DETERMINATION OF THYROID SECRETION RATES IN RAINBOW TROUT, SALMO GAIRDNERII, USING RADIOACTIVE IODINE

Thesis for the Degree of M. S.

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Jack Russell Hoffert

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DETERMINATION OF THYROID SECRETION RATES IN RAINBOW TROUT, *SALMO GAIRDNERII*, USING RADIOACTIVE IODINE

By

JACK RUSSELL HOFFERT

AN ABSTRACT

Submitted to the College of Science and Arts
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ABSTRACT

The control and regulation of metabolic activity by hormonal factors has been established in warm blooded vertebrates. The existence of similar hormonal systems in poikilothermic vertebrates and invertebrates has also been established. The functional and anatomical characteristics of these hormonal systems may vary from those found in higher warm-blooded vertebrates.

Thyroid tissue in the teleost is known to produce iodinated amino acids identical to those of mammals. The utilization of thyroidal compounds by the teleost does not follow the same pattern as in higher vertebrates since it has been shown that thyroidectomy does not change oxygen consumption in the teleost.

Estimation of glandular activity will aid in understanding the function of the thyroid hormone in teleosts. This may in turn strengthen our understanding of the thyroidal systems in other organisms.

The following thesis presents findings on the determinations of thyroid activity of rainbow trout, Salmo gairdnerii. The secretion rate determinations were based on changes produced on the I-131 output rate of thyroid tissue by administration of exogenous thyroxine. The assay for thyroid secretion rates has been satisfactorily applied to several species of warm-blooded vertebrates. Determination of thyroid activity by this method, has to the author's knowledge, never been applied to poikilothermic vertebrates.

Thyroid secretion rates for Salmo gairdnerii were found to range from 0.190 to 0.415 µgm. l-thyroxine/100 gms. body wt./ day. No significant difference in the mean thyroid secretion rates were found for any

of the experimental groups. Determinations run on two-year old and one-year old trout yielded mean secretion rates of 0.302 and 0.243 µgms. l-thyroxine/100 gms body wt. /day respectively. One-year old fish at 13°C. and 3°C. gave rates of 0.243 and 0.139 µgm./100 gm./day respectively.

Radioactive uptake and output studies indicated that the peak accumulation of approximately 20% of the injected dose occurred within 24 hours after a plerupoeritoneal injection of I-131. In vivo counting under the geometry described in the thesis, indicated a two component output curve. The first component ($t\frac{1}{2}$ = 9 days) is followed by a much slower output rate ($t\frac{1}{2}$ = 37 + days). The first component of this output curve may possibly be the result of the high accumulation of non-thyroidal I-131 activity occurring immediately after the injection of I-131. The site of maximal accumulation of non-thyroidal I-131 activity was shown to gradually shift anteriorly for 4 days following injection of I-131 until it reached the thyroidal area. After 4 days there was no further change in the position of maximal activity.

The mean values of oxygen consumption at 13°C. and 3°C. were found to be 0.087 and 0.040 ml./gm./hr. respectively. Operculum rates at 13°C. and 3°C. had values of 117 and 61 movements per minute respectively. Q_{10} values indicate that the oxygen consumption of trout follows the pattern of a normal thermochemical type of reaction (Q_{10} = 2.0) for the temperature range of 3°C. to 13°C. Oxygen consumptions and operculum rates yielded the following Q_{10} values: oxygen consumption = 2.17; operculum rates = 1.92. The Q_{10} value of the mean thyroid secretion rates for this temperature range was 1.72.

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INTRODUCTION

General Remarks

Animal physiology, a science of function, attempts to explain how an animal or its organs, tissues and cells perform their varied functions. The study of animal physiology has laid the foundations for our present understanding of the physiology of the higher forms of life, including man. Scientists dealing with more advanced living organisms have often found it necessary and helpful to turn to the study of either related or more primitive forms of life for a more complete understanding of a complicated biological phenomena.

Endocrinology, a field of physiology, has advanced rapidly in recent years. As with many fileds of science dealing in some way with medicine, endocrinology got its start through clinical medicine. That the control or regulation of cellular structures of an organism by internal secretions was a very early phylogenetic development has been shown through the study of various invertebrate phyla.

Investigations of animals, including vertebrates and protochordates, show that thyroid tissue exists in all vertebrates and in some of the protochordates (Lynn and Wachowski, 1951). Gudernatsch (1911) discovered that amphibian metamorphism was controlled by the thyroid gland. This opened wide the field of investigation dealing with thyroid tissue in lower vertebrates.

Literature Review

Anatomy and Embryological Development of the Teleost Thyroid.

The thyroid of fishes should not be regarded as a gland since in most cases thyroid follicles enclosed in a connective tissue capsule do not occue. A few discrete thyroid glands in fish have been described.

In the teleost Xiphias gladius (swordfish) a compact well circumscribed mass of thyroid tissue is found near the cephalic end of the ventral aorta (Addison and Richter, 1932). This gland is very vascular and deep red in color. Matthews and Smith (1948) found a compact thyroid gland in the parrot fish, Sparisoma.

Thyroid tissue develops early in teleosts. Stratified epithelium enlarges to form an unpaired structure on the ventral side of the pharynx, between the 1st. and 2nd. gill pockets. At this time the thyroid tissue is located near the tubular heart but a subsequent shift in position of the heart and ventral aorta causes a removal of the thyroidal tissue from its site of origin to its scattered location in mature fish. In adult fish thyroidal tissue is generally most dense in the location of the ventral aorta and/or branchial arteries. The thyroid tissue is brownish yellow in color, however, in most cases it is impossible to make a definite identification in teleosts without microscopic examination (Gudernatsch, 1911).

Through the use of radioautographs employing radioiodine Chavin (1956) showed that functional thyroidal material occurred in the throat and also in the lymphoidal pronephric remnants (the head kidney) of goldfish (Carassius auratus L.). Under thyroid hyperplasia follicles may appear in tissues adjacent to the throat regions and even in the kidney. Ninety percent of normal goldfish have thyroid follicles in the head kidney (Pickford and Atz, 1957) and this is not a pathological condition.

Histology of the Teleost Thyroid

There is a great variability in the histology and cytology of the thyroid of different individuals taken from the same environment. In warm-blooded vertebrates there is much less variation. Because of the

great variance among individuals, one must exercise caution with regard to conclusions concerning the functional state of the thyroid which are based on histological evidence alone.

The structural form of the thyroid follicle changes as the fish ages. The thyroid follicle cell of teleosts has a very prominent nuclei with scanty cytoplasm, and the cell membranes are usually hard to see. The shape of the nuclei vary with the condition of the gland. The thyroid follicle is supported by loose connective tissue (Hoar, 1939).

Blood Supply to the Thyroid Tissue

Gudernatsch (1911) reports that the thyroid artery arises from the dorsal branch of the united right and left fourth commissural arteries. This vessel is believed to serve the main mass of follicles located in this area, while follicles more widely scattered may, and probably do receive capillaries from vessels other than the thyroid artery. Blood is removed from the thyroid follicles by the thyroid vein, a vessel which also drains the musculature below the ventral aorta. The thyroid vein enters directly into the sinus venosus. The lymphatic system may also play an important role in drainage of the thyroid tissue.

Iodine Metabolism in Mammals

The general scheme of iodine metabolism leading to the formation of thyroxine in the mammal is as follows: Most ingested iodine is reduced to iodide during digestion and absorption and it is in this form that most of the element is found in the blood. Iodide is concentrated in the thyroid gland where it is enzymatically oxidized to iodine by the thyroid cells. The iodine replaces two hydrogens of the benzene nuclei of tyrosine forming 3,5 diiodotyrosine. The 3,5 diiodotyrosine next undergoes oxidation and condenses to form thyroxine, with extrusion of

aminopropionic acid. A peroxidase system having sufficient potential to promote oxidation of iodide has been found in thyroid tissue (Werner, 1955).

The use of I-131 in studies of thyroxine formation has confirmed the classic scheme of thyroid hormone formation. But these studies, along with chromatographic separation techniques, have demonstrated the presence of very appreciable quantities of monoiodotyrosine and triiodothyronine in the normal gland. The synthesis of thyroxine is a rapid process and is believed to take place on the protein molecules of the colloid. Diiodotyrosine and thyroxine cannot be readily removed by dialysis of homogenates of thyroid tissue.

The intermediate biochemistry of monoiodtyrosine and triiodothyronine production has not been worked out. These substances could be formed by iodination or by deiodination. Triiodothyronine has been demonstrated in thyroid extracts and in certain tissues such as the liver and kidney of the higher vertebrates (Werner, 1955). Triiodothyronine is more potent in producing a metabolic response than either diiodotyrosine or thyroxine. It has been suggested that triiodothyronine is the active cellular form of the thyroid hormone, however, its site of formation is not definitely known. Thyroxine is the major thyroidal hormone component found in the blood.

Iodine Metabolism in Pisces

Gorbman and associates have done much work on the biochemistry of the thyroid hormones found in fish. Berg and Gorbman (1954) found a peak thyroidal accumulation of 3 per cent of the injected dose of I-131 in goldfish, Carassius auratus. Injections of thyrotropic hormone (TSH) prior to I-131 injections increased the uptake of I-131 to 9 per cent

of the injected dose and keeping goldfish in water of low iodine content also caused an increased I-131 uptake. A relatively slow rate of thyroxine synthesis was indicated by the fact that one week after injection of radioiodine most of the I-131 was in the form of monoiodotyrosine and diiodotyrosine, with little, if any as thyroxine. TSH caused the thyroid tissue of the goldfish to produce small amounts of thyroxine as early as 24 hours after injection of I-131.

Again Gorbman et al., (1952) working with Scyliorhinus canicula (shark) showed that the gross uptake of injected I-131 was irregular but rapid, with the peak uptake of 20 to 34 per cent of the injected dose in 6 to 17 hours. The output half-life was said to be rapid. Monoiodotyrosine and diiodotyrosine were produced first, and small but significant amounts of labelled thyroxine were formed within 17 hours after injections of the I-131. No appreciable amounts of inorganic iodine were found after 24 hours. Radioautographs showed the I-131 to be localized in the colloid one hour after injections with no significant amount of radioiodine in the epithelial cells at this time.

By use of chromatograms Gorbman and Berg (1955) were able to find monoidotyrosine, diiodotyrosine and thyroxine in Fundulus diaphanus and F. heteroclitus. He found no triiodothyronine. The iodine compounds found, their order of appearance in the chromatograms, and their relative proportions did not differ in any significant way from the thyroxineogenic cycle of mammals but the rate of syntheses of the thyroxine is not known.

In various species of mammals including man, and as far as is known also in other classes of vertebrates, the iodinated amino acids derived from hydrolysis of colloid are the same (Pickford and Atz, 1957). She states that the available data thus provides no grounds for believing that the

teleostean thyroid releases a special hormone different from those secreted in higher vertebrates.

Control of Iodine Metabolism in the Teleost - Hypophysectomy and Its Effect

Chavin (1956) stated that the basic endocrine mechanism for control of thyroid function in goldfish is similar to that of mammals. His conclusions were based on the following results:

(1) The thyroidal I-131 uptake of intact goldfish was 8-10% of the injected dose. TSH increased the uptake some 234 per cent. Injected NaI-131 was rapidly excreted for within 24 hours after injection 65% of the radioactivity was found in the aquarium water.

(2) Hypophysectomy cut the uptake of I-131 to less than 1% and hypophysectomized fish showed a 3,000% increase in I-131 uptake when treated with TSH.

(3) Thyroxine, cortisone, thiouracil and the stress of saline immersion decreased thyroid activity in intact goldfish but hypophysectomized fish were unaffected.

Using F. diaphanus maintained in fresh running water Gorbman-Berg(1955) showed that seven days after injection the fish were still accumulating I-131 in the thyroid. There was a slight plateau at 22% of the injected dose. Since no recycling of excreted I-131 could occur he believed that the tracer iodine must have come from depots located in the peripheral tissue. It has never been shown in higher vertebrates that the peripheral tissues could retain iodine in storage form for periods longer than a week so that it could be fed into the blood stream.

Pickford (1953b)found that in hypophysectomized F. heteroclitus the

thyroid follicle cells were relatively flat with no cells over 12 microns in height. Intact fish fed an iodine deficient diet exhibited thyroid hyperplasia but in hypophysectomized fish iodine deficiency had no effect. She has summarized the effects of hypophysectomy on the male killifish as follows:

(1) Did not grow in length. Weight changes are irregular.

(2) Increased liver size

(3) Testes undergo complete regression.

(4) Fish may develop renal calculi and urinary duct obstruction.

(5) Loss of osmoregulatory capacity.

(6) Thyroid glands are inactive.

(7) Fish become anemic.

(8) No effect on pancreatic islets, stannius corpuscles, or adrenal (Giacomini) tissue in the area of the posterior cardinal vein.

Relation of Thyroid to General Metabolism

Reports prior to 1956 (see Fromm and Reineke, 1956) generally agree that the piscine thyroid has no influence on the oxygen consumption. The three papers discussed below present additional data on this problem.

Matty (1957) surgically thyroidectomized parrot fish (Pseudoscarus guacamaia) and he measured the oxygen consumption of individual fish before and after thyroidectomy. No thyroidectomized fish showed changes in oxygen consumption for periods up to 124 days after thyroidectomy. Intraperitoneal injections of dried parrot fish thyroid, l-thyroxine and 0.7 per cent saline had no effect on the oxygen consumption, however, extracts of parrot fish thyroid was shown to elevate markedly the oxygen consumption of rats. The possibility that teleosts may respond metabolically only to thyroid extracts of teleost origin was not demon-

strated in this work.

Fromm and Reineke (1956) used radiothyroidectomy to destroy the thyroid tissue in rainbow trout (Salmo gairdnerii). The radiothyroidectomy was accomplished by a single injection of 250 µc. of I-131 as well as by higher doses. It was shown that radiothyroidectomy did not decrease the oxygen consumption of trout fingerlings below that of the control animals.

Hoar (1958) has presented data showing that both thyroxine and gonadal steroids affect the metabolism of goldfish. Only the steroids affect oxygen consumption while both steroids and thyroxine increase nitrogen excretion. Hoar stated "On the basis of these and of a great many in vitro studies it has been suggested that the primary effect of this hormone (thyroxine) is one of accelerating the splitting of protein and that the products so produced lead to an increased oxygen consumption." He also suggests that the hormone acts in maintaining an equilibrium between protein anabolism and protein catabolism.

It has been shown that the metabolism of poikilotherms varies directly with environmental temperature. Bullock (1955) points out that trout (Trutta iridea) removed from a given temperature and placed in one 10°C. higher will have an increased oxygen consumption. The mechanism of this increased oxygen consumption is not known.

The Effects of Anti-Thyroid Drugs

Platypoecilus maculatus and Xiphophorus belleri immersed in solutions of thiourea showed marked inhibition of growth. Histological examination of the thyroid tissue of the thiourea treated animals showed a definite hyperplasia when compared to normal controls (Goldsmith, 1949). Thiourea produces hypothyroidism through inhibition of synthesis

of thyroid hormone.

Many reports state that during prolonged treatment with antithyroid drugs a gradual restoration of thyroid activity may occur. Pickford and Atz (1957) stated that antithyroid drugs at high doses can kill an animal by general inhibition of cell oxidation. It has also been pointed out that the direct action of antithyroid drugs on other organs cannot be wholly ignored. Pickford found that high dosage levels, needed for complete inhibition of the thyroid produced toxic effects not seen after surgical thyroidectomy.

Some investigators have found that antithyroid materials such as thiourea decreases the demand for oxygen in teleosts. Since thiourea is a strong antioxident the reduced oxygen consumption might be due to the antioxident property of thicurea rather than the decreased amount of thyroid hormone.

Migration and the Thyroid

After about two years, young rainbow trout of Michigan waters undergo a color transformation following which the silver trout or "steelhead" migrates to the Great Lakes. These changes are believed to be analogous to the changes from parr to smolt in salmon. Hoar (1953) found seasonal changes in the histological picture of the thyroid of salmon parr. His interpretation was that the thyroid was more active in the spring than in the summer or fall. Robertson (1948) found similar evidence in rainbow trout. Examination of the thyroid glands of the rainbow trout smolt revealed markedly increased "functional activity" when compared with the parr thyroids.

Migrating trout taken from streams leading to Lake Michigan and Lake Superior showed marked hyperplasia of the thyroid in the sexually ripe

or spent fish in contrast to the relatively quiescent state found in immature trout (Robertson, et al., 1953). The hyperplasia of sexually maturing Lake Michigan trout is believed due to the low iodine content of the water causing hypofunction during the time of increased demand for the thyroid hormone. The egg mass of the mature trout was shown to contain more iodine than the combined total of all the other tissues, including the thyroid. Iodine must be of great importance in the development of the embryo.

In studying the effects of thyroid preparations on Salmonidae LaRoche and Leblond (1952) report that when Salmo salar in fry, parr and smolt stages were given thyroid preparations the following changes took place:

(1) Thickening of the integument (slight in fry and parr) intense in the smolt.

(2) Broadening of the head produced in Salvelinus fontinalis (Brook trout) at parr stage. Believed to be due to hyperplasia of interorbital connective tissue.

(3) Administration of iodide to salmon parr produced a similar, though less pronounced, pallor than that due to thyroid extract.

Estimation of Glandular Activity

There have been many varied means by which workers have tried to measure thyroid activity. Much of the early work was done by histological examination of thyroid follicles. Active follicles are described as having increased colloid material, more prominent cells with rounded nuclei, increased cytoplasm and large dark nucleoli; the lack of these characteristics would indicate an inactive gland. Neither the size nor the histological appearance of a gland is necessarily correlated with

the amount of secretion into the circulation (Swift, 1955). In the case of an animal on an iodine deficient diet, the TSH mechanism of the anterior pituitary will respond to the decreased thyroxine blood level causing an increased growth (simple goiter) of the thyroid but there would be no ultimate increase in the rate of hormone secretion.

It has been shown by Nadler and Leblond (1955) that differences in colloid diameter when statistically examined could be shown to be identical to cross sections taken at different planes through the spherical follicle. Changes in the histological appearance of a given gland will show whether or not the tissue is being stimulated but cannot be used as a means of indication of thyroid activity in terms of the amount of hormone being used by the organism.

One of the major means of studying thyroid function has been to measure the basal metabolic rate (BMR) of the organism. This method cannot be used with fish because thyroid hormone has not been shown to be involved in the regulation of the oxidative metabolism.

There are many indications of increased or decreased thyroidal activity as shown by gross changes in the organism. Changes from parr to smolt stages, migration, sexual maturation, fin regeneration, thinning of the integument and exopthalmos have been correlated with changes in the histological appearance of the thyroid in fish.

With the availability of radioactive iodine more investigators have been using output and uptake half-times as a means of detecting changes in thyroid activity. The rate and amount of I-131 uptake by the thyroid does not indicate the rate at which thyroxine is being produced. The same holds true for measurements of the output rate of I-131. However, these measurements may be used to indicate a relative functional activity

of thyroid tissue.

Chemically the assay for total iodine in the gland has been used as an indication of secretion rate. It has been shown that hypophysectomy fails to affect the iodine content of the thyroid gland. It could be that this is due to a decrease in both the rate of secretion and the ability of the thyroid to concentrate iodine (Wolff, 1951). He injected I-131 into rats and after a short period of time measured the rate at which the I-131 was leaving the gland (biological half-time). Hypophysectomy had the same effect as an addition of thyroxine, that is, it prolonged the biological half-time. Perry (1951) believed that this decline in I-131 removal from the thyroid gland is an indication of a decreased secretion rate of the hormone. If the above were true then any known factors that would increase thyroid secretion rate should also increase the rate of I-131 output from the gland. The major controlling factor of thyroid secretion is the thyrotropin (TSH) from the anterior pituitary. He found an increased output of I-131 following injection of TSH. This supported his basic plan for a method to measure thyroid secretion rates.

Perry, using groups of rats injected with different levels of thyroxine, showed that the inhibition of thyroidal I-131 output was proportional to the dosage of thyroxine given. Reineke and Singh (1955) studying the effects of increased dosage of thyroxine to individual animals, also found that thyroidal I-131 output is proportional to the dosage of thyroxine administered. Based on these results a method for the estimation of thyroid secretion rates was proposed by these investigators.

Statement of the Problem

At the present time our knowledge of thyroid function in the teleosts is slight. The major block to further understanding in this area has been the inability to estimate the true rate of thyroxine utilization by the animal. With the development of a satisfactory method of detecting thyroid secretion rates many of the still unanswered problems of teleost thyroid function may be answered.

The actual rate of thyroid hormone formation in poikilothermic vertebrates has, to the author's knowledge, never been measured. Investigators working with teleosts indicated that on the bases of "histological evidence" such factors as age and environmental temperature may effect thyroid activity. A preliminary examination into these factors has been attempted.

Through secretion rate determinations future workers will be able to clarify existing data and investigate further the problem of thyroid function in the teleosts and other vertebrates.

GENERAL METHODS AND MATERIALS

Experimental Animals

Rainbow trout, Salmo gairdnerii, were obtained from the Wolf Lake Hatchery, which is operated by the State of Michigan, Department of Conservation. The average weight of the fish used was 14.0 gms. with a standard deviation of ± 3.4 gms.

At the laboratory the trout were held in 26 gallon glass aquaria in a constant temperature room. They were kept at 13°±0.5°C. under constant illumination. Each tank was aerated and equipped with an air lift filter which contained gravel, glass wool and activated charcoal. Every third day the fish were transferred to a clean holding tank of aged water. Fish were fed dried trout pellets currently used by the Michigan Department of Conservation. The pellets contained 2% iodized NaCl.

On arrival all fish were dipped in a fresh 15 p.p.m. solution of malachite green for 15-30 seconds. The dip prevented contamination of the stock tanks with rather common pathological conditions of trout such as tailrot, Ichty, and other parasitic infections.

Fish were anesthetized by immersion for approximately 15 seconds in a 0.033% solution of MS-222 (Sandoz Chemical Co.). A syringe connected to a microburet and fitted with a 27 gauge needle was used for all injections. The needle was inserted into the pleuroperitoneal cavity approximately 2.0 cm. anterior to the pelvic fins. All injections were kept to a volume less than 0.2cc. to minimize internal damage caused by osmotic changes or increased pleuroperitoneal pressure.

One of the most convenient ways of marking fish is to clip the fins. In this study usually only one fin was removed and when two fins were

removed one of them was the adipose fin which is not used in swimming. About 24 hours after the clipping, the fish showed signs of tail-rot, and subsequently 2/3 of the experimental group died. Noting that only operated fish developed tail-rot, a strict aseptic operative procedure was next used. The fish were dipped in fresh malachite green (1:1500) for 15 seconds before and after the operation. The fin was removed and the cut surface dried. An antibiotic powder, Ureka Sulmide Powder (Jensen Salsbery Laboratories), was applied to the wound, which was in turn coated with "collodion". The fish showed no tail-rot after three weeks of post operative experimentation.

Radioactive Iodine

Carrier free radiodine as NaI-131 was obtained from the Oak Ridge National Laboratory. The I-131 solution was diluted with distilled water so that 0.1 ml. of solution contained the desired activity.

Thyrotropin (TSH)

The hormone used was obtained from the Armour Laboratories (Veterinary Standard - Lot. No. R377158) in the form of a sterile powder of purified thyrotropic principle of bovine anterior pituitary glands.

Thyroxine

Crystalline l-thyroxine, supplied by Glaxo Laboratories, Greenford Middlesex, England, was purified by Dr. E. P. Reineke. Ten mgs. of the crystallized thyroxine were dissolved in distilled water made slightly alkaline with NaOH. HCl was then added to make the solution slightly cloudy and at this point the monosodium salt of thyroxine was formed. This solution was then diluted to contain 100 µg. of l-thyroxine per ml. of stock solution. Dilutions for injection were made from this stock solution at the start of each experiment.

Radiation Standards and Injected Doses of I-131

Each fish received 12 μc. of I-131, and this activity was contained in 0.1 ml. Twenty per cent (by volume) of the injected dose was placed in a small porcelain cup, six drops of a solution containing casein, NaI and $NaHSO_3$ were added and the mixture evaporated to dryness. This solution produces a more stable mixture minimizing loss of I-131 by chemical or physical means. Standards were counted by placing them in the holding tube (figure 1) and centering them over the scintillation detector. In the holding tube the porcelain cups remained at the same level as the lower jaw of the average trout. Thus the standard was counted at the same geometry as the *in vivo* counts of the trout. Corrections for physical decay and changes in the counting apparatus were made by expressing the activity of the fish as a per cent of the injected dose. Using the activity of the standard, the activity of the total injected dose was calculated.

In-Vivo Counting Method

The apparatus used to maintain a fish in a constant position for the purpose of measuring the gamma radiation from the I-131 is shown diagramatically in figure 1. The refrigerated water ($13 \pm 2^{\circ}$ C.), at a constant pressure and free of air bubbles, passes into the anterior end of the holding tube, leaves the tube by the posterior drain, and is returned to the holding tank.

The holding tube is placed on top of a lead block collimator and is centered over the 2.6 cm. hole. The lead chamber encloses a Nuclear-Chicago Model DS5 Scintillation Detector with a 3/4 x 3/4 inch sodium iodide crystal. Counts were recorded using a Nuclear-Chicago Model 1620 Analytical Count Rate Meter. An Esterline Angus Graphic Instrument was

FIGURE 1

In-Vivo Counting Apparatus - A, refrigerator; B, holding tank; C, pumb; D, pressure control; E, bubble trap; F, holding tube; G, scintillation tube; H, lead chamber; I, 1620 Count Rate Meter; J, Esterline Angus Graphic Instrument.

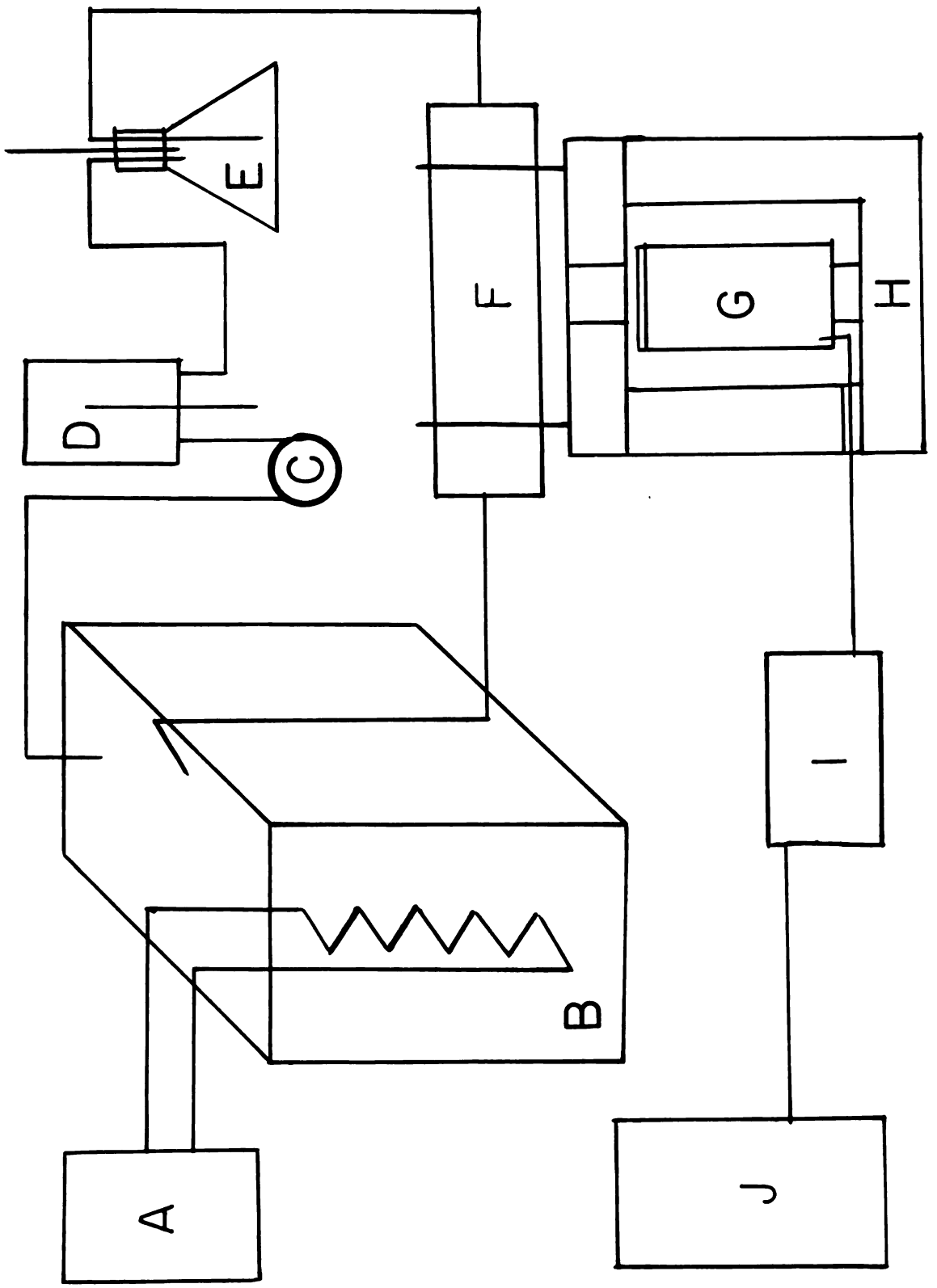


FIGURE 1

used to make a permanent record. The equilibrium time is the time taken by the count rate meter to reach a final reading within the probable error of the true average. The per cent probable error of a single reading of data obtained varied between 0.7% and 5.2%.

The holding tube, containing fish, was centered over the collimator. The activity in the thyroid area was counted by adjusting the position of the fish until the highest count was recorded. A second *in vivo* count was made of each fish. This is the animal background and includes the activity of the stomach, intestines, kidney and gonads. No thyroid tissue is included in this count. The activity in this area is due to I-131 in the blood, intestine, pleuroperitenal cavity, and general body cells, either as free iodine, or as radioactive iodinated amino acids produced by the thyroid or somatic metabolism. The count was continued until a stable horizontal line was recorded on the Esterline Angus Recorder. By this time the "Equilibrium Time" had been reached and the per cent probable counting error was as noted above. Figure 2 shows the gross anatomy of the trout with the injection site and counting area for thyroid and background activity.

Gross Radioactive Mapping

The Model C-100 Actigraph Strip Feeder (Nuclear-Chicago) was used with a shielded scintillation tube, 1620 Count Rate Meter and Esterline Angus Recorder. The strip feeder is attached to the Esterline-Angus Recorder by means of a flexible coupling cable. As the recording chart is fed out of the Esterline-Angus the C-100 Actigraph feeds an aluminum strip table, at constant geometry, over the scintillation tube at a speed of 3.4 inches per minute. Suitable collimation was gained by using a lead shield with a slit running perpendicular to the direction of the

FIGURE 2

The Anatomy of the Trout showing the Injection Site and Counting Area for Thyroid and Background Activity - A, pyloric caeca; B, liver; C, stomach, D, heart; E, large intestine; F, pleuroperitoneal cavity; G, fat; H, transverse septum (false diaphragm); I, pericardial cavity; J, operculum. The area above "thyroid" and "background" is included in the <u>in vivo</u> measurements of each.

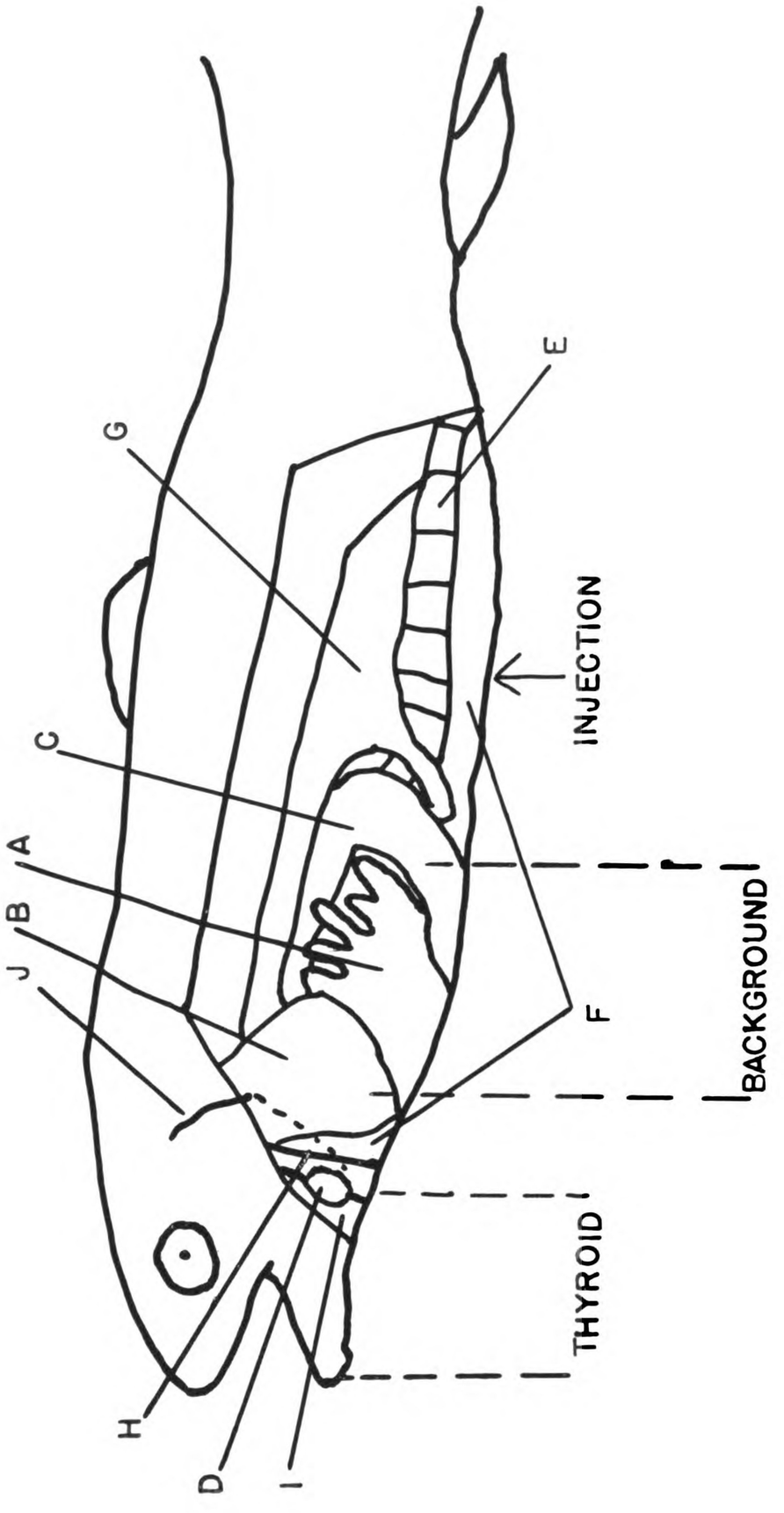


FIGURE 2

aluminum strip table. The fish, after being killed, was placed on the aluminum strip table and fed over the slit collimator while a record of the counting rate was made.

Physical Decay of I-131 Standards

To correct for physical decay of the I-131 all measured activities were expressed as per cent of the total injected dose as calculated from the decaying I-131 standard. The I-131 standard should decay at a rate similar to the decay constant for I-131. Investigating this assumption two standards of 2.0 μc. I-131 prepared as noted above were counted daily for several days. The daily determinations as shown in Table I are the average cpm. of four determinations of the two standards corrected for room background. At time zero (A_0) the standards had an average count of 2,950 cpm. This value when corrected for physical decay by use of a table based on $t\frac{1}{2}$ = 8.06 days gives A_t (physical) values as shown in Table I. Column A_t (measured) shows the activity of the decaying standard as measured with the counting setup.

The line drawn to points A_0, A_t (physical) and A_t (measured) are shown in Figure 3A. Figure 3B shows A_t (measured) values with the line fitted by method of least squares. The $t\frac{1}{2}$ based on values of A_t (measured) taken from the fitted line gave:

A_0 = 2,950 cpm.
A_t = 2,077 cpm.
t = 4 days
$t\frac{1}{2}$ = 7.898 days

The variance between the "$t\frac{1}{2}$" of the standard's count and the decay "$t\frac{1}{2}$" constant was probably caused by changes in the counting sensitivity of the instruments coupled with a slight amount of physical or chemical loss from the prepared standard. Making decay corrections of in vivo

thyroid measurements by expressing the activity as a per cent of the injected dose allows for compensation of the changes in the counting instruments sensitivity. The I-131 standard did show an overall "$t_{\frac{1}{2}}$" slightly less than the "$t_{\frac{1}{2}}$" value taken from physical decay tables.

TABLE I

DECAY OF I-131 STANDARD

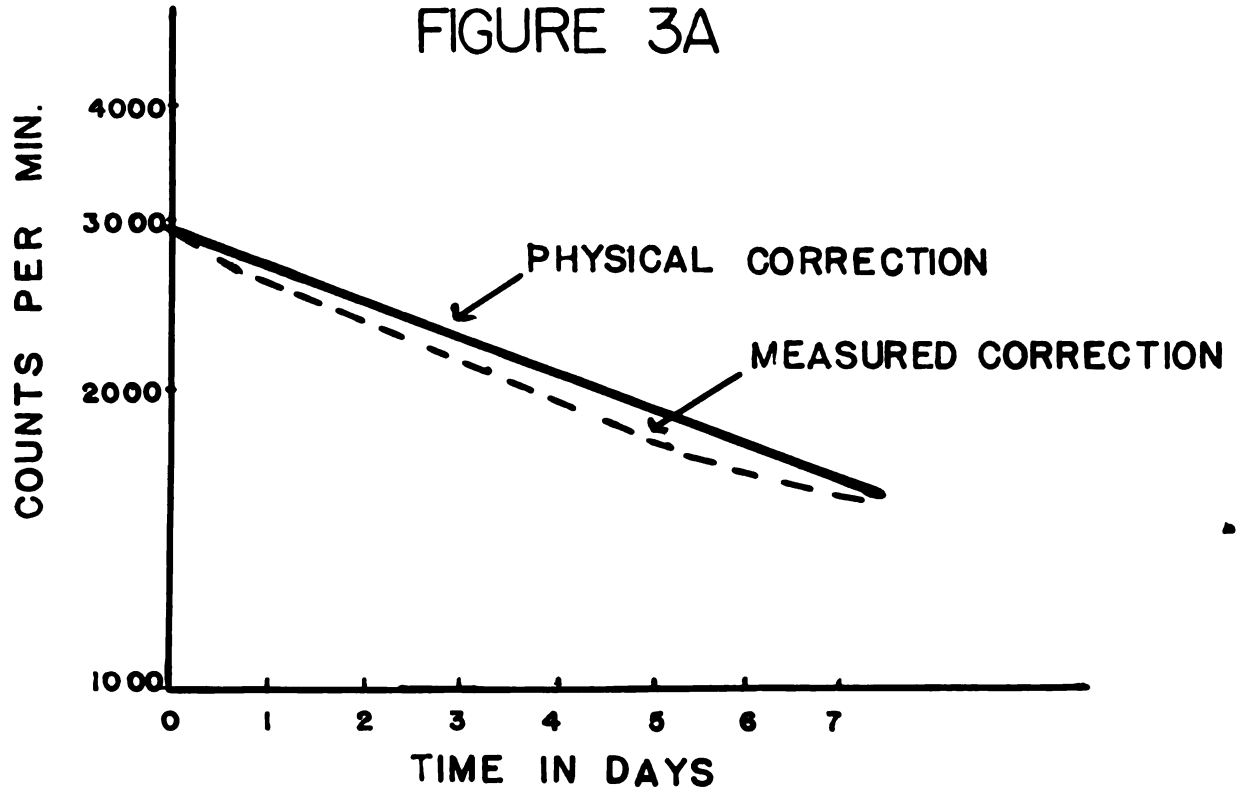
Days of Decay	At (physical)*	At (measured)*
0	2950 cpm.	2950 cpm.
1	2707 cpm.	2700 cpm.
2	2484 cpm.	2460 cpm.
3	2279 cpm.	2197 cpm.
5	1905 cpm.	1795 cpm.
6	1761 cpm.	1716 cpm.
7	1617 cpm.	1591 cpm.

* Counts per minute (cpm.) of injected dose corrected for room background.

Body Background Correction

Because of the inability of the counting apparatus to count only thyroid I-131 activity a certain per cent of the animal background was included in the total activity of the *in vivo* thyroid measurements. The problem of correcting for body background was approached as follows: Nine fish were injected with I-131. The *in vivo* activity of the thyroidal area and body background of three fish was determined 1 day after injection. After these measurements were made the fish were sacrificed and a portion of the lower jaw was removed. The activity of these lower jaws was determined at the same geometry as the *in vivo* counts. Similar procedures were carried out on 3 fish for two succeeding days.

In Table II the values under "T" refer to the activity in the iso-



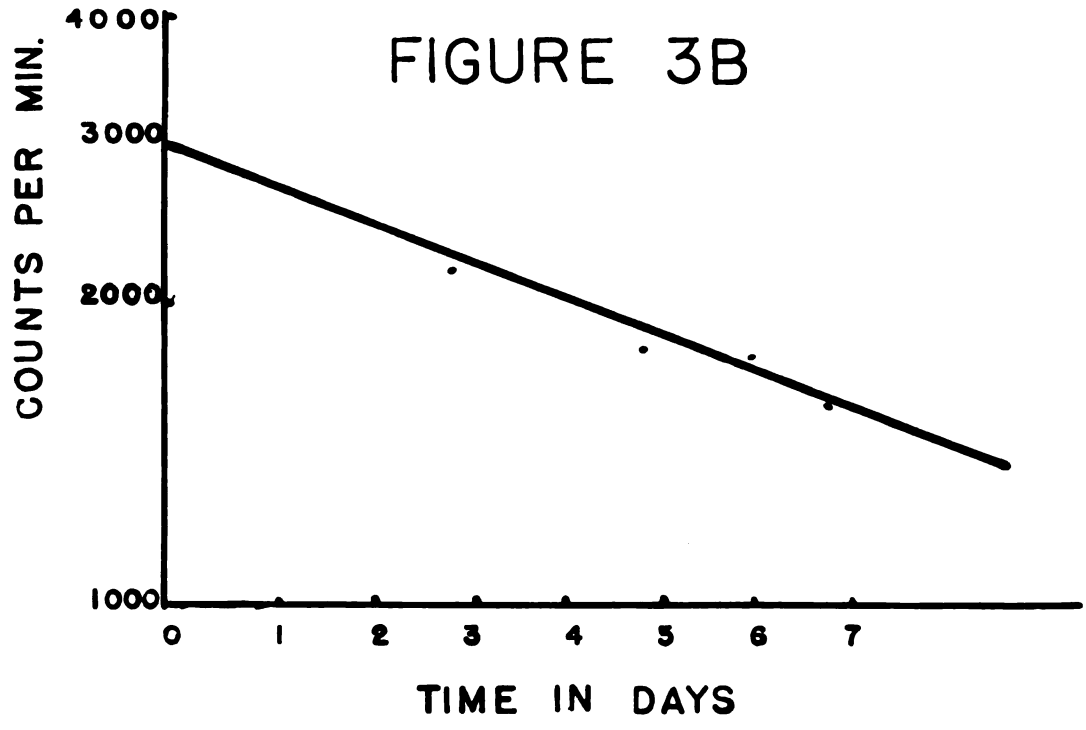


Fig. 3A. A semi-log plot of the standards activity corrected by physical decay tables based on the zero time activity. Measured correction is a plot of the measured activity of the standard.

Fig. 3B. A semi-log plot of the measured activity with line fitted by method of least squares. "$t_{\frac{1}{2}}$" = 7.89 days.

lated lower jaws of the fish. The values in column "A" are the *in vivo* counts of the thyroidal area minus total body background. The "B" values are the *in vivo* counts of the thyroidal area minus one-half the total body background. All values are in terms of per cent of total injected dose. The fact that values for "T" were in all cases smaller than those for either "A" or "B" may indicate that all of the thyroidal tissue was not contained in the excised low jaws or that complete correction for body background was not being made.

R_a and R_b are ratios of A/T and B/T respectively. These ratios are less on days 2 and 3 than on day 1 indicating that both "A" and "B" values are coming closer to the "T" value. A_r and B_r are values for the per cent change in R_a or R_b from day 1 to day 3. A value of 100 for either A_r or B_r would indicate that we have reached a true estimate of the thyroid activity based on the "T" values. Since the B_r value falls closer to 100 than the A_r value it was concluded that correction of thyroid activity count for body background was most accurately accomplished by subtraction of one-half the total body background.

Animal background shortly after injection of I-131 is quite high. This activity decreases rapidly during the following 3 to 4 days. Thus the importance of making corrections for body background becomes relatively less as the interval between time of injection and time of measurement increases.

Since data for the determination of thyroid secretion rates were obtained starting 4 days after injections of I-131, corrections of these values for body background is less important than corrections needed in the determination of uptake and early output rates.

TABLE II

BODY BACKGROUND CORRECTION

Time*	T'	A'	B'	R_a	R_b	A_r	B_r
1	8.245	16.363	23.514	1.985	2.852	-------	-------
2	6.874	7.476	9.611	1.088	1.398	54.811	49.018
3	6.891	8.751	9.970	1.270	1.446	116.728	103.433

* Days after injection of I-131
' Per cent injected dose (average of three animals)
Note: See above text for explanation of values found in this table.

EXPERIMENTAL RESULTS

Uptake and Outnut Rates of Body Background and Thyroid

A group of eight fish were each injected with 12 μc of I-131 and counts of the thyroid and animal background activity made over a period of 8 days. The average activity of the thyroid and of the tissues counted for the animal background determinations are presented in Table III.

TABLE III

MEAN ACTIVITY OF THYROID AND OF TISSUES COUNTED FOR BODY BACKGROUND FOLLOWING INJECTION OF I-131

Days after injection	Number of Observations	Thyroid*		Body Background	
1	7	23.2	± 11.9**	8.9	± 7.2**
2	8	23.1	12.8	7.6	2.7
3	8	20.4	11.6	4.8	1.5
4	8	19.9	11.5	4.6	2.5
6	8	20.3	12.1	3.9	0.8
7	8	18.0	11.5	4.4	1.9
8	8	18.6	12.8	4.6	1.2

* Activity as per cent of injected dose
** Standard deviation

The corrected values for thyroid activity plotted in Figure 4A indicate that during the first 4 days following injection there is a rapid loss of I-131 from the thyroid area ($t_{\frac{1}{2}}$ = 9.0 days). This is followed by a slower rate of loss which has a half-time of 37 days.

Figure 4B shows the animal background activity which again appears to be divided into two components. There occurs initially a rapid loss of activity ($t_{\frac{1}{2}}$ = 2.5 days) followed by an increase in activity. ($t_{\frac{1}{2}}$ = 13 days uptake). All lines drawn in Figures 4A and 4B were fitted by visual methods.

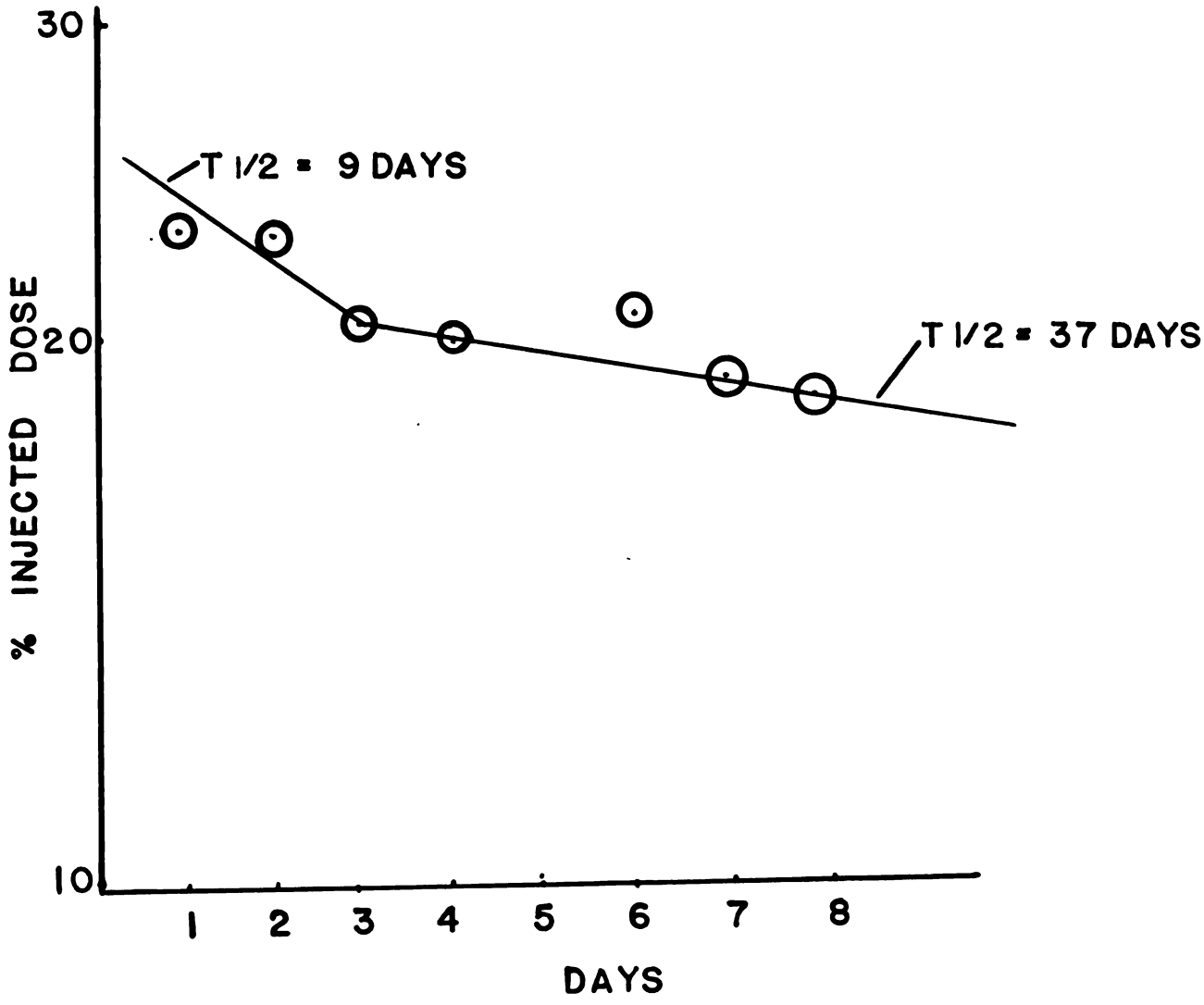


Fig. 4A - Output Curve of Thyroid Activity. Activity expressed as per cent injected dose corrected for animal background, vs. time in days after I-131 injection.

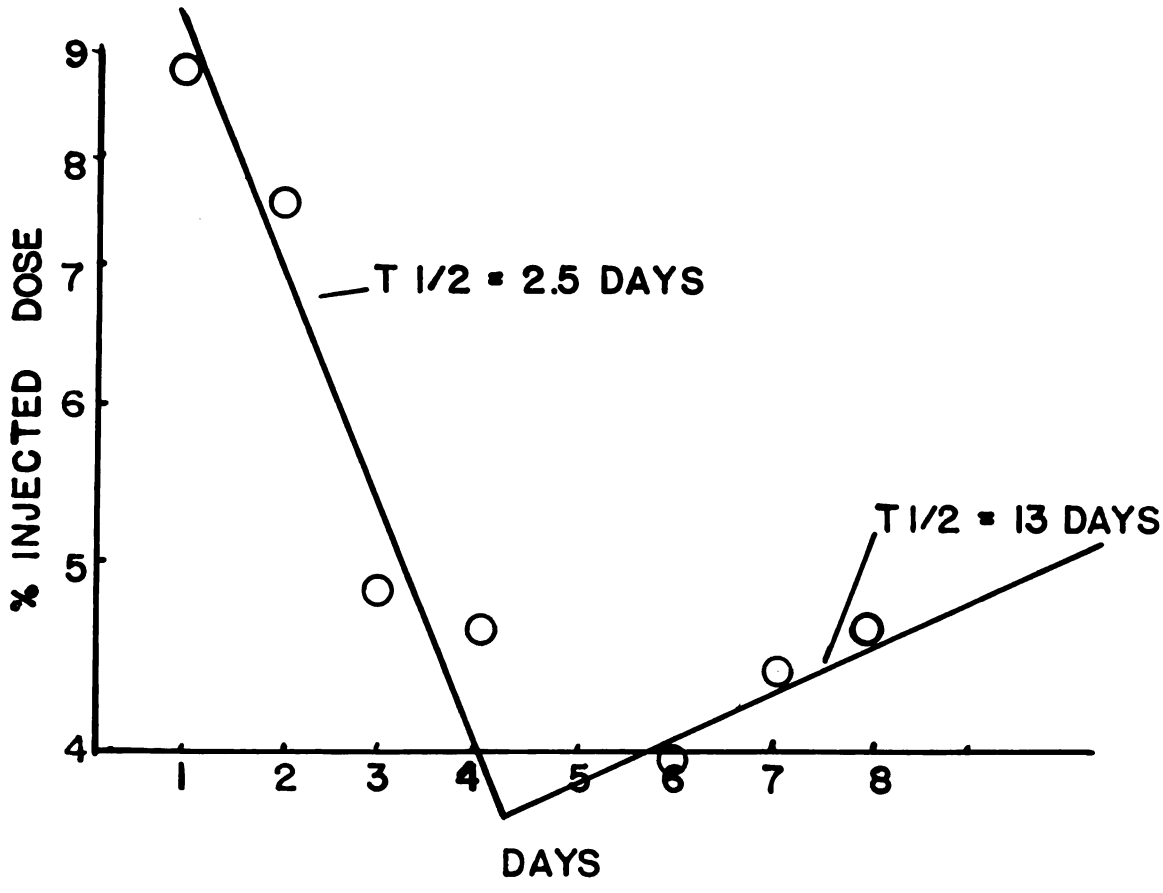


Fig. 4B - Per Cent Injected Dose of Animal Background. Days after I-131 injection shown on "x" axis. Each point is the average of 12 fish.

FIGURE 5

0 - 3K10 - 3/4 : Gross Radioactive Map of Dead Fish Injected with I-131

1 - 3K10 - 3/4 : Gross Radioactive Map of Fish One Hour After I-131 Injection

10 - 3K10 - 3/4 : Gross Radioactive Map of Fish One Day After I-131 Injection

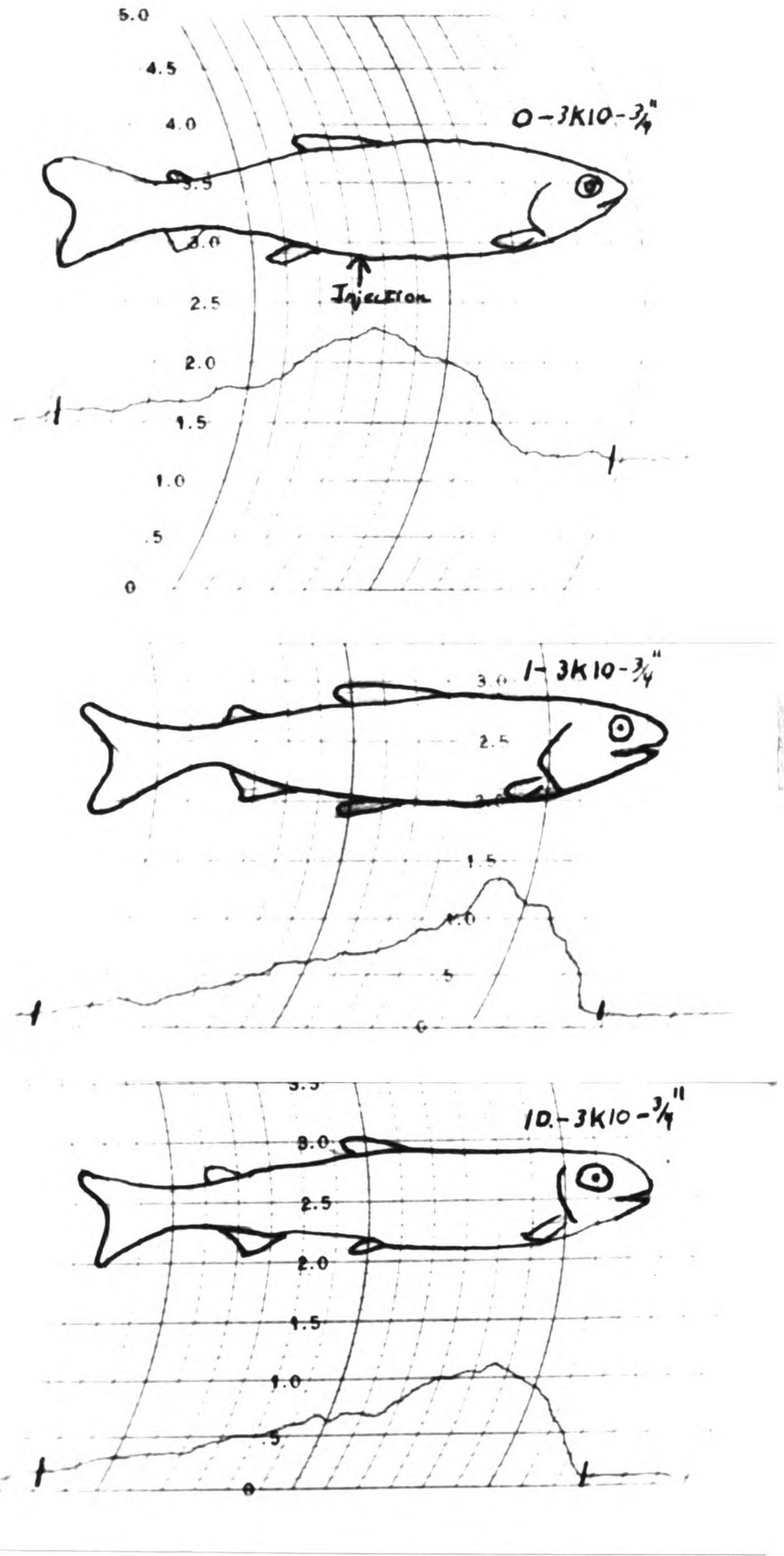


FIGURE 5

FIGURE 6

4D - 3K10 - 3/4: Gross Radioactive Map Made Four Days After Injection of I-131.

14D - 3K10 - 3/4: Gross Radioactive Map Made 14 Days After Injection of I-131.

21D - 3K10 - 3/4: Gross Radioactive Map Made 21 Days After Injection of I-131.

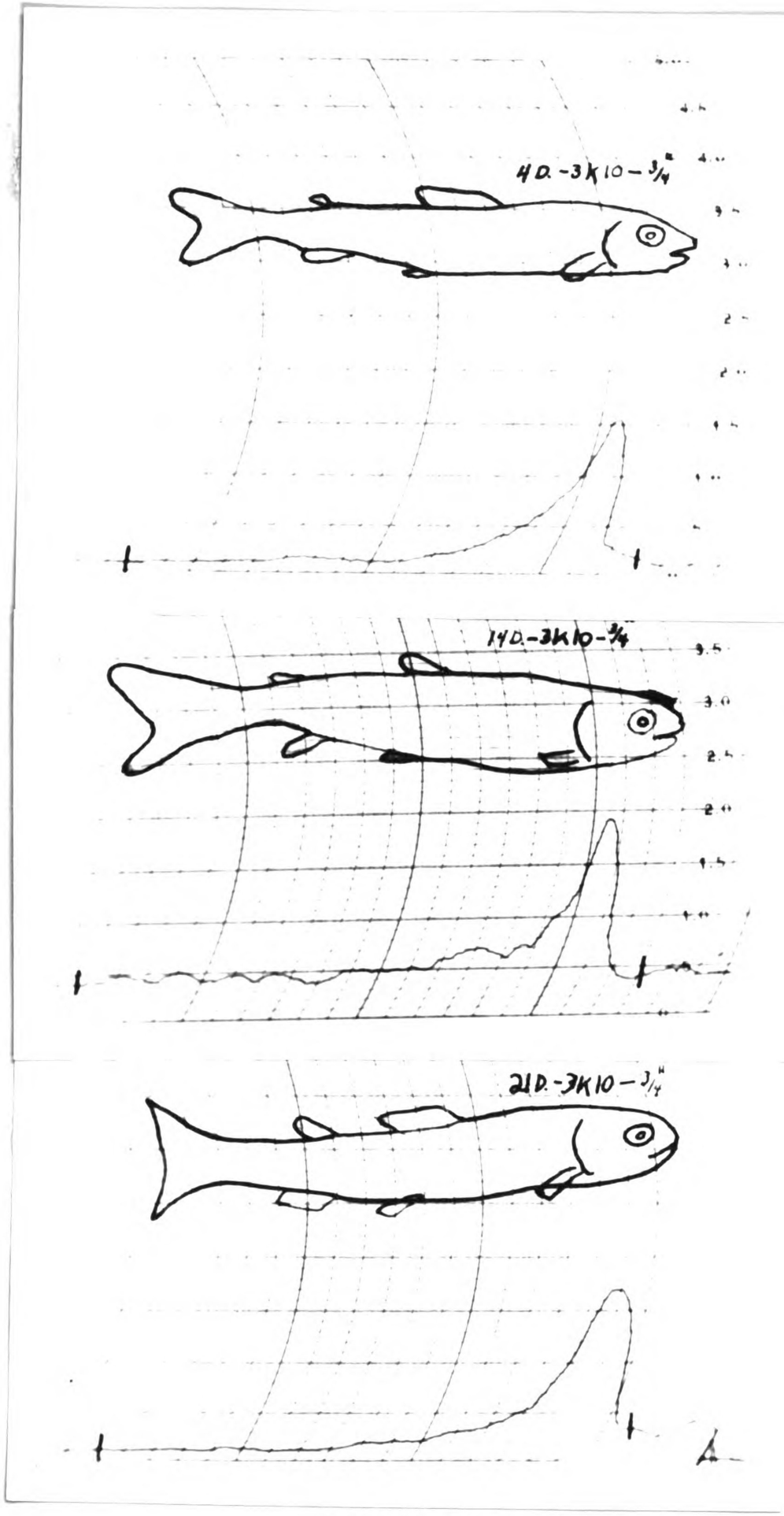


FIGURE 6

Gross Distribution of Injected I-131

For this study one fish was sacrificed and then injected with I-131. Five other fish were injected with I-131 and then sacrificed at varying time intervals. A "gross radioactive map" was made of each fish as previously described. Reproductions of these maps including an outline of the fish drawn to scale are shown in Figures 5 and 6.

Some of the I-131 injected into the dead fish showed a slight migration both posteriorly and anteriorly from the site of injection. After 1 hour the greatest accumulation of I-131 was in an area anterior to the injection site but posterior to the transverse septum. Activity was also present in the thyroidal area at this time. Between 1 and 4 days after injection the greatest accumulation of I-131 shifted to the thyroid area. There was no further change in the position of maximal activity.

Effects of TSH on In Vivo Thyroid Counts

A group of 12 fish were injected with I-131 and counts taken every 24 hours thereafter. On the sixth day after I-131 injections each fish received one mg. of TSH, and counts were continued as before. Data are presented in Table IV and shown graphically in Figures 7 and 8.

TABLE IV

EFFECT OF TSH ON IN VIVO THYROID ACTIVITY

Days after I-131 Injection		% Injected Dose Thyroid and S.D.*		% Injected Dose Background
1		19.1	9.1*	12.3
2		13.1	7.7	5.3
3		11.7	8.0	2.9
4		11.1	7.3	2.7
5		12.6	6.2	2.8
6	TSH given	10.8	7.4	3.0
7		9.2	5.2	2.7
8		8.7	4.9	3.2
9		9.1	6.2	3.1
10		11.0	6.2	3.2

* Standard Deviation (±)

Injections of TSH caused an increase in the rate of release of I-131 from the thyroid which lasted for approximately 3 days. This increased output rate caused an increase in the animal background as shown in Figure 8.

Thyroid Secretion Rates

Determination of thyroid secretion rates were carried out on several groups of animals under different experimental conditions. Table V (page 39) gives a summary of the data collected for each determination.

The first in vivo count was taken three days after the I-131 injection, and continued every two days thereafter. Starting five days after the injection of I-131 a series of increasing daily thyroxine injections were given. The dose (in µg./100 gm. body wt.) was increased on the day of counting. Figure 9 shows the response of eleven 1-year old trout kept at 13°C. Data for the other experimental groups were treated in a similar fashion. The solid line used to estimate 100% previous count and the secretion rates was fitted by the "method of least squares". The dotted lines are plots of the standard deviations about the fitted line. An explanation of the statistical methods used is given in Appendix one.

Secretion rates were made on both one and two year old fish kept at 13°C. Rates were also determined for one-year old fish which had been cooled from 13°C. to 3°C. in 24 hours and maintained for one week at 3°C. before the secretion rates were determined.

The thyroid secretion rates as determined in both 1 and 2 year old fish at 13°C. show a range from 0.190 to 0.415 µg. l-thyroxine/100 gm. body wt./day. Due to the large variation no significant differences occurred

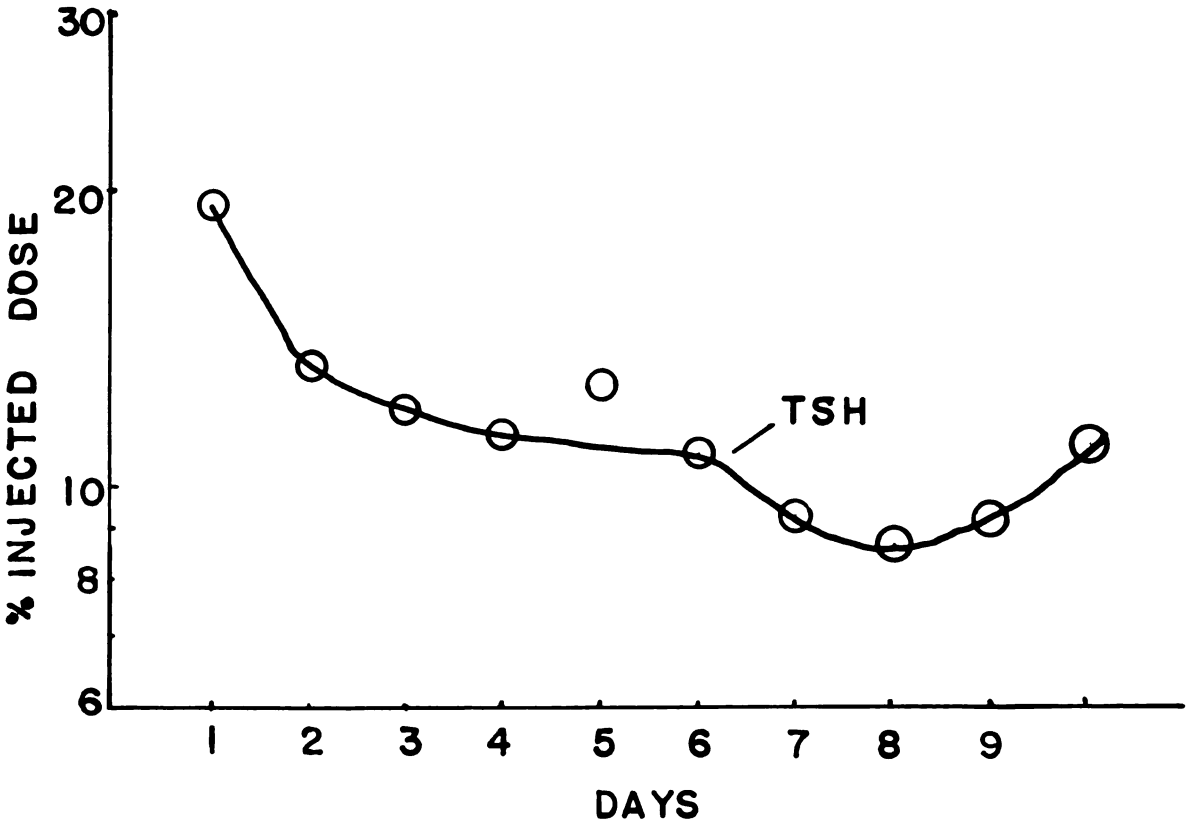


Fig. 7 - Output curve of average *in vivo* thyroid activity. Six days after I-131 injection each of the 12 fish were injected with 1 mg. TSH.

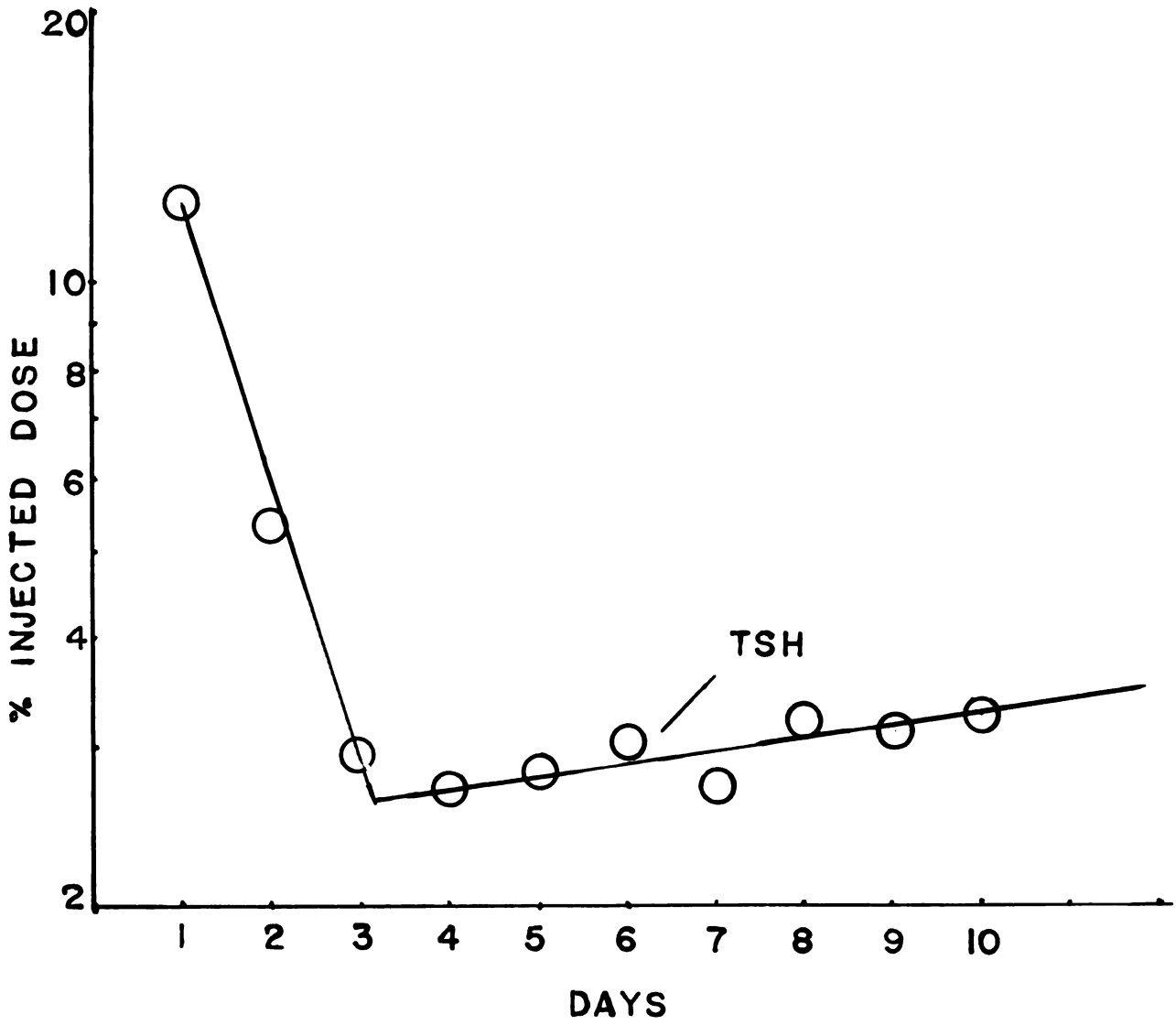


Fig. 8 - Per Cent of Injected Dose of Animal Background. Each point is average of 12 fish. On the sixth day after I-131 injections each fish received 1 mg. TSH.

between these groups (I through IV). For fish kept at 3°C. the thyroid secretion rate was 0.139 µg. l-thyroxine/100 gm. body wt./day. No difference was found between this group and others. The t-test of secretion rate showed no significant differences at the 5% level.

Effect of Temperature on Oxygen Consumption and Operculum Rates

Oxygen consumptions were determined by the analysis for dissolved oxygen using a modified "Micro-Winkler Method" and a constant flow apparatus as described by Fromm (1958). Fish (1 year old) were maintained for at least one week at experimental temperatures before they were used for oxygen consumption determinations. Operculum rates were determined while each fish was in the holding tube during the oxygen consumption experiment. The results are shown in Table VI.

TABLE V

DATA FOR THYROID SECRETION RATE DETERMINATIONS

++	I	II	III	IV	V
Date	8/14/58	8/23/58	9/1/58	10/1/58	10/3/58
Temp	13°C.	13°C.	13°C.	13°C.	3°C.
Age	2	2	1	1	1
N	27	27	21	33	30
Ex	3.375	6.750	2.625	4.125	1.880
Ex^2	0.711	2.817	0.553	0.869	0.200
Exy	341.292	675.118	247.576	392.964	178.041
Ey	2,607.060	2,516.454	1,882.541	2,907.847	2,629.274
Ey^2	260,507.915	239,011.531	170,383.996	262,405.275	238,660.052
a	89.895	83.019	82.830	77.984	77.523
b	53.300	40.731	54.516	83.436	161.474
S. R.	0.190	0.415	0.223	0.264	0.139
$Sy\pm$	17.833	10.200	50.330	9.592	14.730
$Sx\pm$	0.335	0.250	0.130	0.115	0.091
SE	0.066	0.049	0.029	0.020	0.017
cy^2	9,323.447	8,686.613	8,036.226	7,764.607	7,681.295
$r\pm$	0.966	0.647	0.411	0.232	0.260
r = sig.	1% level	1% level	1% level	1% level	1% level

++ See Appendix one.

TABLE VI

MEAN VALUES AND STANDARD DEVIATION OF O_2 CONSUMPTIONS AND OPERCULUM RATES OF 1 YEAR OLD TROUT AT 3°C. and 13°C.

Temp. (°C.)	No. of Observations	O_2 Cons. (ml/gm/hr)	Operculum Rate (number/minute)
13°C.	16	0.087 ± 0.032	117 ± 7.28
3°C.	8	0.040 ± 0.032	61 ± 4.44

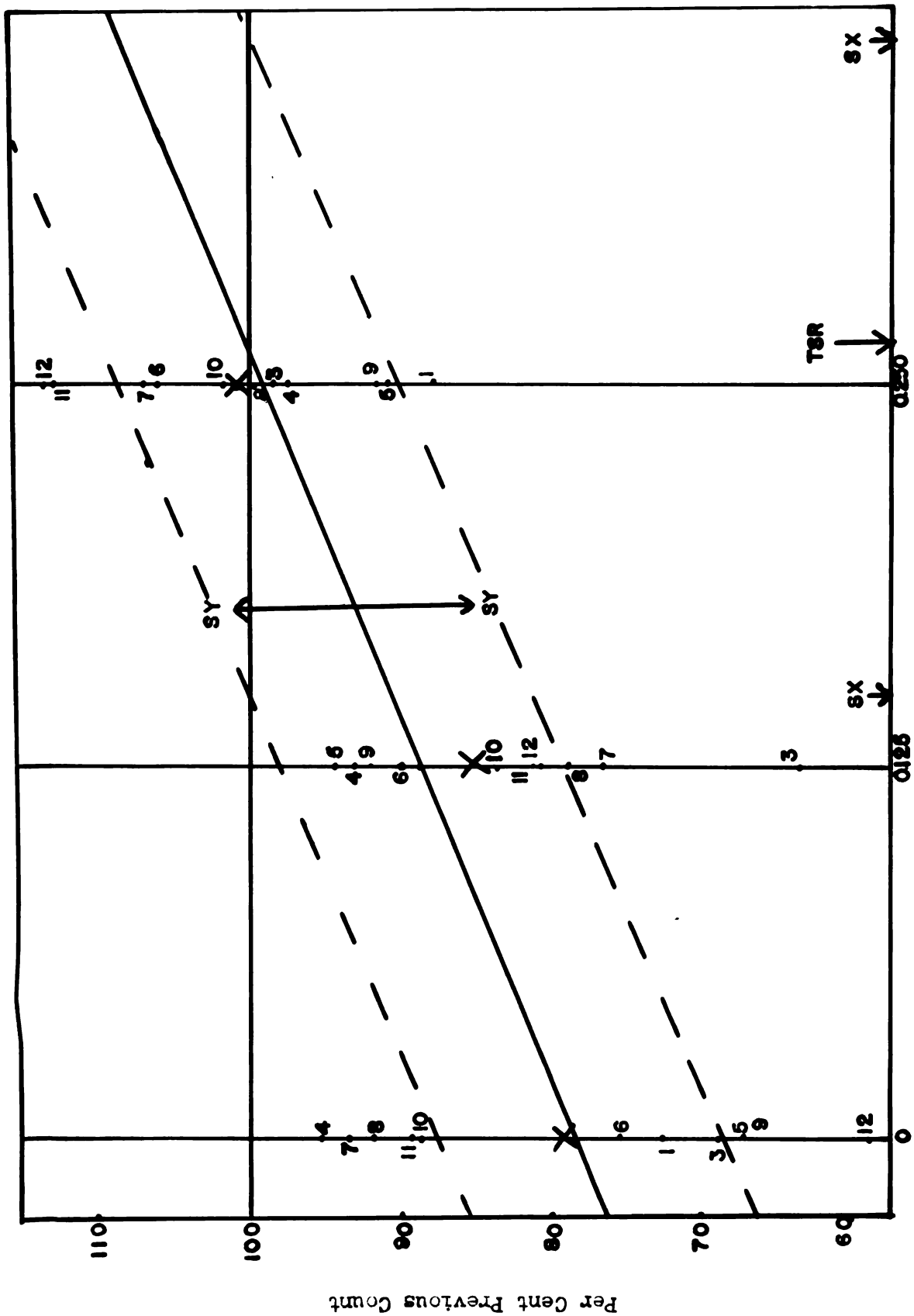


Fig. 9 - µgms. l-thyroxine/100 gms. body wt./day

DISCUSSION

The thyroid secretion rate determinations made on rainbow trout ranged from 0.1 to 0.4 ug. l-thyroxine/100 gm./day. No comparable data for cold-blooded vertebrates have been found in the literature. A few values for thyroid secretion rates have been reported for warm-blooded animals. Flamboe (1956) reported a value of 0.278 mg. l-thyroxine/100 lbs./day (S.d. = 0.036) for non-lactating goats. Amin (1956) working with several strains of mice reported values of the order of 2.5 ug. l-thyroxine/100 gm/day. In work by Reineke and Singh (1955) values for rats averaged around 2.0 ug. l-thyroxine/100 gm./day. All of these data were obtained using the similar techniques as those used with rainbow trout. The thyroid secretion rate of trout appears to be significantly below that of warm-blooded vertebrates. On the basis of histological appearance of the trout thyroid one would expect this to be the case. Histological sections of the trout thyroid (not included in this thesis) show the follicles to have an extremely low epithelium. The over-all picture was very similar to sections of rat thyroid which had undergone atrophy as the result of hypophysectomy.

Secondly the extremely slow output of I-131 from the thyroid of trout ($t_{\frac{1}{2}}$ = 37 days) when compared with output values ($t_{\frac{1}{2}}$ = 6 days) for mice (Amin, 1956) would indicate a slow rate of release of iodinated amino acids from the gland.

Irrespective of the large amount of research that has been done on thyroid function in teleosts no definite continous function of thyroxine has been established. There may be periods during which the thyroid is

quite inactive. Hoar (1953), for example, has described seasonal variations in the activity of the teleost thyroid based on differences in the histology of thyroidal tissue.

The data obtained from the radioactive mapping procedure and uptake-output studies with the geometry described herein can be used to explain some of the variation in uptake-output data reported in the literature.

The measurement of thyroidal accumulation and output of I-131 in fish made by injecting large numbers of fish, sacrificing at appropriate time intervals, and determining the activity in excised thyroid tissue introduces the factor of experimental variability between animals. When in vivo counting methods are used each animal serves as its own control and individual animal variance is reduced, giving more valid results. The possibility of not surgically removing all thyroid tissue is eliminated by the in vivo counting procedure.

In vivo estimations of the I-131 content of thyroidal tissue may be erroneous due to the accumulation of injected I-131 in the anterior part of the pleuroperitoneal cavity. This non-thyroidal activity amounts to a significant per cent of the injected dose of I-131 for three days following injection but is relatively unimportant thereafter. The great differences in per cent uptake as well as the uptake and output rates of I-131 appearing in the literature probably reflect differences in the amount of animal background (non-thyroidal above) included in the thyroid counts. The previous literature dealing with the metabolism of I-131 by the teleost thyroid show no corrections for animal background.

As an example of the influence of animal background on in vivo counts was shown by Fromm and Reineke (1956). After radiothyroidectomy considerable amounts of tracer doses of I-131 accumulated in the head

region of trout. This non-thyroidal activity was shown to leave the area rapidly having a half life of 1.8 days. This value compares favorably with the rapid decrease in animal background found in the present study.

The rapid decrease in animal background is probably the result of an accelerated rate of excretion of excess I-131. During the period of accelerated excretion of I-131 some 65% of the total injected dose was excreted into the aquarium water. Much of the excreted I-131 was removed from the water by the charcoal filter and changes of the aquarium water every 3 days prevented most recycling of the excreted I-131.

For an accurate estimation of thyroid secretion rates it is important that the in vivo thyroid activity be a measure of the true activity of the thyroid gland. The interference of animal background in these studies can be minimized in three general ways: (1) By injecting larger doses of I-131 the determination of secretion rates could be started at a much later time. This increased interval between the injection and the beginning of secretion rate determinations would result in a low body background while the thyroid would still have sufficient activity to give a relatively low counting error. The effects of the beta and gamma radiation must be taken into account when increasing the dosage. (2) Suitable collimation could be developed which would enable counts to be taken with minimal animal background activity. Because of the penetrating power of gamma radiation from I-131 appropriate collimation is very important. (3) If the rate of output of the animal background is known, along with values for in vitro activities, a mathematical expression could be derived to correct in vivo thyroid activity for animal background. In this first attempt to estimate the thyroid secretion rate of rainbow

trout all of the above methods were used to some degree.

On the basis of the following statistical approach no significant differences were found between the thyroid secretion rates for each experimental group. A single standard deviation (Sy) (see Fig. 9, page 41) was plotted as parallel lines to the fitted line. At the intercept of the Sy lines with the 100% previous count line perpendiculars were dropped from the "100% line" to the x axis. The distance from these intercepts with the "x" axis (2Sx) is equivalent to two standard deviation of the estimated thyroid secretion rate. Therefore the standard deviation of the secretion rate is given by Sx. "Sx" will be valid if Sy is a measure of the true variance about the fitted line. Since two-thirds of the observations fall within one standard deviation of the fitted line the assumption that Sy is a valid measure of the standard deviation is strengthened. From the standard deviation (Sx) the standard error of the thyroid secretion rate was calculated. Using these data, tests for significant differences between experimental groups were made using the t-test.

It is well known that one of the main functions of thyroid secretions in homoiotherms is the control or regulation of oxidative metabolism. It is also known that exposure of warm-blooded animals to lowered environmental temperatures causes increased thyroidal activity. It has been reported that when fish are exposed to low temperatures thyroid function is maintained or slightly enhanced as shown by histological examination of thyroidal material (Pickford and Atz, 1957).

The rate of reactions in the organism will increase as the temperature increases. For meaningful comparisons of the effects of temperature on the rates of various biologic processes, the rates of reaction

may be compared for some given temperature interval. The ratio of the rate of activity at one temperature to the rate at a temperature 10°C. lower is called the "temperature coefficient" (Q_{10}). Thermochemical types of reactions have Q_{10} values of about 2.00 (Giese, 1957). Appendix 1 gives the equation for obtaining Q_{10} values.

Trout at 13°C. and 3°C. have oxygen consumptions and operculum rates that yield the following Q_{10} values: oxygen consumption = 2.17; operculum rates = 1.92. The Q_{10} values indicate that the respiratory metabolism decreases by about one-half per 10°C. drop in temperature. The Q_{10} value of the mean thyroid secretion rates at the two temperatures was found to be 1.72, indicating that thyroidal activity had neither remained the same nor increased as previously postulated but that it had followed a proportional decrease in metabolic activity similar to that of the general oxidative metabolism. In the face of the above data it seems rather unlikely that thyroxine plays an essential role in the control of oxidative metabolism of the teleost at low temperatures.

SUMMARY AND CONCLUSIONS

1. A method for the measurement of thyroid secretion rates of fish was developed. Thyroid secretion rates for a poikilothermic vertebrate, Salmo gairdnerii, was found to range from 0.190 to 0.415 µgm. l-thyroxine per 100 grams body weight per day.

2. No significant differences in the thyroid secretion rate was found between fish kept at 3°C. and 13°C. For this temperature interval the thyroid secretion rates had a Q_{10} value (1.72) characteristic of a thermochemical type of reaction.

3. One-year old and two-year old trout showed no significant difference in their mean thyroid secretion rates.

4. Under the counting geometry and corrections described the peak uptake of I-131 by the thyroid tissue occurred within 24 hours after pleuroperitoneal injection of I-131. A two component output curve was found. The first component (rapid output rate) may possibly be the result of incomplete animal background corrections. Approximately 20% of the injected dose was accumulated in the thyroidal area within the first 24 hours.

5. The site of maximal accumulation of non-thyroidal I-131 activity was shown to gradually shift anteriorly for 4 days following injection of I-131 until it reached the thyroidal area. After 4 days there was no further change in the position of maximal activity.

6. Q_{10} values indicate that the oxygen consumption of trout follows the pattern of a normal thermochemical type reaction (Q_{10} = 2.0)

for the temperature range of 3°C. to 13°C. Oxygen consumptions and operculum rates yielded the following Q_{10} values: oxygen consumption = 2.17; operculum rates = 1.92.

7. It is suggested that complete or standardized animal background corrections are needed before *in vivo* counting data from different workers may be compared.

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APPENDIXES

APPENDIX I

STATISTICAL TREATMENT OF DATA

STATISTICAL TREATMENT OF DATA

Thyroid Secretion Rate

1. Method of Least Squares

N = number of observations
Ex = Sum of thyroxine dose X(N)
Ex^2 = Sum of thyroxine dose squared (N)
Exy = Sum of thyroxine dose (% preceding count)
Ey = Sum of % preceding count
Ey^2 = Sum of % preceding count (% preceding count)
y = % preceding count
x = thyroxine dose in mg./gm. body weight/day.

$$y = a + b(x)$$

$$E(y) = N(a) + b(E(x))$$
$$E(xy) = E(x)(a) + b(Ex^2)$$

solve for (a) and (b) where a = intercept and b = slope of line.

2. Standard Deviation and Error of Least Square Line.

$$Sy^2 = \frac{Ey^2 - a(Ey) - b(Exy)}{N-2}$$

Sy = ± Standard deviation of least square line.

Variance of the estimated secretion rate on the x axis is defined as Sx and calculated by the following:

$$Sx = \pm Sy/b$$

The standard error of the stimated secretion rate is found by:

$$SE = \pm Sx/\sqrt{N-1}$$

3. Coefficient of Correlation "r".

$$CY^2 = (Ey/N)^2$$

$$r^2 = \frac{a(Ey) - b(Exy) - N(CY^2)}{E(y^2) - N(CY^2)}$$

"r" values found in Barnes and Noble (1957) page 140.

4. Test for Significant Differences "T"

To determine the existence of significant differences between

the mean secretion rates of any two groups the following calculations were made:

$$T = d/SE_d$$

where T = test statistic (Barnes and Noble (1957))
d = differences between secretion rate "1" and secretion rate "2"
SE_d = standard error of the difference.

$$SE_d = \sqrt{(SE_1)^2 + (SE_2)^2}$$

degrees of freedom (df) = $N_1 + N_2 - 2$

Method of Least Squares Used in Determination of "$t_{\frac{1}{2}}$" values

$$Ex_1y_1 = E(xy) - \frac{Ex\,(Ey)}{N}$$

$$Ex_1^2 = Ex^2 - \frac{(Ex)^2}{N}$$

$$b = \frac{Ex_1y_1}{Ex_1^2}$$

where b = slope of line passing through the mean of the x and y axis.
x = time axis
y = activity axis
Ex = sum of time (N)
Ey = sum of activity
Exy = sum of activity (time)
Ex^2 = sum of $(\text{time})^2(N)$
Ey^2 = sum of $(\text{activity})^2$
N = total observation

Method of Determinating Standard Deviation of "$t_{\frac{1}{2}}$" fitted line

$\pm$ s = standard deviation of activity

$$S^2 = \frac{Ey2 - (Ey)^2 / N}{N-1}$$

Method Used to Calculate "$t_{\frac{1}{2}}$"

$$A_t/A_c = e^{-0.693(t)/t_{\frac{1}{2}}}$$

where A_o = activity at time zero
A_t = activity at time "t"
t = time
$t_{\frac{1}{2}}$ = half-life

Calculations of Q_{10} Values

$$Q_{10} = (K_2/K_1)^{10/t_2 - t_1}$$

where k_1 = rate at temperature "1"
k_2 = rate at temperature "2"
t_2 = temperature 2 (°C. + 273)
t_1 = temperature 1 (°C. + 273)
Q_{10} = ratio of activity per 10 degree change in temperature

APPENDIX II

THYROID SECRETION RATE DATA FOR GROUPS

I - II - III - IV - V

OF TABLE V

GROUP I

Thyroid Secretion Rate Data for 2 Year Old Fish at 13°C.*

Fish #	7/13/58, X= base**	7/15/58, X = 0.00	7/17/58, X = 0.125	7/19/58, X = 0.250
1	---.---	86.646***	94.728	93.795
2	---.---	---.---	--.---	--.---
3	---.---	---.---	--.---	--.---
4	---.---	64.950	79.840	131.755
5	---.---	99.268	67.135	105.824
6	---.---	79.807	112.396	92.543
7	---.---	102.099	91.311	98.583
8	---.---	--.---	--.---	--.---
9	---.---	84.945	102.028	87.358
10	---.---	69.008	100.288	105.699
11	---.---	68.443	125.610	82.786
12	---.---	95.480	91.122	128.464
		$\bar{X}$ =83.405	$\bar{X}$ = 96.058	$\bar{X}$= 102.979

* See Appendix I and Table V

** X = µg. l-thyroxine/100 gm. body wt./day

*** Per cent previous count
7/13-7/15 $t_{\frac{1}{2}}$=7.64 days

GROUP II

Thyroid Secretion Rate Data for 2 Year Old Fish at 13°C.*

Fish #	8/19/58, X = base**	8/21/58, X = 0.00	8/23/58, X = 0.250	8/25/58, X = 0.500
1	---.---	88.533***	91.489	105.416
2	---.---	83.679	95.658	94.405
3	---.---	81.550	104.815	105.545
4	---.---	--.---	--.---	--.---
5	---.---	--.---	--.---	--.---
6	---.---	63.660	90.851	88.533
7	---.---	78.090	79.080	113.329
8	---.---	79.699	114.943	107.443
9	---.---	64.758	99.765	98.585
10	---.---	100.127	87.300	101.428
11	---.---	--.---	--.---	--.---
12	---.---	94.093	100.158	103.522
		$\bar{X}$ = 81.577	$\bar{X}$ = 96.007	$\bar{X}$ = 102.023

* See Appendix I and Table V

** X = µg. l-thyroxine/100 gm. body wt./day

*** Per cent Previous Count

8/19-8/21 $t_{\frac{1}{2}}$ = 6.82 days

GROUP III

Thyroid Secretion Rate Data for 1 Year Old Fish at 13°C.*

Fish #	9/1/58, X = base**	9/3/58, X = 0.000	9/5/58, X = 0.125	9/7/58, X = 0.250
1	---.---	78.093***	92.421	84.196
2	---.---	93.368	78.292	104.962
3	---.---	78.552	92.703	93.401
4	---.---	--.---	--.---	--.---
5	---.---	77.932	89.528	93.393
6	---.---	88.696	84.689	108.393
7	---.---	87.455	93.394	90.621
8	---.---	--.---	--.---	--.---
9	---.---	--.---	--.---	--.---
10	---.---	78.321	88.612	105.517
		$\bar{X}$ = 83.202	$\bar{X}$ = 88.520	$\bar{X}$ = 97.212

* See Appendix I and Table V
** X = µg. l-thyroxine/100 gm. body wt./day
*** Percent Previous Count
9/1-9/3 $t_{\frac{1}{2}}$ = 7.59 days

GROUP IV

Thyroid Secretion Rate Data for 1 Year Old Fish at 13°C.*

Fish #	9/1/58, X = base**	9/3/58, X = 0.00	9/5/58, X = 0.125	9/7/58, X = 0.250
1	---.---	72.682***	88.501	87.925
2	---.---	--.---	--.---	--.---
3	---.---	69.047	63.888	98.774
4	---.---	95.402	93.883	97.752
5	---.---	66.738	99.829	91.059
6	---.---	75.735	89.459	105.109
7	---.---	94.323	77.193	107.435
8	---.---	92.991	78.707	99.146
9	---.---	66.808	93.912	91.638
10	---.---	88.759	83.883	101.157
11	---.---	88.810	81.021	112.704
12	---.---	58.964	81.188	113.425
		$\bar{X}$ = 79.115	$\bar{X}$ = 84.679	$\bar{X}$ = 100.557

* See Appendix I and Table V
** X = µg. l-thyroxine/100 gm. body wt./day
*** Per cent Previous Count
9/1-9/3 $t_{\frac{1}{2}}$=5.01 days

GROUP V

Thyroid Secretion Rate Data for 1 Year Old Fish at 3°C.*

Fish #	10/3/58, X = base**	10/5/58, X = 0.00	10/7/58, X = 0.063	10/9/58, X = 0.125
1	---.---	73.838***	87.800	105.416
2	---.---	56.582	63.855	123.473
3	---.---	86.073	103.766	95.831
4	---.---	--.---	--.---	--.---
5	---.---	94.155	70.621	101.587
6	---.---	89.691	115.683	99.864
7	---.---	95.386	81.932	98.764
8	---.---	44.995	94.290	84.252
9	---.---	--.---	--.---	--.---
10	---.---	70.664	89.781	79.838
11	---.---	66.634	100.124	104.659
12	---.---	79.187	82.712	76.691
		$\bar{X}$ = 75.822	$\bar{X}$ = 90.064	$\bar{X}$ = 97.039

* See Appendix I and Table V
** X = µg. l-thyroxine/100 gm. body wt./day
*** Per Cent Previous Count
10/3-10/5 t = 5.00