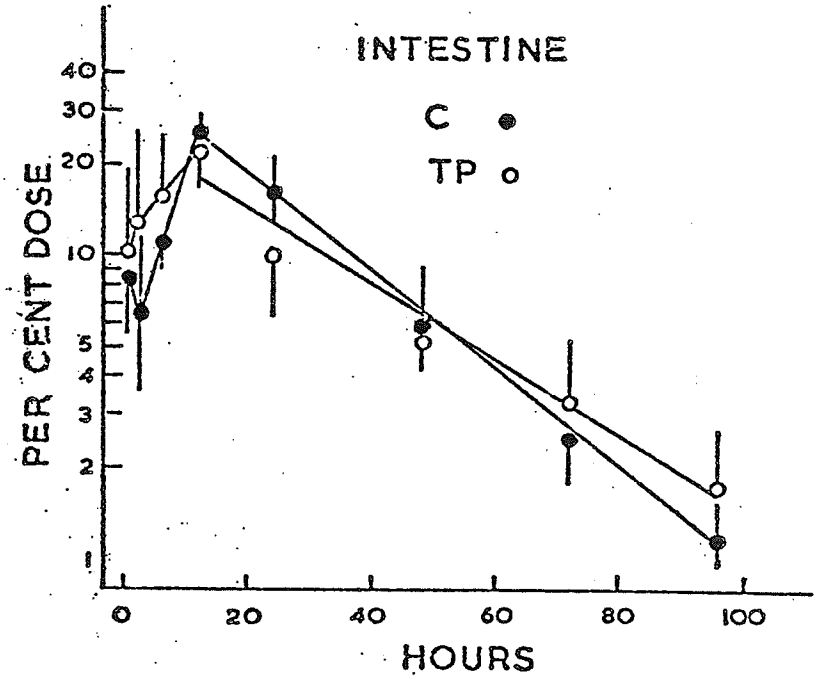
g. Enterohepatic excretion

^{125}I-radioactivity increased until 6 hr pi in the gall bladder of fish in both groups (Figure 12). Control fish accumulated significantly ($p < 0.05$) more radioactivity by 6 hr pi than TP-injected fish (Table 5). Loss of radioactivity between 12 and 96 hr pi appeared exponential in both groups. There was a great amount of variability associated with the loss of ^{125}I-radioactivity from the gall bladder of the TP-injected trout. No attempt was made to compare rates of uptake or loss from this organ because of the variability in the data.

^{125}I-radioactivity increased in the intestine up to 12 hr following ^{125}I-T_4 injection. This was followed by an exponential loss of radioactivity in both groups (Figure 12). Analysis of covariance revealed a significantly greater rate of loss of ^{125}I-radioactivity from the intestine of the control fish (Appendix Table 14). Fractional rates of loss/hr (k_1) were 0.0370 (control) and 0.0283 (TP-injected). Regression lines were extrapolated to the y axis and intercepts of 38.6% (control) and 24.8% (TP-injected) were obtained.

Figure 12. Percentage of the injected dose in the gall bladder and intestine of control (●) and TP-injected (o) trout at various times following ^{125}I-T_4 injection. Geometric means with 95% confidence intervals are given.


GALL BLADDER
C
TP
PER CENT DOSE
5
4
2
1
0.5
0.4
0.3
0.2
0.1
0.05
0
20
40
60
80
100
HOURS


INTESTINE
C
TP
PER CENT DOSE
40
30
20
10
5
4
3
2
1
0
20
40
60
80
100
HOURS

Rate constants (k2) for intestinal uptake/hr of ^{125}I-radioactivity were 0.0817 (control) and 0.1834 (TP-injected). The percentage of the injected dose of ^{125}I-T_4 present in the intestine at time zero was 21.1% for control fish and 21.0% for TP-injected fish.

Total enterohepatic (gall bladder plus intestine) ^{125}I-radioactivity increased until 12 hr pi before declining in an exponential manner (Figure 13). Fractional rates of loss/hr (k1) were 0.0361 (control) and 0.0283 (TP-injected). Regression lines were extrapolated to the *y* axis and intercepts of 39.0% (control) and 28.3% (TP-injected) were obtained. Analysis of covariance revealed that the rate of radioactive loss was significantly greater in the control fish (Appendix Table 15).

Rate constants (k2) for enterohepatic uptake/hr of ^{125}I-radioactivity were 0.1386 (control) and 0.1847 (TP-injected). It was determined that 28.9% and 24.0% of the injected ^{125}I-T_4 dose would have been present in the enterohepatic organs of the control and TP-injected fish, respectively, at zero time.

h. Liver

The hepatosomatic index (HSI) was significantly ($p < 0.05$) greater in control fish (Table 5). Loss of ^{125}I-radioactivity from liver is presented in Figure 14. The livers of the control fish initially (0.5 hr pi)

Figure 13. Percentage of the injected dose in the enterohepatic organs (gall bladder plus intestine) of control (●) and TP-injected (o) trout at various times following ^{125}I-T_4 injection. Points represent geometric means with 95% confidence intervals for 7-10 fish.

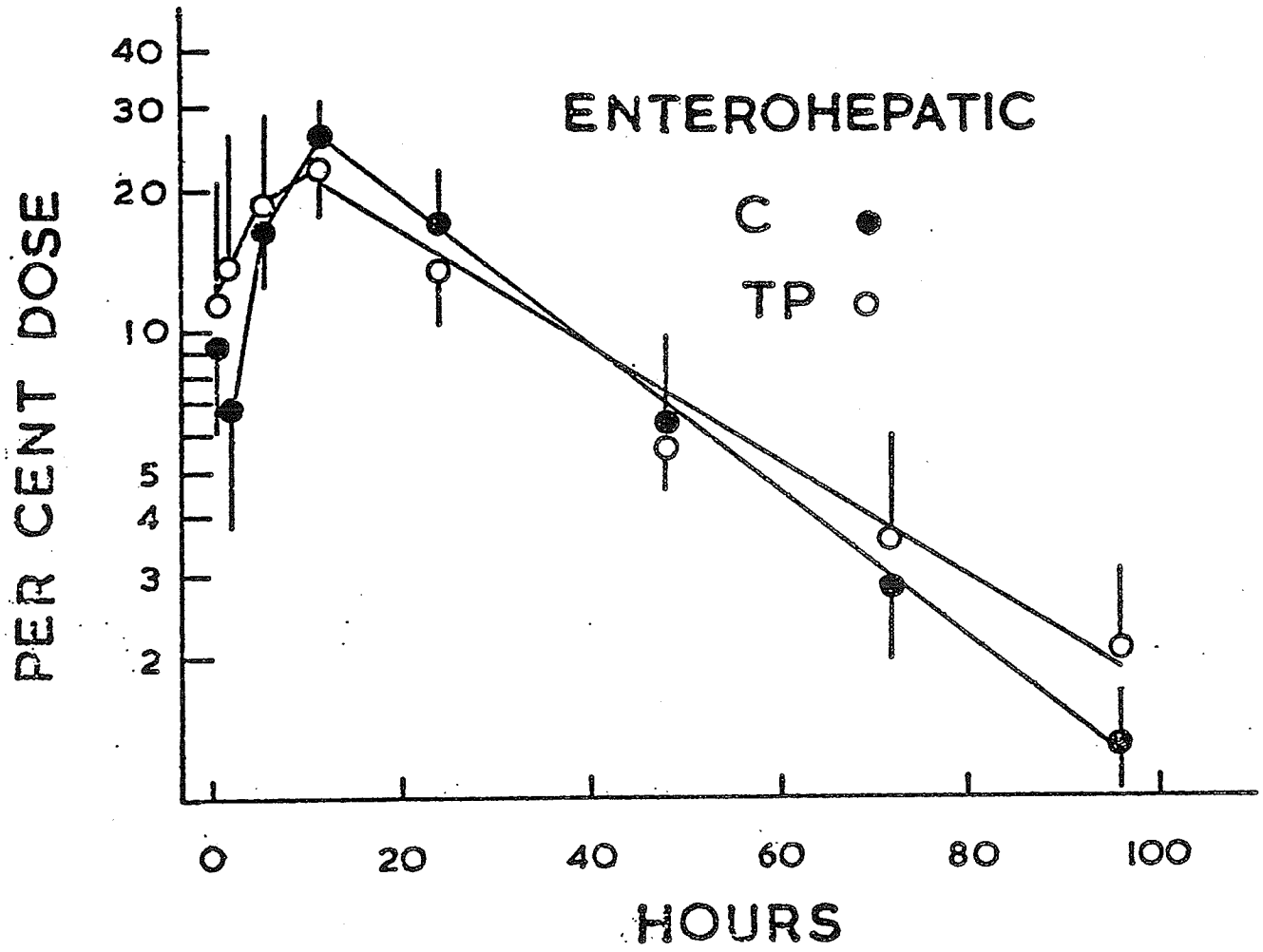
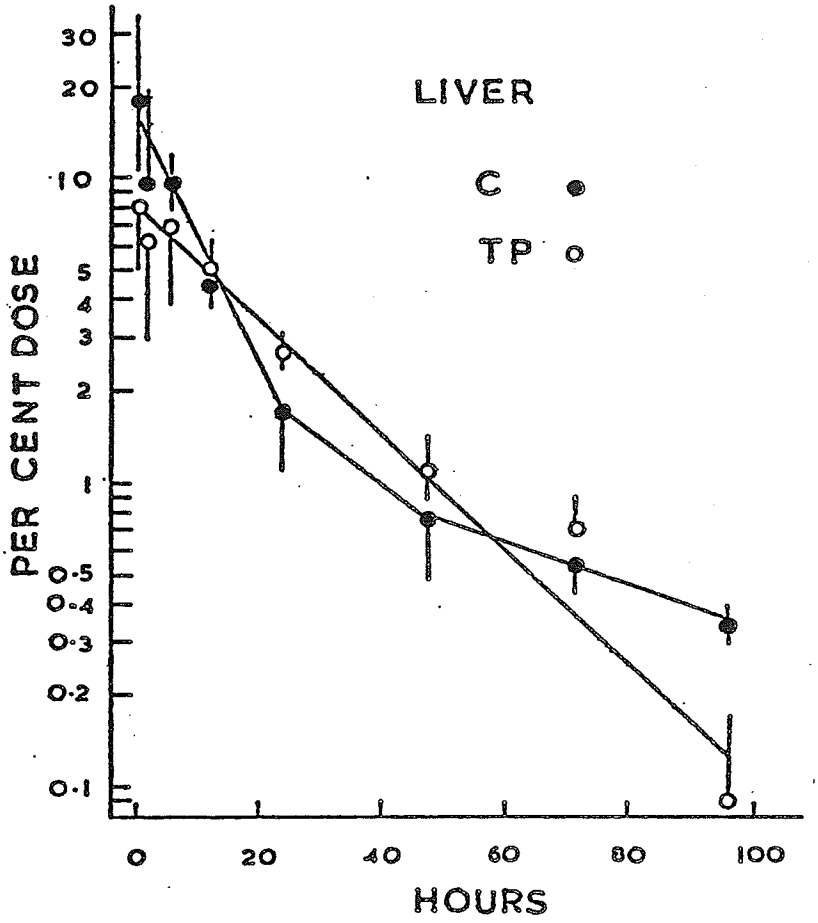
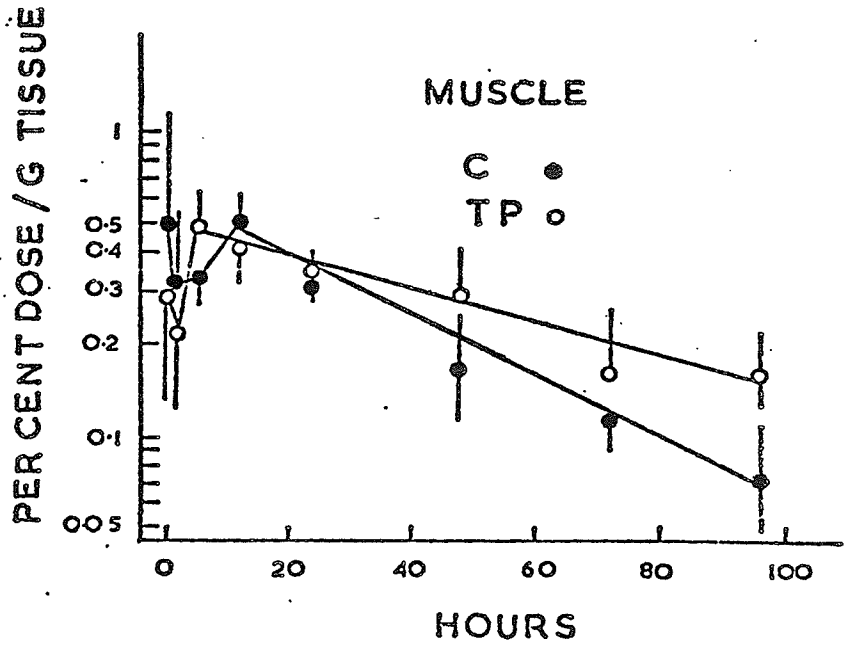

ENTEROHEPATIC
C
TP
PER CENT DOSE
40
30
20
10
5
4
3
2
0
20
40
60
80
100
HOURS

Figure 14. Percentage of the injected dose in the liver and muscle of control (●) and TP-injected (o) trout at various times following ^{125}I-T_4 injection. Points represent geometric means with 95% confidence intervals for 7-10 trout.


LIVER
C
TP
PER CENT DOSE
30
20
10
5
4
3
2
1
0·5
0·4
0·3
0·2
0·1
0
20
40
60
80
100
HOURS


MUSCLE
C
TP
PER CENT DOSE / G TISSUE
1
0·5
0·4
0·3
0·2
0·1
0·05
0
20
40
60
80
100
HOURS

took up a significantly ($p < 0.05$) greater percentage of the injected ^{125}I-T_4 dose than the TP-injected fish (Table 6). Radioactivity decreased in a multi-exponential fashion from the livers of the control fish while loss appeared exponential in the TP-injected fish.

i. Muscle

^{125}I-radioactivity decreased between 0.5 and 2 hr pi in the muscle of both groups, before rising to maximal uptake levels at 6 hr pi for the TP-injected fish and 12 hr pi for the control fish (Figure 14). Radioactivity declined in an exponential fashion after maximal uptake had been reached. Analysis of covariance revealed no significant difference between the slopes of the regressions for the loss of ^{125}I-radioactivity from muscle and $^{125}I^-$ loss from plasma between 24 and 96 hr pi in either group (Appendix Tables 16 and 17). It appears that the majority of ^{125}I-radioactivity retained in muscle after 24 hr following ^{125}I-T_4 injection is inorganic iodide. Loss of ^{125}I-radioactivity from muscle was shown to be more rapid in the control trout (Appendix Table 18).

DISCUSSION

A. Effects of TP on the trout thyroidal system

1. Histology

The thyroid tissue of the TP-injected trout appeared more active than that of control trout.

Histological activation was most marked in the small thyroid follicles. van Overbeeke and McBride (1971) noted a similar effect in gonadectomized male sockeye salmon injected with methyltestosterone or 11-ketotestosterone. Activation of the thyroid was noted in yearling coho salmon which had received methyltestosterone in the diet (Higgs et al., 1977).

2. Plasma T_4 and T_3

The series of TP doses used in Experiments 1 and 2 generally produced elevations in plasma T_4 or T_3 levels.

Inconsistencies in plasma T_4 response to TP may be due to differences in body weight or fish stock. The larger fish used in Experiments 2 and 4 appeared more responsive to the TP doses than fish in Experiment 1, especially in regard to elevations in plasma T_3 levels. Chan and Eales (1976) found that the thyroid of larger brook trout

was more responsive than that of small fish of the same age, to a TSH dose standardized for body weight. Although TP doses were not standardized for body weight in my study, the TP doses appeared to have a greater effect on plasma T_4 and T_3 levels in larger trout.

Increased plasma T_4 levels may occur as the result of thyroidal stimulation. T_4 appears to be the major iodothyronine secreted by the thyroid tissue of rainbow and brook trout (Chan and Eales, 1975; Hunt, unpublished data). Elevation of plasma T_4 following TP treatment may indicate a direct stimulation of the thyroid or the thyroid-pituitary axis. Singh (1969) showed that thyroid iodide uptake was significantly greater 24 hr following $Na^{131}I$ injection in a fresh water catfish (Mystus vittatus) and that this effect was not mediated by the pituitary gland. Sage and Bromage (1970) demonstrated that methyltestosterone directly stimulated the thyroid in Poecilia reticulata.

T_3 is formed in peripheral tissues in trout by removal of an iodine atom from the 3' or 5' position of the phenolic ring of T_4. Deiodination of T_4 readily occurs in brook and rainbow trout (Higgs and Eales, 1977; Eales, 1977b). Elevations in plasma T_3 levels may indicate increased conversion of T_4 to T_3. Higher TP doses tended to produce corresponding elevations in plasma T_3 levels. This was observed at the 300 ug dose level in Experiment 1, at all dose

levels in Experiment 2 and with 30 ug TP in Experiment 4.

3. Plasma Protein Binding of Thyroid Hormones

Increases in plasma T_4 or T_3 levels may result from an enhanced binding to plasma proteins. T_4 and T_3 are bound to plasma proteins in trout (Falkner and Eales, 1973). To investigate if elevated plasma T_4 and T_3 levels may be due to increased hormone binding capacity of plasma; plasma protein concentration, T_4-binding, and T_3-binding were measured in Experiment 2.

An increase in plasma T_3 or T_4 may result from increased specific or non-specific hormone binding produced by the elevation of plasma protein concentration. No separation of plasma proteins was made to demonstrate if enhanced plasma T_4 and T_3-binding was the result of increased concentrations of specific or non-specific binding proteins. There was no correlation between plasma T_4 concentrations and T_4-binding plasma protein levels. The increase in plasma T_3 levels seemed correlated with elevated T_3-binding and plasma protein levels.

Androgens have been shown to have little or no effect on plasma protein levels in some fish (Aida et al., 1973; Baily, 1957). Estrogens produce marked elevations in plasma protein levels in some teleosts (Aida et al., 1973; Baily, 1957; Ho and Vanstone, 1961). Methyltestosterone significantly increased total plasma solids (protein and lipid) in the mullet (Peterson and Shehadeh, 1971). There was also an increase in certain plasma protein fractions in these fish. Hirose and Hibiya (1968) found that total serum protein levels were elevated in sexually inactive rainbow trout injected with 4-chlorotestosterone. The albumin/globulin ratio was reduced in these fish. Serum protein concentrations were elevated in female spiny-tailed lizards (Uromastix hardwickii) receiving 19-nortestosterone (Rao and David, 1967). Alterations of plasma protein levels could account for variation in plasma T_4 and T_3 concentrations.

Experiment 3 investigates the possibility that the observed increased levels of plasma T_3 and T_4 in previous experiments was secondary to elevated plasma protein binding capacity of thyroid hormones. Plasma T_4 levels were significantly elevated in fish 24 hr following the injection of 30 ug TP. Plasma T_3, T_3-binding, T_4-binding, or protein levels were unaltered in these fish. TP appears to stimulate

T_4 release from the thyroid without significantly altering plasma binding of thyroid hormones.

4. Iodide Metabolism

TP altered various parameters of iodide metabolism in rainbow trout. Plasma inorganic iodide levels were significantly greater in TP-injected fish. Huang and Hickman (1968) demonstrated that male whitefish and northern pike plasma had a significantly higher iodide binding capacity than the plasma of female fish. Increased plasma iodide concentrations in the TP-injected fish may have been due to an enhanced iodide binding capacity created by elevated plasma protein levels. Huang and Hickman (1968) showed that there was no significant positive correlation between iodide binding capacity and plasma albumin-like protein concentration in whitefish or northern pike. Gregory (1973) demonstrated that plasma protein affinity for iodide or plasma iodide levels were unrelated to plasma protein concentrations in the brook trout. Iodide binding capacity is correlated with plasma protein concentrations in Atlantic salmon, Salmo salar (Leloup, 1970). TP could enhance the synthesis of the specific iodide-binding protein iodurophorine which is present in the plasma of species of the order Clupeiformes (Perrier et al., 1976). Plasma levels of this particular protein were not determined in my study. TP may

enhance iodide uptake from the gut or from the water. Leloup (1970) found that T_3 and T_4 could stimulate the uptake of iodide from the water by the gills, T_3 being more potent than T_4.

Estimates of the $^{131}I^-$ distribution space and fractional turnover were slightly lower in trout receiving TP. TP-injected fish cleared slightly more iodide/day from the plasma (3.82 ug versus 3.55 ug) than the control fish. This was due to the greater plasma iodide pool size in these fish. Iodide excretion rates greatly exceed the hormonal iodide degradation rates for these fish. Iodide excretion rates have not been reported previously for teleosts.

5. Thyroid Iodide Uptake

Rates of thyroid $^{131}I^-$ and $^{125}I^-$ uptake were greater in the TP-injected trout. Although the thyroid accumulated $^{131}I^-$ at a greater rate, early sampling periods (0.5 - 12 hr pi) revealed a smaller percentage of the injected dose present in the thyroid of these fish. This may be due to rapid recycling of iodide by the gland, indicated by the presence of significant amounts of ^{131}I-T_4 and ^{131}I-T_3 in the plasma. It is conceivable that small, active thyroid follicles may be responsible for rapid turnover of iodide while larger, less active follicles account for the overall accumulation and storage of iodide. van Overbeeke and McBride (1971) showed that the major effect exerted by

11-ketotestosterone and 17 α-methyltestosterone on the thyroid of gonadectomized male sockeye salmon was noted in small follicles. The large follicles were no different than those of the control fish. A similar effect was noted in Experiment 1. In my study it appears that thyroidal turnover and accumulation of iodide is enhanced by TP.

Singh (1969) showed that thyroid ^{131}I uptake was significantly elevated 24 hr following $Na^{131}I$ injection in hypophysectomized Mystus vittatus which had been treated with TP for three weeks.

Lowenstein and Stebbins (1969) found that thyroid $^{131}I^-$ uptake was much greater in male lava lizards (Tropidurus albemarkenis) than in females. TP significantly elevated thyroid $^{131}I^-$ uptake and serum PBI in castrated male Indian garden lizards (Calotes versicolor) (Chandola et al., 1973). Androgens appear to greatly influence thyroid activity in some reptiles (Chandola et al., 1973).

6. Production ^{131}I-labeled T_3 and T_4

Plasma analysis revealed a significant production of ^{131}I-T_4 and ^{131}I-T_3 in both groups shortly following $Na^{131}I$ injection. It appears that the thyroid tissue of rainbow trout can rapidly cycle iodide. Iodide is taken up from the plasma by the thyroid, incorporated into iodothyronines and then released as T_4 which is rapidly converted to T_3.

Bibor and Leray (1973) reported significant $PB^{125}I$ levels in the plasma of the yellow perch shortly following $Na^{125}I$ injection.

The plasma of the TP-injected trout contained more $^{131}I\text{-}T_3$ but less $^{131}I\text{-}T_4$ than the plasma of the control fish at most sampling times. This strengthens the findings revealed by other measurements that TP enhances deiodination in these fish. Lower plasma $^{131}I\text{-}T_4$ levels in the TP-injected fish may be due to its more rapid degradation and conversion to T_3.

7. T_4 Metabolism

T_4 was cleared more rapidly from the plasma of trout injected with TP than from the plasma of control fish. Although plasma T_4 concentrations were virtually identical, compartmental analysis revealed a greater fractional turnover (*Kob*) and plasma pool size (*Qa*) in the TP-injected fish. The extravascular T_4 pool size (*Qb*) was smaller in the TP-injected trout but was exchanged much more rapidly with the plasma (*Fab*). The initial or "fast" phase describing $^{125}I\text{-}T_4$ loss from plasma was similar for both groups. However, the "slow" phase appears to make a greater contribution to the overall T_4 kinetics in the TP-injected fish. The physiological basis of an increased emphasis on the "slow" component in these fish is unclear.

Although increased thyroid activity has been indicated in teleosts receiving androgens, no estimates

of T_4DR have been made for these fish (Matty, 1960; Singh, 1969; van Overbeeke and McBride, 1971; Higgs et al., 1977).

Kumaresan and Turner (1968) showed that thyroid hormone secretion rate (TSR) was increased by 25% in normal male rats injected with TP. Gonadectomy of similar rats resulted in a significant reduction of TSR. T_4SR of male mice was shown to be significantly higher than that of virgin female mice (Wills et al., 1970). Fisher and Oddie (1968) demonstrated a 10% increase in the fractional turnover rate of T_4 in adult male humans receiving methyltestosterone. T_4 turnover rate decreased 13% due to a decrease in plasma T_4 levels created by a drop in TBG T_4-binding capacity. The anabolic steroid norethandrolone decreased serum PBI, but increased the fractional rate of T_4 turnover in humans (Braverman and Ingbar, 1967). Total daily T_4 disposal was unaltered because of the decrease in PBI.

8. Deiodination and T_3 Generation

T_4 deiodination rate was greater in trout injected with TP. This was reflected in increased levels of plasma $^{125}I^-$ and ^{125}I-T_3 shortly after the injection of ^{125}I-T_4. In addition, the thyroid $^{125}I/^{131}I$ ratio was greater in the TP-injected trout. Estimates of T_3 generation determined by plasma radioiodide kinetics

were 4.04 ng/hr (control) and 6.40 ng/hr (TP-injected). The higher T_4 deiodination rate of the TP-injected trout appears responsible for increased plasma T_3 levels in fish treated with TP.

Androgens are anabolic hormones which produce a 'positive nitrogen balance' in the organism (Kruskemper, 1968). They have been used as growth promoters in salmonid culture (Higgs et al., 1977; McBride and Fagerlund, 1973; Yamazaki, 1970). An elevation in plasma T_3 levels may be the consequence of an increased demand for thyroid hormone in the 'androgen-activated' fish. There is increasing evidence that conversion of T_4 to T_3 is necessary in order to activate the hormone in mammals. T_3 may contribute 85-90% of the activity of thyroid hormones in the euthyroid rat (Surks and Oppenheimer, 1977). In my study it is impossible to discern if elevated T_3 levels are the consequence of an increased requirement for T_3 produced by a stimulating action of TP on the metabolism of peripheral tissues or by a direct effect of TP upon tissues which catalyze the conversion of T_4, or by both pathways.

9. Enterohepatic Excretion

TP altered the kinetics of ^{125}I-radioactive uptake and disappearance in the enterohepatic organs of the trout. Interpretative problems arise in this area since the relative proportions of $^{125}I\text{-}T_3$, $^{125}I\text{-}T_4$,

and $^{125}I^-$ in these tissues are unknown. The enterohepatic organs (gall bladder and intestine) of both groups took up a large percentage of the injected dose of ^{125}I-T_4. Bilary excretion is the major pathway for radiothyroxine excretion by the book and rainbow trout (Sinclair and Eales, 1972). T_3 is also lost via this route in brook trout (Eales et al., 1971). The gall gladder of the control fish accumulated significantly more ^{125}I-radioactivity by 6 hr pi than the TP-injected fish. Radioactivity dropped rapidly between 6 and 12 hr pi in both groups. This decrease was most likely associated with feeding at 8 hr pi. Feeding causes the discharge of radiohormone from the gall bladder of brook trout (Eales and Sinclair, 1974).

^{125}I-radioactive loss from the enterohepatic organs was greater in the control fish. Maximum tissue uptake was also greater in these fish. However rate constants for intestinal and total enterohepatic uptake of radioactivity were greater in the TP-injected trout. The relative amounts of radioactivity associated with gut contents or with tissues was unknown. The intestine may be a target site for thyroid hormone action in fish. Collicutt and Eales (1974) showed that intestinal tissue contained ^{125}I-radioactivity 96 hr following the injection of ^{125}I-T_4 into the eye of starved channel catfish with a ligated bile duct. Higgs (1974) suggested that thyroid

hormones may influence the activity of intestinal cells in brook trout. Homogenates of brook trout intestine readily deiodinate T_4 (Law and Eales, 1973). TP could enhance intestinal uptake of thyroid hormones from plasma. Diminution of the rate of ^{125}I-radioactive loss 12-96 hr pi from the enterohepatic organs of the TP-injected trout may have been due to reduced transfer of ^{125}I-T_3 and ^{125}I-T_4 from the liver. ^{12-}I-T_3 produced from ^{125}I-T_4 may be excreted at a proportionately slower rate by the enterohepatic organs of the TP-injected fish because of its greater dilution within the plasma T_3 pool. Less ^{125}I-T_3 would be lost/hr via this route unless the fractional turnover of T_3 was greatly increased or the T_3 distribution space greatly reduced. The absolute quantity of T_3 lost per hr may actually be greater in the TP-injected trout. This argument cannot be substantiated since no separation of T_4 or T_3 in these organs was made. A separate study examining T_3 kinetics in TP-injected fish could clarify some of these points.

10. Muscle and Liver

Skeletal muscle accumulated a moderate percentage of the injected ^{125}I-T_4 dose in both groups. Considering the large muscle mass of fish, muscle takes up a considerable proportion of the injected dose. Radioactivity declined between 0.5 and 2 hr pi in both groups before rising to maximal uptake values at 6 hr pi

in the TP-injected fish and 12 hr pi in the control fish. The basis for the decline of muscle ^{125}I-radioactivity between 0.5 and 2 hr pi was undetermined. ^{125}I-radioactive loss from muscle 24 - 96 hr pi paralleled $^{125}I^-$ loss from plasma in both groups. The majority of ^{125}I-radioactivity retained in muscle at later sampling times appears to be inorganic iodide.

Rapid uptake (2-6 hr pi) of radioactivity by muscle of the TP-injected fish may indicate an increased requirement for thyroid hormone in these fish. Muscle appears to be a target site for thyroid hormones in teleosts. Brook trout muscle homogenates readily deiodinate T_4 (Law and Eales, 1973). Thyroid hormones stimulate muscle protein synthesis in fish (Thornburn and Matty, 1963; Jackim and LaRoche, 1973).

The anabolic steroids norethandrolone and dimethazine stimulated the incorporation of ^{14}C-1-leucine into skeletal muscle and accelerated the growth rate of rainbow trout (Cheema and Matty, 1977). Although thyroid or androgenic hormones may separately enhance protein synthesis in skeletal muscle, increased amounts of thyroid hormone may be required in fish treated with androgens in order to optimize tissue responses.

The hepatosomatic index (HSI) was significantly

reduced in TP-injected fish. No qualitative analysis was made of the livers but TP may increase utilization of liver glycogen. Hoar (1958) reported that gonadal steroids stimulated oxygen consumption in the goldfish. Depletion of liver glycogen may occur in response to increased energy requirements of these fish. Dickson and Kramer (1971) demonstrated that active metabolism of hatchery rainbow trout was highest at spawning and was consistently higher in males than females throughout the year. Hirose and Hibiya (1968) showed that the anabolic steroid 4-chlorotestosterone acetate caused liver hypertrophy in sexually immature rainbow trout by increasing glycogen content of liver cells. Deposition of liver glycogen in these fish perhaps was facilitated by the food ration (5% wet body weight/day) used.

The pattern of ^{125}I-radioactive loss from the liver was distinct for each group. The initial uptake of ^{125}I-radioactivity was greater in the control fish. No explanation for differences in the disappearance curves is evident but TP may alter the fashion in which the liver treats thyroid hormone. Anabolic steroids may reduce the excretory ability of the liver (Kruskemper, 1968). Chronic administration of 11-ketotestosterone or 17 α-methyl-testosterone produced degenerative features in the livers of gonadectomized male sockeye salmon (McBride

and van Overbeeke, 1971). Fisher and Oddie (1968) noted a mild impairment of liver function and T_4 turnover rate in humans treated with 17 α-methyltestosterone.

B. Mechanism and Significance of Thyroidal Activation by TP

Despite the presence of many TP-induced alterations in thyroid activity, the mechanisms underlying these changes are unclear. The basis of these changes may relate to a direct effect of TP upon the thyroid-pituitary axis or could occur secondarily to a general stimulation of the metabolism of the fish.

In Experiment 3, plasma T_4 levels were significantly increased 24 hr following a single injection of 30 ug TP. Plasma T_3 levels and protein binding were unaltered in these fish. TP can elicit a relatively rapid release of T_4 from the thyroid, indicating sensitivity of the trout thyroid system to TP.

TP increased plasma protein binding of T_4 and T_3. This effect was poorly correlated with plasma T_4 levels while increases in plasma T_3 levels generally paralleled elevations in plasma protein binding. Enhanced protein binding of thyroid hormones could account for elevations in plasma T_4 or T_3 levels but not for an increase in hormone turnover.

Androgens may indirectly stimulate thyroid

activity by increasing the general metabolism of peripheral tissues. Increased thyroid activity may be the result of an elevated requirement for thyroid hormones in the androgen-treated trout.

It is postulated that the control of thyroid function evolved from that of the gonad (Sage, 1973). The presence of cycles of thyroid activity associated with reproduction in teleosts supports this idea. Sex steroids similar to those found in high concentrations in the plasma of maturing fish can stimulate thyroid activity in experimental fish (Singh, 1968; van Overbeeke and McBride, 1971; Higgs et al., 1977). However the thyroid gland of anadromous salmonids such as Pacific salmon (Robertson and Wexler, 1960), steelhead trout (Robertson and Chaney, 1953), and Atlantic salmon (Fontaine and Leloup, 1962) may exhibit low activity during the time up to and during reproduction. These fish undertake arduous migrations and rarely feed during the latter stages of maturation. Reduced thyroid activity in these fish may be due to fasting and the stress of migration and not to high plasma levels of sex steroids. The thyroid of spawning non-migratory

rainbow trout may appear histologically normal or even slightly activated. These fish feed up to and during spawning (Robertson et al., 1961). Feeding prevented the degeneration of thyroid function frequently observed in sexually mature sockeye salmon (McBride, 1967).

It is often difficult to correlate laboratory results with observations from nature. Fed fish held under controlled conditions may respond more positively to an anabolic agent than sexually maturing, starved fish fending against the elements. Activation of thyroid function may not have occurred in the TP-injected trout if they had been starved. Recent studies indicate that feeding greatly influences thyroid function in fish (Higgs, 1974; Brown, 1977; Higgs and Eales, 1977, 1978).

An increase in the uptake of ^{125}I-radioactivity by skeletal muscle in the TP-injected trout may indicate an increased requirement for thyroid hormone by this tissue. Thyroid hormones may participate in anabolic events originally precipitated by TP.

C. Observations on Some Basic Concepts of Trout Thyroid Function

A number of observations obtained in this study but not directly related to the effects of TP may lend themselves to a fuller understanding of thyroid function in the trout.

1. Comparison of Various Parameters for the Evaluation of Thyroid Status in the Trout

The use of a number of methods for evaluating TP-induced changes in trout thyroid activity in Experiment 4 provides for an assessment of the relative validity of each procedure.

Measurement of plasma T_4 and T_3 levels indicates little of their rate of turnover. In Experiment 4 plasma T_4 levels were identical in the control and TP-injected fish although T_4DR was greater in the TP-injected fish. Determination of T_4DR requires large numbers of fish and lengthy sampling periods but an accurate estimation of thyroid secretion is obtained.

Histological assessment of thyroid activity in fish may not correspond well with radiochemical methods (Drury and Eales, 1968). In my study there appears to be little correlation between thyroid cell height and plasma T_4 levels. Plasma T_3 levels appear somewhat correlated with thyroid cell height.

The percentage of the injected dose in the thyroid, 24 hr following radioiodide injection, is frequently used to assess thryoid activity in fish. My study revealed that thyroid $^{131}I^-$ uptake values were similar in each group 24 hr following $Na^{131}I$ injection, although the slopes of the regressions for $^{131}I^-$ uptake were significantly different. This indicates

the danger of using only one sampling period to compare thyroid iodide uptake between groups. The overall uptake of iodide by the thyroid appears related to T_4 secretion.

Measurement of plasma labeled thyroid hormones at various times following radioiodine injection gives little information concerning rates of hormone turnover, although it may indicate if a tendency towards a prominence of plasma T_3 or T_4 exists in a group of fish. This method parallels the PBI technique except that hormonal protein bound iodine is divided into T_4 and T_3 fractions.

Deiodination studies indicate the percentage of T_4 undergoing conversion to T_3 and when combined with the T_4DR a rate of T_4 deiodination is obtained. The rate of T_4 to T_3 conversion is an important measure of thyroid status, since T_3 may be the active form of thyroid hormone in fish and all T_3 must be produced from T_4. The existence of elevated plasma T_3 levels in the TP-injected fish may be directly related to the greater T_4 deiodination rate in these fish.

Measurement of plasma protein-binding of thyroid hormones provides little information concerning hormone turnover. Protein-binding of T_4 and T_3 as measured in this study may be a function of plasma protein concentration.

Analysis of various tissues and organs following ^{125}I-T_4 injection indicates the rate at which ^{125}I-radioactivity may be taken up or lost. Although some treatments may alter the fashion in which the enterohepatic organs, liver, or muscle of the fish handles thyroid hormone, some reservations must be made in the interpretation of this information since the relative proportions of ^{125}I-T_4, ^{125}I-T_3, and $^{125}I^-$ in these tissues are unknown.

2. Iodide Excretion Rate and Relation to Thyroidal Iodine Demand

The iodide excretion rate greatly exceeded the hormonal iodine degradation rate for control and TP-injected trout. The trout thyroid system appears to be somewhat inefficient in its utilization of inorganic iodide for thyroid hormone production. It is conceivable, however, that some iodide may be required in other areas of the fish rather than solely in the thyroid. High levels of iodide relative to organic iodine have been noted in the plasma of rainbow trout (Fontaine and Leloup, 1959; McNabb, 1963; La Roche et al., 1965).

3. Thyroid Iodide Uptake

The pattern of thyroid $^{131}I^-$ uptake was distinct for each group. Early sampling periods revealed that the thyroid tissue of the TP-injected fish accumulated

less $^{131}I^-$ than that of control fish. This appears to be related to rapid cycling of iodide in the TP-injected trout as indicated by the presence of significant quantities of ^{131}I-T_4 and ^{131}I-T_3 in the plasma. In latter sampling periods, the thyroid tissue of the TP-injected fish accumulated more $^{131}I^-$ than the control fish.

The existence of apparent 'fast' and 'slow' components for the uptake of $^{131}I^-$ by the thyroid could be due to the presence of two or more forms of the iodoprotein thyroglobulin (TGB) in the thyroid follicles. The major form of TGB in the vertebrate thyroid has an ultracentrifugatory coefficient of 17-19s (Roche et al., 1968). Small proportions of 3-8s (5s), 12s, and 27s TGB forms exist in vertebrates (Lachiver et al., 1965). Roche et al. (1968) showed that the smallest protein present in soluble thyroid extracts is the first to be iodinated following a single pulse-label with radioiodine. Heavier TGB components were iodinated at a slower rate but accumulated a greater proportion of the label. Rapid or long term turnover of thyroidal iodide could be explained by the presence of various TGB forms possessing inherent iodide handling capacities. It is unknown whether each TGB form is contained within separate follicles or contained within each follicle.

4. Rapid Cycling of Iodide

The existence of rapid cycling of iodide has implications for any study of radioiodide or

radioiodide-labeled T_4 kinetics in trout. Earlier studies on channel catfish (Collicutt and Eales, 1974) and brook trout (Higgs and Eales, 1976; 1977) assumed that negligible errors in ^{125}I-T_4 kinetics arose due to recycling of $^{125}I^-$ derived from ^{125}I-T_4 monodeiodination. My study indicates that iodide can be rapidly utilized by the thyroid and released as labeled hormone. Addition of thyroidal ^{125}I-T_4 to the plasma would alter ^{125}I-T_4 degradation kinetics.

The presence of ^{131}I-T_3 in the plasma indicates that ^{131}I-T_4 deiodination occurs shortly after its release into the plasma. If ^{131}I-T_3 is formed by the loss of a ^{131}I-labeled atom from the 3' or 5' position of T_4, $^{131}I^-$ will be added to the plasma. This could confound ^{131}I kinetics by increasing plasma $^{131}I^-$ levels. For these reasons, it may be advisable to conduct this type of study on fish in which iodide recycling has been blocked by perchlorate or some other competitive inhibitor.

5. A "Ligand" Approach to T_4 Kinetic Analysis

A two-compartment model describing T_4 loss from plasma assumes that T_4 is secreted into, and cleared from the plasma compartment "a". Some two-way exchange of T_4 occurs between compartment "a" and compartment "b", the extravascular pool, and that T_4 is eventually lost from compartment "b" via degradation and excretion pathways (Eales, 1977). However, once T_4 has left the plasma it may be

deiodinated to form T_3 or excreted in the bile as T_4 or its glucuronide conjugate (T_4G) (Sinclair and Eales, 1972). Negligible reabsorption of bilary-excreted T_4 or T_4G occurs in fed brook trout (Eales and Sinclair, 1974). T_4 deiodination has been demonstrated in vitro in several brook trout tissues (Law and Eales, 1973). Conversion of T_4 to T_3 readily occurs in vivo in brook and rainbow trout (Eales, 1977; Higgs and Eales, 1977; this study). As a consequence of deiodination and bilary excretion only small amounts of T_4 may be available for tissue-plasma exchange.

The obtained T_4 loss curves may conform to a kinetic model based on T_4 interactions with plasma proteins. Eales (unpublished data) has shown that T_4 is bound to two plasma proteins; one of high affinity and low capacity T_4-binding sites, and one of low affinity and high capacity T_4-binding sites. Following injection, T_4 is distributed between the proteins in a manner associated with the relative binding affinities of the proteins. There is also a small proportion of T_4 (0.5%) which exists in the unbound or free form (Falkner and Eales, 1973). T_4 may be more rapidly lost from the protein with the low affinity high capacity binding sites than from the high affinity low capacity protein. T_4 loss from these proteins may comprise fast and slow components.

On the basis that such a model exists, the T_4 distribution between proteins can be determined by extrapolating the fast and slow component lines to the y axis. The relative proportion of the individual intercepts to their total sum indicates the percentage of plasma T_4 bound to each protein. The amount of T_4 bound by each protein is obtained by multiplying T_4 pool size ($Q\alpha$) by the relative T_4 distribution values. T_4 loss from each protein is the product of the rate constant (α or β) and the protein-bound T_4 concentration. T_4DR is the sum of rate loss from each protein.

Using this procedure, T_4DR values were 9.89 ng/hr (control) and 13.21 ng/hr (TP-injected). Although these values differed little from those obtained by compartmental analysis, the framework of the analysis is radically altered. Differences in the <u>configuration</u> of T_4 loss curves may be explained by variations in plasma T_4-binding protein concentrations rather than by ill-defined compartments. Fisher and Oddie (1968) reported that methyltestosterone significantly altered the electrophoretic distribution patterns of ^{131}I-T_4 among T_4-binding proteins in male humans. The steroid enhanced T_4-binding to thyroxine-binding prealbumin (TBPA) while reducing binding to thyroxine-binding globulin)TBG). Androgens increase the synthesis of TBPA in humans (Braverman <u>et al.</u>, 1968).

TP elevated total plasma protein levels in my study, but it is unknown whether the synthesis of specific T_4-binding proteins was altered. Peterson and Shehadah (1971) reported an increase in certain plasma protein fractions as well as total plasma solids in the mullet (Mugil cephalus) injected with methyltestosterone.

6. Thyroid $^{125}I/^{131}I$ Ratio for the Evaluation of T_4 Deiodination

Collicutt and Eales (1974) suggested that the thyroid ratio of $^{125}I/^{131}I$ following simultaneous ^{125}I-T_4 and $Na^{131}I$ injection could be used as an index of ^{125}I-T_4 deiodination. The T_4 deiodination rate for control fish obtained by this method compares favourably with that derived from plasma radioiodide kinetics. However this method appeared to overestimate T_4 deiodination in the TP-injected trout. No explanation is evident for this discrepancy. The relative stability of the thyroid $^{125}I/^{131}I$ ratio from 24-96 hr pi sampling times allows for the determination of an index of T_4 deiodination. An index of T_4 deiodination for a small number of fish could be obtained by sampling the thyroid tissue 1-4 days following simultaneous ^{125}I-T_4 and $Na^{131}I$ injection.

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Appendix Table 1

Percent recovery (% R) of ^{125}I-T_3 added to a series of T_3 concentrations (0 - 800 ng%) and incubated with T_3-antibody for 30 min on G-25 Sephadex columns. Each determination is the mean of triplicates.

Added T_3 (ng%)	% R
0	98.5
200	97.0
800	96.3

Appendix Table 2. Mean ($\bar{x}$) original and final body weights with standard error of the mean (SEM) for trout in Experiment 1. Percentage increases from original body weight are given.

Group	Original Body Weight		Final Body Weight		% Change
	$\bar{x}$ (g)	SEM	$\bar{x}$ (g)	SEM	
Control	49.3	2.97	52.0	3.19	5.5
3 ug TP	47.9	2.36	49.7	2.96	3.8
30 ug TP	49.4	2.07	52.3	2.16	5.9
300 ug TP	53.7	1.89	56.3	2.69	4.8

Appendix Table 3. One-way ANOVA for plasma T_4, T_3 and thyroid epithelial cell heights, and original and final body weights in trout from Experiment 1.

	Bartletts X^2	Significance	ANOVA F-value	Significance
T_4	8.95	NS	3.58	*
T_3	1.11	NS	4.21	*
Cell height	12.00	NS	122.75	**
Original Wt.	2.37	NS	1.12	NS
Final Wt.	1.12	NS	0.99	NS

* Level of significance 0.05

** Level of significance 0.01

Appendix Table 4. Mean ($\bar{x}$) original and final body weights with standard error of the mean (SEM) for fish in Experiment 2. Percentage increases from original body weight are given.

Group	Original Body Weight		Final Body Weight		% Change
	$\bar{x}$ (g)	SEM	$\bar{x}$ (g)	SEM	
Control	94.9	2.17	113.8	4.21	19.9
3 ug TP	92.9	2.71	101.4	1.69	9.2
30 ug TP	99.4	3.53	109.6	2.95	10.3
300 ug TP	95.1	5.74	110.9	6.07	16.6

Appendix Table 5. One-way ANOVA for plasma T_4, T_3, T_4 (B/F), T_3 (B/F), protein, and original and final body weights in trout from Experiment 2.

	Bartletts X^2	Significance	ANOVA F-value	Significance
T_4	7.74	NS	10.03	**
T_3	1.82	NS	7.26	**
T_4 (B/F)	6.27	NS	15.68	**
T_3 (B/F)	9.38	NS	13.83	**
Protein	1.42	NS	14.64	**
Original Wt.	9.27	NS	0.53	NS
Final Wt.	12.80	NS	1.41	NS

** Level of significance 0.01

Appendix Table 6. Covariance analysis of regression lines for the correlation of the percent of the injected dose $Na^{131}I$ in the thyroid region versus time (0.5 - 96 hr pi) in 77 control and 73 TP-injected rainbow trout.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Control	32.35	0.97	33.38	0.95	0.34	0.0194	0.68	65.1**	1,75
TP-injected	28.78	1.18	32.40	1.55	-0.12	0.0453	0.95	594.0**	1,71

Tests: 1) Homogeneity of within group variances

F = 1.88 (df = 75,71) NS

2) Comparison of slopes

F = 70.7** (df = 1,146)

** Level of significance 0.01

Appendix Table 7. Covariance analysis of regression lines for the correlation of the percentage of the injected dose $^{125}I\text{-}T_4$ in the thyroid region versus time (24 - 96 hr pi) in 39 control and 31 TP-injected trout. Y values have been multiplied by two to correct for labeling of $^{125}I\text{-}T_4$.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Control	59.08	0.69	26.89	0.58	0.06	0.0106	0.49	11.7**	1,37
TP-injected	58.07	1.67	28.24	1.26	-0.63	0.0395	0.89	105.3**	1,29

Tests: 1) Homogeneity of within group variances

F = 1.35 (df = 37,29) NS

2) Comparison of slopes

F = 35.31** (df = 1,66)

** Level of significance 0.01

Appendix Table 8. Covariance analysis of regression lines for the correlation of percent dose $Na^{131}I$ and $^{125}I\text{-}T_4$ in the thyroid region versus time (24 - 96 hr pi) in 39 control fish.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
$^{125}I\text{-}T_4$	59.08	0.69	26.89	0.58	0.06	0.0106	0.49	11.7**	1,37
$Na^{131}I$	59.08	1.51	26.89	1.07	0.47	0.0176	0.44	9.0**	1,37

Homogeneity of within group variances

F = 3.61** (df 37,37)

Variances are not homogeneous and it is invalid to compare regressions

** Level of significance 0.01

Appendix Table 9. Covariance analysis of regression lines for the correlation of percent dose $Na^{131}I$ and $^{125}I\text{-}T_4$ in the thyroid region versus time (24 - 96 hr pi) in 33 TP-injected fish.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
$^{125}I\text{-}T_4$	58.07	1.67	28.24	1.26	-0.63	0.0395	0.89	105.3**	1,29
$Na^{131}I$	57.46	2.42	28.10	1.60	-0.52	0.0511	0.90	128.8**	1,31

Tests: 1) Homogeneity of within group variances

F = 1.45 (df = 31,29) NS

2) Comparison of slopes

F = 3.79 (df = 1,60) NS

3) Comparison of elevations

F = 21.20** (df = 1,61)

** Level of significance 0.01

Appendix Table 10. Covariance analysis of regression lines for the correlation of ln plasma $^{131}I^-$ and $^{125}I^-$ versus time (12 - 96 hr pi) in control fish.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
$^{131}I^-$	49.47	1.35	30.66	0.59	2.03	-0.0137	-0.71	47.1**	1,47
$^{125}I^-$	49.47	-0.03	30.66	0.64	0.54	-0.0115	-0.55	20.6**	1,47

Tests: 1) Homogeneity of within group variances

F = 1.60 (df = 47,47) NS

2) Comparison of slopes

F = 0.49 (df = 1,94) NS

3) Comparison of elevations

F = 200.0** (df = 1,95)

** Level of significance 0.01

Appendix Table 11. Covariance analysis of regression lines for the correlation of ℓn plasma $^{131}I^-$ and $^{125}I^-$ versus time (12 - 96 hr pi) in TP-injected fish.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
$^{131}I^-$	46.88	1.59	31.29	0.45	2.18	-0.0125	-0.88	135.01**	1,41
$^{125}I^-$	46.83	0.44	31.61	0.42	0.86	-0.0091	-0.69	35.49**	1,39

Tests: 1) Homogeneity of within group variance

F = 1.95 (df = 41,39) NS

2) Comparison of slopes

F = 3.41 (df = 1,80) NS

3) Comparison of elevations

F = 391.7** (df = 1,81)

** Level of significance 0.01

Appendix Table 12. Covariance analysis between regression lines for Zn plasma $^{131}I^-$ versus time (12 - 96 hr pi) in 49 control and 43 TP-injected trout.

	Means		Standard Deviation		Regresstion		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Control	49.47	1.35	30.66	0.59	2.03	-0.0137	-0.71	47.1**	1,47
TP-injected	46.88	1.59	31.29	0.45	2.18	-0.0125	-0.88	135.0**	1,41

Comparison of homogeneity of variances

F = 3.78** (df = 47,41)

∴ Variances are not homogeneous and it is invalid to compare regressions

** Level of significance 0.01

Appendix Table 13. Covariance analysis between regression lines for ^{125}I-Zn plasma versus time (12 - 96 hr pi) in 49 control and 41 TP-injected trout.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Control	49.47	-0.027	30.66	0.64	0.54	-0.0115	-0.55	20.6**	1,47
TP-injected	46.83	0.435	31.61	0.42	0.86	-0.0091	-0.69	35.5**	1,39

Comparison of homogeneity of variances

F = 3.10** (df = 47,39)

** Level of significance 0.01

Appendix Table 14. Covariance analysis between regression lines for the correlation of ln percentage of the dose ^{125}I-T_4 versus time (12 - 96 hr pi) in the intestine of 48 control and 42 TP-injected trout.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Control	49.0	1.84	30.80	1.21	3.65	-0.0370	-0.95	397.0**	1,46
TP-injected	46.3	1.90	31.42	1.01	3.21	-0.0283	-0.88	140.5**	1,40

Tests: 1) Homogeneity of within group variances

F = 1.50 (df = 46,40) NS

2) Comparison of slopes

F = 8.55** (df = 1,86)

** Level of significance 0.01

Appendix Table 15. Covariance analysis of regression lines for the correlation of ln percentage of the dose ^{125}I-T_4 versus time (12 - 96 hr pi) in the enterohepatic organs of 48 control and 41 TP-injected trout.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Control	49.0	1.90	30.80	1.18	3.67	-0.0361	-0.94	351.1**	1,46
TP-injected	46.8	2.02	31.61	0.97	3.34	-0.0283	-0.92	209.5**	1,39

Tests: 1) Homogeneity of within group variances

F = 1.09 (df = 39,46) NS

2) Comparison of slopes

F = 8.15** (df = 1,85)

** Level of significance 0.01

Appendix Table 16. Covariance analysis of regression lines for the correlation of ℓn plasma $^{125}I^-$ and ℓn muscle ^{125}I-radioactivity versus time (24 - 96 hr pi) in 39 control trout.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Plasma	59.08	-0.13	26.89	0.65	0.57	-0.0118	-0.49	11.73**	1,37
Muscle	59.08	-1.90	26.89	0.69	-0.72	-0.0199	-0.78	58.15**	1,37

Tests: 1) Homogeneity of within group variances

F = 1.75 (df = 37,37) NS

2) Comparison of slopes

F = 3.47 (df = 1,74) NS

3) Comparison of elevations

F = 228.4** (df = 1,75)

** Level of significance 0.01

Appendix Table 17. Covariance analysis of regression lines for the correlation of ln plasma $^{125}I^-$ and ln muscle ^{125}I-radioactivity versus time (24 - 96 hr pi) in 31 TP-injected trout.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Plasma	68.07	0.36	28.24	0.40	0.96	-0.0104	-0.73	32.7**	1,29
Muscle	58.07	-1.42	28.24	0.46	-0.73	-0.0118	-0.73	33.4**	1,29

Tests: 1) Homogeneity of within group variances

F = 1.25 (df = 29,29) NS

2) Comparison of slopes

F = 0.26 (df = 1,58) NS

3) Comparison of elevations

F = 551.8** (df = 1,59)

** Level of significance 0.01

Appendix Table 18. Covariance analysis of regression lines for ln muscle ^{125}I-radioactivity (% dose ^{125}I-T_4/g tissue/100 g body weight) versus time (24 - 96 hr pi) in 39 control and 31 TP-injected trout.

	Means		Standard Deviation		Regression		Correlation Coefficient	F-value	df
	x	y	x	y	a	b			
Control	59.08	-1.90	26.89	0.65	-0.72	-0.0199	-0.78	58.2**	1,37
TP-injected	58.07	-1.42	28.24	0.46	-0.73	-0.0118	-0.73	33.4**	1,29

Tests: 1) Homogeneity of within group variances

F = 1.88 (df = 29.37) NS

2) Comparison of slopes

F = 5.72* (df = 1,74)

* Level of significance 0.05

** Level of significance 0.01