UPTAKE, DISTRIBUTION AND EXCRETION OF THE RADIONUCLIDE I^{131} BY RAINBOW TROUT (SALMO GAIRDNERI)

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This is to certify that the

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ABSTRACT

UPTAKE, DISTRIBUTION AND EXCRETION OF THE RADIONUCLIDE I^{131} BY RAINBOW TROUT (SALMO GAIRDNERI)

by Joseph Bruce Hunn

Rainbow trout (Salmo gairdneri) were exposed to various solutions (distilled water, tap water, 0.6% NaCl and 66% Ringer's) containing carrier free I^{131} for 24 hours. At the end of the exposure period, the fish were anesthetized with MS-222 and samples of blood, heart, gill, lower jaw (thyroid), liver, spleen, caeca, head kidney, kidney, intestine, stomach, muscle and eye were taken. The relative accumulation and distribution of I^{131} in these tissues was determined.

The uptake of I^{131} by the gills of trout is inhibited by NaSCN, reduced by chloride, inversely related to the calcium level of the medium and is not affected per se by osmotic gradient or thiouracil.

I^{131} in the blood of the trout is more than 90% ionic and its biological half-life at $14^{\circ}C$ is 1.7 days.

Iodine storage capacity of trout tissues was determined using an equilibrium technique. Results indicate that muscle may contain 30% of the total body iodine.

Joseph B. Hunn

Iodine is lost from the body of trout via the gills and urine. Fecal loss was not measured. About four times as much I^{131} is lost via the urine as via the gills. Measurements of the renal clearance of I^{131} and urine to plasma concentration ratios indicate that I^{131} is filtered and reabsorbed and that I^- clearance is greater than Cl^- clearance.

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By

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INTRODUCTION

The nuclear age has brought many advances in science and technology but has also introduced a number of perplexing problems not the least of which is the problem of radioactive waste disposal and its control. Atomic power plants located adjacent to large bodies of potable fresh water, as in Michigan, pose a problem as a potential source of radionuclides which might contaminate this aquatic habitat. Whether or not these radionuclides become an actual danger to aquatic life depends upon the type and quantity of radionuclide(s) released. Our knowledge of the biological role of many radionuclides is rather meager. Additional knowledge is, however, vital in determining the quantities of isotope(s) that may be released into a body of water without having potentially harmful effects on aquatic life.

The present study was undertaken to broaden our knowledge about the iodine metabolism in fishes and the possible involvement of I^{131} in the life of aquatic animals. Rainbow trout (Salmo gairdneri) were chosen as the experimental animal because (1) of my interest in fish, (2) they could be obtained gratis from the Michigan Department of Conservation,

(3) adequate holding facilities for cold water fish were available and (4) these fish inhabit the Great Lakes and many adjacent bodies of water.

The problem investigated covered three general areas: (1) the direct uptake of iodine from the habitat by fish, (2) the distribution and storage capacity for iodine in fish tissues and (3) the rate of turnover of iodine in the fish.

HISTORICAL SURVEY

Iodine

Iodine was discovered by Courtois in 1811 and proven one of the four halogen elements by Gay-Lussac. Shortly thereafter Prout in England introduced iodine as a treatment for goitre.

Early reports of iodine being present in fish or fish products were by Jonas (1838) in herring, Gmelin (1839) in cod liver oil, and Girardin and Preisser (1842) in the liver of the ray. In 1850 Raboudin published an analytic method for the determination of iodine which was to become the basis for the extensive work of Chatin (1850-1876). Chatin improved the method and was able to detect as little as 0.1 gamma of iodine. This investigator was the first to associate the occurrence of goitre and cretinism with geographical areas having insufficient iodine in the water, soil, air and foodstuffs; however, his idea was severely criticized by his fellow scientists (Elmer, 1938).

In 1896 Baumann showed conclusively that iodine is found in the thyroid and that it was strongly linked to protein, yet, it was not until 1914 that Kendall extracted and crystallized thyroxine. Kendall and Osterberg (1919) concluded

from analytical data that thyroxine was a tryptophan derivative, a claim disproved by Harington and Barger (1927) who showed by direct synthesis that thyroxine was a derivative of tyrosine (Elmer, 1938).

Baumann's work stimulated research on the biochemistry of iodine and the use of iodine in treatment of goitre lead to an extensive search for dietary sources of this material. Many reports contain information on the occurrence of iodine in fish or fish products in addition to a wealth of information on the iodine content of plants and other animals. References to this work are given in appendix G.

Ion Regulation in Fresh Water Fish

In aquatic animals hyperosmotic regulation, that is, maintaining the body fluids at a higher osmotic concentration than the surrounding environment, is characterized by processes for the uptake of ions and for the expulsion of water. The total energy required for the maintenance of a hyperosmotic state in higher animals, is utilized almost exclusively for the active transport of ions. The energy is consumed either entirely at the external surface in direct contact with the surrounding water or shared between this surface and tubule cells of the excretory organ. Where the

osmotic work is shared, the proportioning of work between the surface membranes and the excretory organ depends upon both the relative permeability of the body surface to water and salts, and on the efficiency of the excretory tubule cells in removing ions from the urine (Shaw, 1960).

One of the first studies on the uptake of ions by fish from the surrounding medium was done indirectly by Marine and Lenhardt (1910 a, b) in their study of goitre prevention in brook trout. They found that adding I^- to the water which flowed past the fish resulted in a reduction of the goitrous condition of the fish. Later Krogh (1937b, 1938, 1939) was able to show uptake of Cl^- and Br^- but not of I^- by goldfish. He concluded that I^- penetrates the gills of the goldfish very slowly by diffusion. Further work on anion uptake will be discussed later.

The absorption of an anion (Cl^- or Br^-) by the gills of fish need not be accompanied by any absorption of cation, however, when a cation is absorbed simultaneously with the anion, the rate is higher and the total quantity of anion absorbed is definitely larger (Krogh, 1939). This uptake of anion is against a rather large concentration gradient especially in the case of the fresh water fish and the actual mechanism for this uptake is not known.

Ion movement against a concentration gradient may be due to active transport, an energy-requiring process, facilitated diffusion which requires no direct expenditure of energy or differential permeability. Solvent drag may also play a role in ion movement.

The role of the gastro-intestinal tract in the uptake of ions in fresh water fish is rather a question mark. Some authors believe that aquatic animals get most of their salt requirement from food (Wikgren, 1953). Apparently little water is drunk by fresh water fish and it is assumed that the uptake of ions from this source is minimal.

The urine of fresh water fish is hypoosmotic to the blood but always contains some salts. Calculations of Krogh's (1939) data give urinary chloride loss for rainbow trout ranging from a minimum of 0.11 mEq/Kg/day to a maximum of 1.25 mEq/Kg/day. For the goldfish, urinary chloride loss ranges from a minimum of 0.10 mEq/Kg/day to a maximum of 0.41 mEq/Kg/day. Wikgren (1953) gives as the urinary Cl^- loss 0.25, 0.12 and 0.10 mEq/Kg/day for the lamprey, salmon and carp respectively. Fromm (1963) finds that the rainbow trout has an average urinary chloride of 10.2 $\pm$ SE .9 mEq/l and a urine flow of 101 $\pm$ SE 8 ml/Kg/day, thus a urinary chloride loss of about 1 mEq/Kg/day. The loss of chloride

via the integument is probably equal to or less than the urinary loss. Krogh (1939) gives the rate of loss in trout as 0.1 to 0.2 $\mu M/cm^2/hr$ and Wikgren (1953) reported the level of chloride loss in the goldfish as 0.07 and 0.05 $\mu M/cm^2/hr$ as computed from Krogh's (1937b) and Smith's (1929) data respectively.

Loss of ions across the gill is difficult to assess. Krogh (1937), in studies of Cl^- loss via the gills of goldfish in distilled water, found that "the loss of Cl^- from the head is very small because it is compensated by absorption." Because ion movement out of fish (outflux) and uptake (influx) may be operating simultaneously, most studies have measured the net movement of ions. With the development of the double isotope technique, flux rates can now be measured in both directions simultaneously.

In freshwater fishes fecal loss of absorbable salts is probably minimal; however, no specific data appear to be available on this subject.

Role of the Gill in Ion Regulation

Since the description of the so-called chloride cells in eel gills by Keys and Willmer (1932), much circumstantial evidence has been brought forth to support their role in

salt regulation. The exact significance of these cells in fresh water fish is still in doubt. Krogh (1939) expressed his thoughts on it by writing "the alternative that the whole of the respiratory epithelium might be also secretory appears to me more likely . . . ." Doyle and Gorecki (1961) after studying gills by use of the electron microscope came to the conclusion that the location in the epithelium and the interspecific distribution of the "chloride cells" casts doubt on their supposed function as the principal site of chloride exchange. Fleming and Kamemoto (1963) report histochemical and isotopic evidence which strongly indicates the so-called chloride cells are not the site of Na exchange in the gills of Fundulus.

The efficiency of the uptake mechanism of the gills can be seen in fish that are starving. In this case the net movement inward through the gill must equal integumentary and urinary losses ($\sim$70 mg/Kg/day in trout) if ionic balance is to be maintained. If the Cl^- content of Michigan trout waters as determined by Urshel and Hooper (1961) is typical (1-30 mg/l) then the gill must be extremely efficient in extracting the Cl^-. Such efficiency speaks strongly in favor of many cells rather than a few specialized cells being involved in maintaining the proper ion level of the blood (4,000 - 5,000 mg Cl^-/l).

Role of Endocrine Glands

The material available on the role of the endocrines in ionic regulation in fish is limited and confusing. The role of the thyroid gland in osmoregulation has been reviewed by Fontaine (1956) and Pickford and Atz (1957). Although much of the work published in this area is contradictory some general conclusions can be drawn. First, stimulation of the thyroid in decreasing salinity and inhibition in the reverse situation is a transitory response in marine fish. Secondly, there is an increase in thyroid activity in fish migrating from fresh water to salt water. This is paralleled by the development of the so-called chloride cells in the gills. The development of the chloride cells, the increase in thyroid activity and other physiological changes represent a phase in the maturing process which enables the fish to continue its life cycle in a marine habitat. Thirdly, the promotion of salinity tolerance by thyroxine may be related to the release of growth hormone or the synergism of growth hormone action by thyroxine. Gorbman and Bern (1962) in summarizing the role of the thyroid in osmoregulation state that if thyroid hormones are involved in osmoregulation they may only modify the actions of the more potent adrenal corticosteroids.

The work of Smith (1956) indicates that growth hormone may play a major role in osmoregulation. Other indirect evidence supports his contention. At sexual maturation the salinity tolerance of trout decreases; a significant increase in salinity tolerance has been shown to occur simultaneously with periods of increased growth rate in the spring and fall. And lastly, the increase in salinity tolerance can be evoked with physiologic levels of growth hormone.

The evidence to support the idea that the corticosteroids are involved in ionic regulation is meager. Sexton (1955) working with goldfish found that DCA slightly inhibited the uptake of radioactive sodium through the gills but didn't effect sodium loss. Holmes (1959) likewise found that DCA suppressed sodium uptake but he noted an increase in total sodium output from saline-loaded trout after injection with DCA or hydrocortisone. His inability to collect urine via cannulation casts doubt on values presented for renal excretion of sodium. Also, values for the extra renal excretion of sodium using data from cannulated fish can not be considered as reflecting the "normal" excretory pattern. Edelman et al. (1960) were unable to show any change in the response of euryhaline and stenohaline marine species to immersion in freshwater following injection with

hydrocortisone. The fact that glucocorticoids may affect ion regulation in terms of suppressed sodium uptake is of interest. There is good evidence that teleost interrenal tissue increases its activity in response to the demands of physical activity both under experimental conditions and during migration. Here, the corticoid role may be that of stimulating gluconeogenesis to satisfy metabolic needs attendant on increased energy output. Any osmoregulatory action is probably subsidiary to its metabolic role.

The influence of aldosterone on ion regulation has only recently been investigated. Edelman et al. (1960) could find no change in response following injection of aldosterone. Bentley and Follet (1962) have demonstrated that aldosterone reduces the net loss of Na in the lamprey. Chester Jones et al. (1962) indicate that aldosterone inhibits the uptake of sodium in the eel adapted to fresh water but does not affect the renal excretion of sodium. In some experiments the gill inhibition was not recordable until 8 hours had passed. This suggests the possibility that the steroid does not act directly on the gill uptake mechanism. Failure of the kidney to respond to aldosterone treatment by increasing sodium reabsorption may be due to its lack of capacity to respond. That is to say, probably the kidney was functioning

to its maximal capacity for sodium retention without, or prior to, the aldosterone treatment. Holmes and Butler (1963) show aldosterone can significantly reduce plasma sodium in rainbow trout but the route of loss of this sodium was not ascertained. Even though aldosterone has been identified in the plasma of all groups of fishes examined exclusive of the cyclostomes, no clear picture of its function in fish has been elucidated. Its presence in the blood does suggest that it may serve some regulatory function possibly, as in higher vertebrates, in the regulation of Na and K at the kidney level.

Work on the neurohypophyseal hormones in fish has seen two phases: (1) isolation and identification and (2) experimentation on the possible role of these hormones in ion regulation. The early work on the role of the neurohypophyseal hormones is reviewed by Fontaine (1956), and Pickford and Atz (1957). Work in the 1930's showed that pituitary extracts and pituitrin caused neither a decrease in urine volume nor an increase in body weight in freshwater fishes. Fontaine and co-workers were unable to show any measurable weight gain in fish following injection of pituitary extracts. Sexton (1955) found that pitressin increased urine flow in the goldfish and significantly

reduced the loss of Na via the gills; however, there was no change in the rate of Na uptake. Since 12 of 16 of his experimental fish died within one day following pitressin injections, the results on the urine flow probably indicate a stress reaction. Maetz and Juien (1961) have shown that neurohypophyseal extracts of Carassius as well as oxytocin stimulate the influx of Na across the gills of Carassius while the outflux of Na remains constant. Bentley and Follett (1962) show that oxytocin, arginine vasotocin and pitressin are all effective in promoting a net Na loss in the lamprey. Whole mammalian neurohypophyseal extract also increased net Na loss. Meier and Fleming (1962) have recorded a number of effects of pitocin and pitressin on water and Na movements in the killfish. Pitocin affected water influx and outflux while comparable doses of pitressin had no effect. Both pitocin and pitressin stimulated Na uptake from tap water. The renal loss of Na was affected by neither pitocin nor pitressin while the gut loss showed a five-fold increase. By tying off anus, renal papilla or both, the amount of Na loss via various routes was estimated; gill 65%, kidney 25% and gut 10%. Also survival time of fish transferred from tap water to high concentrations of NaCl was increased by pitocin but not by pitressin.

The idea that the gut in fish may play a significant role in ion regulation has been largely ignored. The indication of a five-fold increase in loss of Na by the gut under the influence of posterior pituitary is of interest especially since it is known that these hormones exert an effect on the contraction and tonus of the intestines of several teleosts.

The study of neurosecretory cells in relation to ion regulation has provided circumstantial evidence suggesting hypothalamic control and/or involvement. Fontaine (1956) states that the results obtained in fish are comparable to those described for mammals and amphibians as a result of experimental changes in their osmotic equilibrium. It therefore seems logical to presume these neurosecretory products are similar in function but the intermediary stages through which they act in osmoregulation are still to be identified. Carlson and Holmes (1962) have been able to show an increase in pituitary and hypothalamic neurosecretions in rainbow trout subjected to handling. Transfer of the fresh water fish to 60% sea water was accompanied by a decline in pituitary anti-diuretic material and an increase in oxytocic activity. The disappearance or accumulation of neurosecretory material has been used as circumstantial

evidence indicating an increase or decrease in the rate of release of this material. These observations give little indication as to the rate of synthesis or utilization of the material by the body tissues. The measurements of this and other parameters (blood level of hormones, urine flow, etc.) would do much to integrate the picture of ion regulation in fishes.

The caudal neurosecretory system of fish was first described anatomically by Weber in 1827 as a warty appendix at the end of the spinal cord of the carp. In the 1920's Speidel confirmed the existence of Dahlgren cells in the posterior part of the spinal cord of skates and other fishes. From his cytological studies it became evident that a morphologically discrete material was elaborated by these cells. Work by Enami and co-workers reviewed in 1959 suggests that the urohypophysis (a shortened term for caudal neurosecretory system) has much the same structure as the posterior pituitary. The experimental analysis of the urohypophysis was carried out by subjecting fish (loach Misgurnus) to osmotic stress via salt loading and by surgical removal of the gland followed by injection of various fractions of the gland. After salt loading, the urohypophysis exhibited increased secretory activity. Repeated saline

injections caused complete loss of secretory activity of the cells. Following surgical removal of the urohypophysis, fish were adapted to 50% sea water or fresh water. The fish were then injected with urohypophyseal extracts. Those in sea water decreased in total sodium content while those in fresh water increased in sodium content. This suggests a possible relation between the urohypophysis and certain target organs responsible for sodium regulation. The exact role of this gland is yet to be clearly experimentally defined.

Biology of Iodine in Fish

Marine and Lenhardt's (1910 a,b) reduction of goitre in brook trout by adding Lugol's Solution to the water was the first study which indicated the ability of fish to take iodine directly from water. Krogh (1938) states the following about experiments using goldfish: "A single experiment with 0.1 NaI shows a slight absorption of 0.007 μEq in three hours, but in other experiments with NaI no change whatever could be detected and the gills are no doubt practically impermeable to I^-." Earlier Keys (1937) had suggested the same thing about eel gills. All recent in vivo data on uptake come from studies in which the iodine was

injected (see Leloup and Fontaine, 1960). The only *in vitro* data available is the study by Maqsood, Reineke and Fromm (1961).

The routes and rate of excretion of iodine in fish have been little studied. Bieter (1933) reports work by E. K. Marshall, Jr., in which toadfish were injected with NaI and loss via the gill and kidney measured. At the end of 23 hours the gill loss was 74 and 92% respectively for the two fish used while the urinary loss was 0.67% and 1.1%. The toadfish is a marine, aglomerular fish. Hickman (1959) concludes from his I^{131} injection studies of the starry flounder that "it is evident that a very large proportion of the excreted I^{131}, perhaps as much as 80%, is removed extrarenally in both salt and fresh-water flounder."

The distribution of iodine within the various tissues of the body, with the exception of the thyroid and blood, has received little attention. In the appendix are listed a number of references on the iodine content of fish. Unfortunately few of these papers contain any breakdown as to organ content. Robertson and Chaney (1953) have reviewed the few data available and contributed data on the iodine content of muscle, thyroid, eggs, testes, liver and other organs in Michigan rainbow trout and California steelhead.

Two things stand out in this study; one, that there is a large capacity for iodine in the tissue of rainbow trout and two, that the development of the eggs in the female places a great demand on the body's iodine supply. Leloup and Fontaine (1960) have shown the concentration of iodine in the ovaries of salmon and trout to be about 10 times that of the plasma.

The first work on fish blood iodine is that of Leloup (1949) in which he studied 11 species of marine fish. The total iodine ranged from 12.2 to 91.5 µg%, the Somogyi precipitable iodine 5.1 to 35.7 µg% and the butanol extractable iodine (BEI) 1 to 19.2 µg%. Fontaine and Leloup (1950), studying salmon and shad at the start of their reproductive migration, found total serum iodine values of 77 - 231 µg% in salmon and 345 - 574 µg% in the shad. Robertson and Chaney (1953) give average blood serum values for wild rainbow trout in Michigan ranging from 2.7 - 8.1 µg% whereas hatchery trout had a mean value of 15.5 µg%. They also report an average blood serum iodide of 49 µg% for sea run steelhead in California. Hickman (1962) has suggested that PBI in fish is not a reliable index of circulating thyroid hormone and that only BEI will measure this adequately. His data show that in the starry flounder serum PBI and BEI

values are not well correlated. Leloup and Fontaine (1960) had previously shown that I^{131} will bind to rainbow trout and salmon plasma proteins in vitro. Data on the inorganic iodine in the blood of whitefish in fresh water, Hickman (1962), suggest the whitefish has the ability to take up and retain relatively large amounts of iodine. Hickman (personal communication) has stated that the whitefish is peculiar in this respect as the other species of fish in the same body of water do not exhibit high serum inorganic iodine levels.

The first measurement of the iodine content of fish (elasmobranch) thyroids was recorded by Cameron (1913). Burwash (1929) measured the iodine content in two species of elasmobranchs and one teleost. The more recent work is reviewed by Leloup and Fontaine (1960).

The advent of radioactive iodine in the late 1930's has lead to a tremendous amount of work on the physiology of fish thyroid. Labeled iodine has been used to help identify the various compounds in the thyroid (MIT, DIT, T_3, T_4). Various studies on uptake of injected doses have been used in an attempt to evaluate thyroid function. Estimations of output rates of thyroid hormone have been made using various methods involving labeled iodine (Hoffert and Fromm,

1959; Leloup and Fontaine, 1960). As yet no single method has been put forth which will adequately measure thyroid function in fish. This is partially due to the fact that the exact parameter(s) affected by the thyroid in fish have yet to be elucidated.

MATERIALS AND METHODS

Experimental Animal

Rainbow trout (*Salmo gairdneri*) used in this study were obtained gratis from the Wolf Lake State Fish Hatchery courtesy of the Michigan Department of Conservation. These fish were transported via aerated tanks to East Lansing where they were kept in a temperature controlled room. The water used in the static tanks was aged, aerated tap water. The water temperature in the room was 12 $\pm$ 2^{o}C. The water in the tanks is changed every third day.

The fish were fed trout pellets (Formula 2-B Trout Food made by Zeigler Brothers Feed Mills, Inc., Gardner, Penna.). Trout newly arrived from the hatchery must be acclimated about a week before they start feeding. The feeding regime here differs from the hatchery in that no liver is fed and feeding is every other day.

Trout used in the experiments were approximately of the same body weight for any one experiment. Few of the animals were sexually mature enough to determine the sex without the aid of histologic sections.

Uptake and Distribution Studies

Fish were exposed to various solutions (distilled water, tap water, 0.6% NaCl and 66% Ringer's) containing I^{131} for 24 hours. Two liters of media were placed in covered plastic containers (4 3/4 x 6 3/4 x 10 3/4) with constant aeration. The room was maintained at 13 ± 1°C with constant illumination. The I^{131} solution was added to the media 5 - 10 minutes before the fish were put in. The fish were not acclimated to the various media before being placed into them. Upon termination of the experiment, the fish were anesthetized with MS-222 (Tricaine Methanesulphonate, Sandoz) and samples of blood, heart, gill, lower jaw, liver, spleen, caeca, head kidney, kidney, intestine, stomach, muscle and eye were taken. Blood samples taken using the method of Schiffman (1959) varied from 0.3 to 1.0 ml per fish. After removal the tissues were rinsed three times in fish Ringer's, blotted dry and weighed to the nearest milligram on a Roller-Smith balance. The heart samples included the conus; liver samples included the gallbladder and contents; the gill sample was taken from the upper half of the gills to exclude thyroid tissue; the lower jaw is that piece of tissue from the 1st to the 4th gill arch cleaned of gill and muscle; the stomach sample was taken from the upper stomach while the

intestine sample was the whole lower gut (rectum); the muscle sample was taken on the right side of the fish immediately under the adipose fin and the eye sample was the entire right eye. Upon being weighed the samples were placed in five dram plastic vials and wet-ashed with nitric acid overnight. A ten ml media sample was also placed into a similiar vial. After dilution to the counting volume, all samples were counted using a two-inch thallium treated NaI well scintillation detector, Model PHA-ICA pulse height analyzer, and Model DS-1A decade scaler used as a slave scaler. Counting was done at the 5% level of error or less (according to the method of Calvin, 1949).

Injection Studies

Three different experiments were carried out using injected doses of I^{131}. A 24 hour uptake experiment was made injecting unanesthetized trout with I^{131} and I^{131} plus 20 mg NaSCN. These trout (5 per group) were placed into 40 liter aquaria containing 36 liters of aged tap water after injection. The room conditions were as previously described. After 24 hours water, blood, gill and lower jaw samples were taken and treated as in the distribution studies.

A second injection study was made as above except that

no NaSCN was injected and water from each aquarium was circulated through a separate charcoal filter. A sample of five fish was taken at 24, 72 and 120 hours after injection. The blood samples were centrifuged at 2500 rpm for 20 minutes and the plasma drawn off. The plasma was then precipitated using $ZnSO_4$ and NaOH and washed twice with 10 ml of glass distilled water. The precipitate and all washes were counted as well as the aquaria water and lower jaw.

An iodine equilibrium study was made by placing 9 trout in an 80 liter aquarium containing 10 μg of I^{127} per liter of aged tap water and injecting them daily with a constant volume of a decaying I^{131} solution (start .1 ml = 0.50 μc I^{131}). The water was changed three times a week and the I^{127} renewed each time. These fish were starved except for one feeding on day 15. The fish were sacrificed as follows; 1 on day 25, 2 on day 26, 2 on day 27, 2 on day 28, and 2 on day 29. The fish were anesthetized with MS-222 a blood sample drawn and all other tissues taken and prepared as in distribution studies except the lower jaw. Plasma protein precipitates and the lower jaws were prepared for iodine determination (for method see appendix E). After the iodine determination, aliquots from the precipitates and lower jaws were counted along with the other tissues.

Effect of NaSCN and Thiouracil on I^{131} Uptake

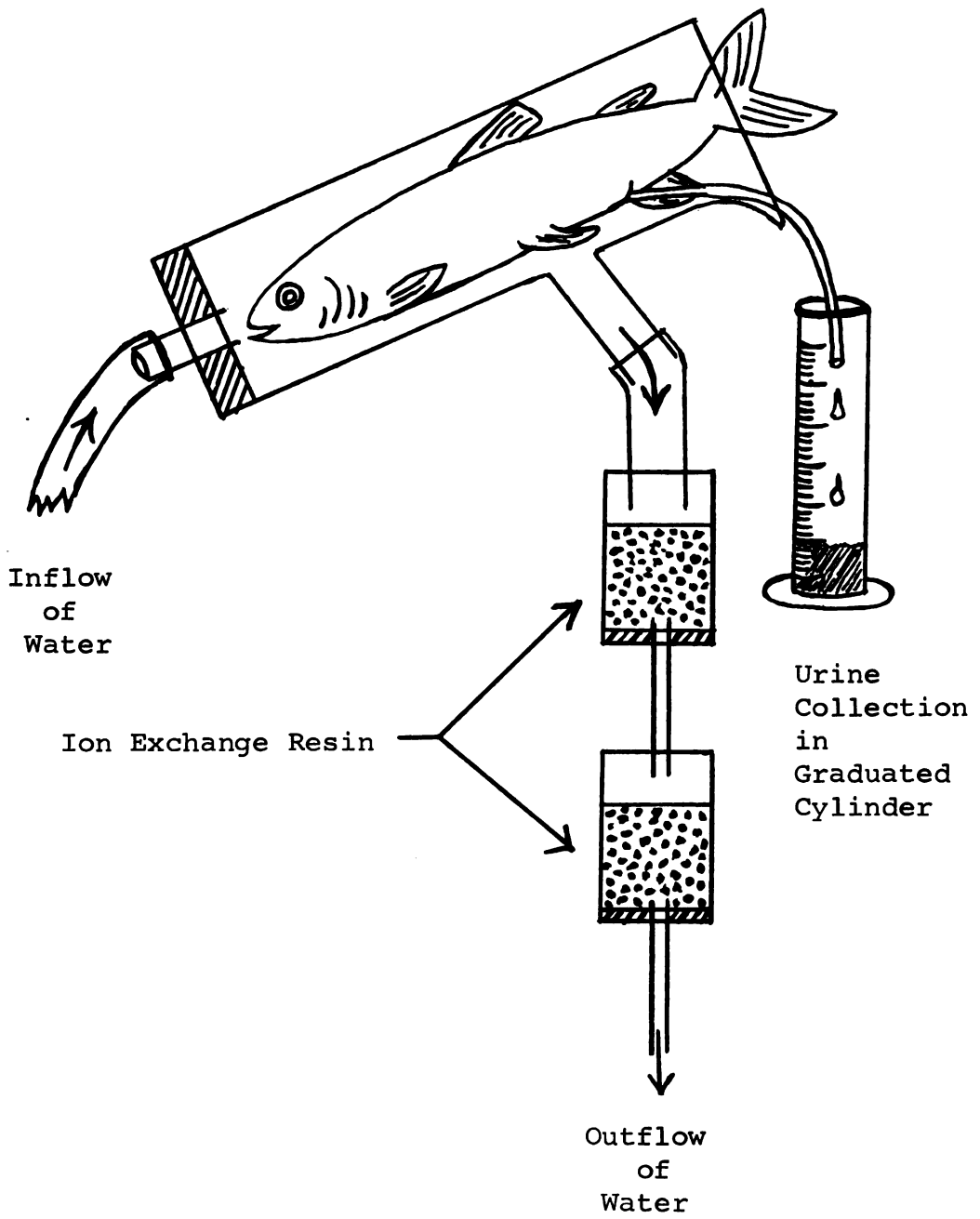
Fish were exposed to three levels of NaSCN 0.1%, 0.01% and 0.001% made up in distilled water. The procedure was the same as in the distribution studies except that only water, blood, gill and lower jaw samples were taken. The other groups were injected and placed into distilled water containing I^{131}. One group of fish was injected with 10 mg NaSCN per fish while the second group was injected with 10 mg thiouracil per fish. Sampling of tissue was as above.

Excretion Studies

Excretion of I^{131} was studied in two groups of fishes. The first group was exposed to I^{131} in distilled water for 24 hours. At the end of the exposure period the fish were anesthetized with MS-222 and a cannula (Intramedic polyethylene tubing PE 60) was inserted into the urinary bladder. The cannula had a flared tip and could be inserted under a slight pressure. Once the cannula was in place, a suture was made through the body wall and the cannula anchored. The cotton thread was then wrapped around the base of the cannula a number of times and again tied. The fish was then placed into the urine collecting apparatus (see fig. 1) head first. The body was held in place by damp cotton. Distilled

Figure 1

Urine Collection Apparatus

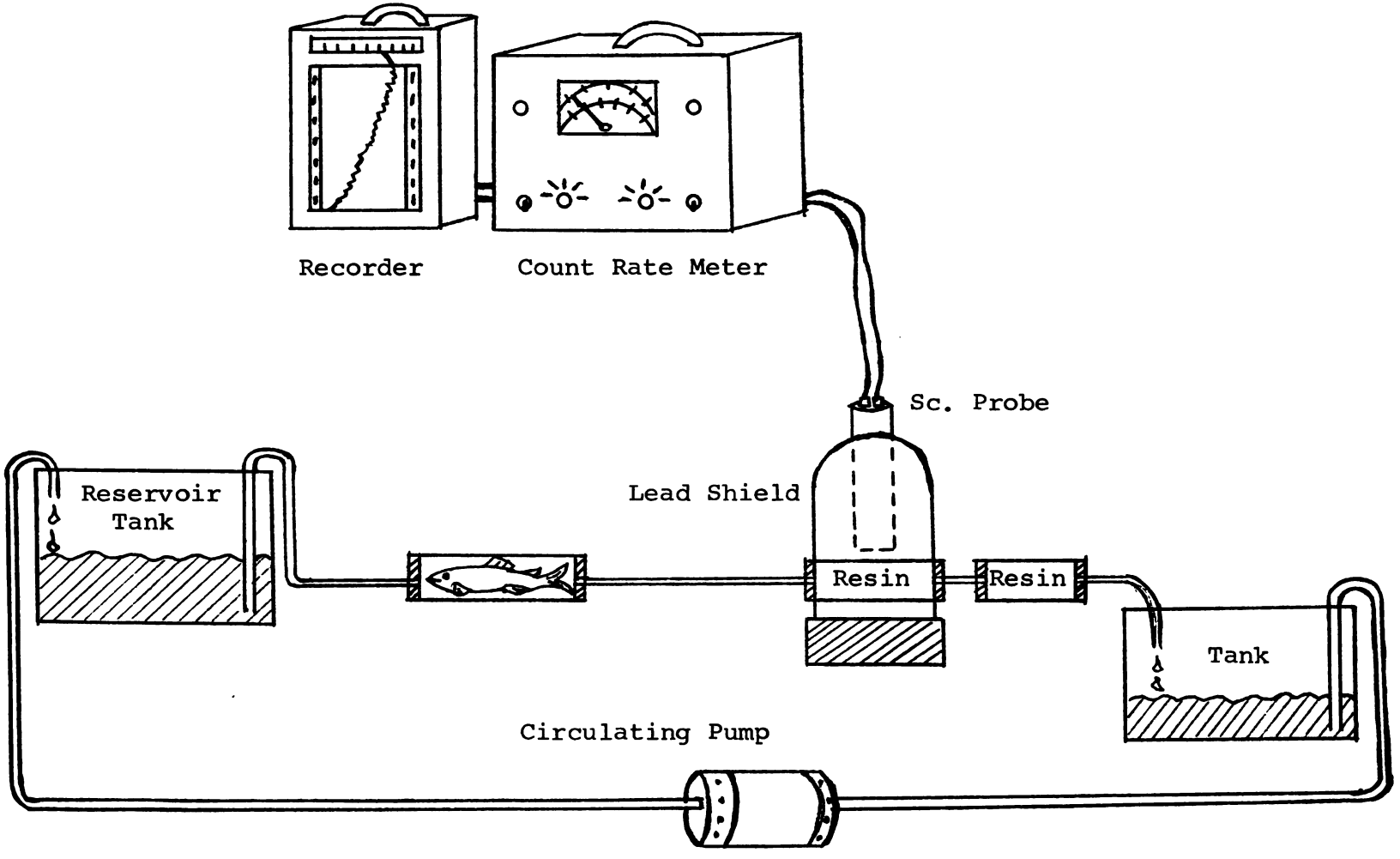

Inflow
of
Water
Ion Exchange Resin
Urine
Collection
in
Graduated
Cylinder
Outflow
of
Water

water, pumped over the head of the fish, flowed out of the side arm into two columns in series containing 10 gm of ion exchange resin IRA 400. The cannula delivered the urine into a 10 ml graduated cylinder. At the end of the urine collection period, the fish was again anesthetized with MS-222, a blood sample drawn and the lower jaw taken out and weighed. The blood was then centrifuged and the plasma drawn off. The freezing point depression of the plasma was determined with a Model C Fiske Osmometer using a small sample probe. The plasma sample was then readied for counting as well as the lower jaw and the exposure media. The second group was injected ip with an I^{131} solution and placed into tap water for 24 hours. The remainder of the procedure is as for the first group except that tap water was used.

Whole body excretion was studied using the apparatus pictured in fig. 2. Fish were exposed for 24 hours in distilled water containing I^{131} as previously described. After 24 hours they were anesthetized with MS-222 and placed into the tube heading into the flow. Distilled water was then circulated past the fish at about 150 ml/min. The water then flowed through two tubes in series containing 30 gm of IRA 400. The tubes were packed in such a manner

Figure 2

Flow-by Apparatus for Whole Body Excretion Study


Recorder
Count Rate Meter
Sc. Probe
Lead Shield
Reservoir Tank
Resin
Resin
Tank
Circulating Pump

as to hold the ion exchange resin in place between two plugs of glass wool. One tube was placed in a lead shield so that the resin would be immediately under the scintillation detector. The scintillation detector was a one-inch thallium treated NaI crystal. The scintillation tube was powered by a Model 1620 count Rate Meter (Nuclear-Chicago) and the activity was recorded via a Model AW Esterline-Angus Graphic Ammeter. The activity was recorded over a 24 hour period. Upon termination, the fish was anesthetized with MS-222 and the blood and lower jaw sampled. Samples of the exposure media, blood and lower jaw were then counted in the well counting apparatus as previously described.

Binding of I^{131} in the Blood

Since the amount of binding of I^{131} in the blood will affect the distribution and the excretion of I^{131}, it was thought necessary to determine the amount of free ionic I^{131} in the blood. Fish were exposed as described in the uptake and distribution section. Blood samples were drawn, centrifuged and the RBC's and plasma separated. The plasma was then precipitated with TCA or $ZnSO_4$ and NaOH or was run through a column containing two cc of IRA 400. The relative activity of each fraction was then determined. Fish were

also injected with I^{131} and samples of the plasma chromatographed on one-inch Whatman 3 MM paper in an ammonia-collidine system (see appendix F). After drying, the strips were placed on Kodak no-screen medical X-ray film and the film exposed for 48 hours. At that time the film was developed. Upon drying the film was placed on an X-ray viewer and the spots marked on the strips. The strips were then cut up according to the activity pattern and placed into plastic vials for counting. The relative activities of the various portions of the strip were then determined.

RESULTS

Other than the work of Marine and Lenhardt (1910 a,b), there are no data showing uptake of iodine from water by fish. The only comprehensive data on the uptake of iodide by fish tissues is the in vitro study of Maqsood, Reineke and Fromm (1961). Therefore it was decided that the I^{131} uptake data be expressed in a form comparable to that of the in vitro study. The 24 hour $\frac{T}{M}$ values were calculated as follows: cpm per unit wet weight of tissue divided by the cpm per unit of medium. The blood $\frac{T}{M}$ values were derived by dividing the cpm per ml of blood by the cpm per ml of exposure media. All other $\frac{T}{M}$ values were calculated using blood as the medium.

The tissue/blood ratio of iodine has been used to demonstrate the iodine concentrating ability of the thyroid gland in many animals. The discrete nature of the thyroidal tissue in most vertebrates makes exact weighing of the gland possible. In the trout, as in most fishes, the thyroid tissue is diffuse, with the greatest concentration of thyroidal tissue being in the lower jaw region. The use of the lower jaw region in the determination of thyroidal iodine uptake resulted in very low values due to the presence of

much extraneous tissue. Quiring (1950, 1962) has estimated that the thyroidal tissue of rainbow trout ranges from 0.60 to 6.07 mg/100 gm in fish weighing from 2,750 grams to 42.8 grams. If this estimate had been used then the thyroidal $\frac{T}{M}$ values, assuming the portion of the lower jaw removed contained all the thyroid tissue, would have been in the thousands. In order to keep the values comparable to the *in vitro* study, all thyroidal $\frac{T}{M}$ values were calculated using the weight of the trimmed lower jaws.

Uptake of I^{131} from Media of Different Osmolarities and Ionic Makeup

In general the water surrounding the fresh-water fish is of low osmolarity compared to the body fluids of fish and the diffusion gradient for salt is from the fish to the surrounding medium. Therefore the water gradient is from the surrounding medium to the fish and the salt gradient from the fish to the medium.

The media, in which the trout were exposed to I^{131}, ranged from a distilled water hypoosmotic to the trout's "normal" habitat (tap water) to 1.27% NaCl which is hyperosmotic both to the normal habitat and the fish (see Table 1).

The ionic make-up of the media was such that the concentration of all ions was less than the concentration in

TABLE 1

EFFECT OF OSMOLARITY ON I^{131} UPTAKE BY RAINBOW TROUT

Media	Osmolarity milliosmoles /Kg $\bar{x}$	24 hour $\frac{T}{M}$ values	
		$\frac{\text{Blood}}{\text{Medium}}$ $\bar{x} \pm SE$	$\frac{\text{Thyroid}}{\text{Blood}}$ $\bar{x} \pm SE$
Distilled water n = 10	6*	6.34 ± 1.12	1.49 ± .19
Tap water n = 10	8**	1.59 ± .28	2.03 ± .43
0.6% NaCl n = 10	192	1.40 ± .31	2.75 ± .41
66% Ringer's*** n = 10	212	0.50 ± .06	1.30 ± .06
1.27% NaCl n = 8	401	0.79 ± .13	4.29 ± .84

*11 mOs/kg after 24 hours

**10 mOs/Kg after 24 hours

***Ringer's after Schiffman (see appendix G)

Note: Osmolarity of rainbow trout plasma normally ranges from 280 to 300 in mOs.

TABLE 2

UPTAKE OF I^{131} AS AFFECTED BY THE CALCIUM AND CHLORIDE LEVEL OF THE SURROUNDING MEDIUM

Medium	n	Cl^- content mEq/l*	Ca^{++} content mEq/l*	Blood / Medium
Distilled water	10	-	-	6.34 ± 1.12**
Tap water	10	<1	2.6	1.59 ± .28
0.6% NaCl	10	102	-	1.40 ± .31
66% Ringer's	10	114	8.6	0.50 ± .06
1.27% NaCl	8	217	-	0.79 ± .13

*Normal plasma calcium (total) in rainbow trout is 5.3 mEq/l; chloride 136 mEq/l.

**Standard error of the mean.

the body fluids of the trout with two exceptions: (1) in 1.27% NaCl the fish were in a "positive" sodium and chloride balance and (2) in Ringer's solution the calcium concentration was greater than that of the blood.

The relative uptake from the various media and tissue distribution of I^{131} are presented in Tables A 1-4. A one-way analysis of variance indicates that there is no statistically significant difference between the $\frac{T}{M}$ values for the four treatments in the case of the spleen, caeca, kidney, intestine and muscle. In fish exposed in distilled water, the heart and head kidney $\frac{T}{M}$ values are significantly higher than those of fish exposed in the other media, while the $\frac{T}{M}$ values for gill, lower jaw, liver, stomach and eye are significantly higher in fish exposed in 0.6% NaCl.

The relative uptake of I^{131} by the trout is reflected in the blood $\frac{T}{M}$ values. It is apparent from the data presented in Table 1 that the osmotic gradient per se does not greatly affect the uptake.

Data in Table 2 indicate that Ca^{++} is involved in the uptake pattern. The highest uptake is noted in fish exposed to I^{131} in distilled water. The uptake of I^{131} from tap water which contained 2.6 mEq Ca^{++}/l and almost no Cl^{-} was statistically significantly less. There is no difference

in the uptake of I^{131} between trout in tap water and in 0.6% NaCl despite the fact there is a 100 fold increase in the amount of a similar halide (Cl^-) in the medium. A significant reduction from "normal" in uptake is noted in trout exposed in Ringer's solution. It is unlikely that the slight increase in either Cl^- content or osmolarity in the Ringer's solution could alone bring about such a change. Davson and Daneilli (1952) have shown that the presence of Ca^{++} and Ca^{++} plus K^+ inhibit movements of certain ions across certain biological membranes. Therefore the presence of a high level of Ca^{++} and some K^+ seems the more likely reason for the reduced uptake in Ringer's solution. The reason for the reduced uptake in 1.27% NaCl is unclear. However two significant changes have occurred: (1) the medium is hypertonic to the fish and (2) the chloride diffusion gradient has been reversed.

In a normal Michigan trout habitat, the level of Ca^{++} in the water may be a definite factor influencing the uptake of available I^- from the water by the fish. The low level of Cl^- (1 mEq/l or less) probably rules out Cl^- as a significant influence in I^- uptake.

Distribution of I^{131} in the Blood

Movement of ions into and out of the blood is affected by the amount of binding of these ions to plasma proteins and/or red blood cells.

The relative distribution of I^{131} in the blood following a 24 hour uptake period is listed in Table 3. Paper chromatography of *in vitro* I^{131}-tagged plasma and of plasma from I^{131}-injected fish also showed some 94-97% of the I^{131} to be in the ionic form. Twelve hour dialysis of I^{131}-tagged plasma at $3^{o}C$ showed 1-5% of the I^{131} to be non-dialyzable. The plasma (0.5 - 1.0 ml) was dialyzed against a liter of fish Ringer's (Stokes, 1963; see appendix G).

It appears that the role of binding of I^{131} is minimal in controlling the movement of I^{131} into or out of the vascular compartment of trout.

Uptake of I^{131} and the Effect of Known Inhibitors of Iodine Uptake Mechanisms

The high uptake of I^{131} from distilled water by trout suggests that an active mechanism may be involved. To test this hypothesis, the uptake of I^{131} was measured in the presence of a known competitive inhibitor of the thyroidal iodide trapping mechanism, NaSCN. Since it is possible that uptake could involve organic binding, fish were also treated

TABLE 3

DISTRIBUTION OF I^{131} IN THE BLOOD
FOLLOWING 24 HOUR UPTAKE

Distribution of Activity as % of Total Blood Activity

Treatment	IRA 400		
Fish no.	RBC	PBI	Inorganic
1	2.2	3.3	94.5
2	1.4	0.6	97.9
3	1.7	0.02	98.3
Treatment	TCA Precipitation		
1	3.2	1.6	95.1
2	0.9	3.3	95.8
3	3.4	1.6	94.9
4	0.5	2.4	97.1
5	0.8	3.2	96.0
Treatment	$ZnSO_4$ - NaOH Precipitation		
1	1.1	1.4	97.5
2	0.6	0.6	98.8
3	0.3	0.9	98.8

with thiouracil to prevent organic binding of the iodide.

Trout were exposed to I^{131} in distilled water which also contained three levels of NaSCN. From the results (Table 4) it is evident that thiocyanate competitively inhibited the uptake of I^{131} at the gill level.

Trout weighing from 64 to 124 grams were injected i.p. with either 10 mg of NaSCN or thiouracil and placed into distilled water containing I^{131}. Thiouracil had to be injected as it will not stay in solution at pH's conducive to fish life. At the same dose level, NaSCN is much more effective in reducing I^{131} uptake in the gill and thyroidal regions than is thiouracil. Since thiouracil did not suppress uptake, it is possible that the dose was too small or that organic binding of I^- is not necessary for the transfer of I^- across the gill. The elevated lower jaw $\frac{T}{M}$ value for these fish suggests that thiouracil had little or no effect in 24 hours which is consistent with the supposition that the overall iodination process is slow in the thyroids of fishes.

NaSCN is known to inhibit the uptake of I^- by the thyroid but it is also thought to have the ability to discharge the I^- from the inorganic iodine pool of the thyroid. The mechanism for the latter action is not known. NaSCN is also

TABLE 4

INFLUENCE OF NaSCN AND THIOURACIL ON I^{131} UPTAKE BY RAINBOW TROUT

Medium and treatment	n	24 hour $\frac{T}{M}$ values	
		Blood / Medium $\bar{x} \pm SE$	Thyroid / Blood $\bar{x} \pm SE$
Nandi & Bern	2 or 4	-	1.537 ± 0.82*
Nandi & Bern 0.2 mg/ml NaSCN	2 or 4	-	0.673 ± .033*
Nandi & Bern .02 mg/ml thiouracil	2 or 4	-	1.612 ± .065*
0.1% NaSCN in distilled water	7	0.13 ± .03	0.13 ± .08
0.01% NaSCN in distilled water	8	0.48 ± .04	0.41 ± .01
0.001% NaSCN in distilled water	8	1.12 ± .15	0.94 ± .15
Distilled water 10mg NaSCN injected per fish	6	1.30 ± .62	0.46 ± .02
Distilled water 10mg thiouracil injected per fish	7	6.72 ± 3.24	2.57 ± .94

*In vitro study of Magsood, Reineke and Fromm (1961)

known to increase the excretion of I^- from the body of mammals. It was of interest to determine whether or not NaSCN would affect the loss of I^{131} from trout. In Table 5 are recorded the effects of an injected dose of NaSCN on the uptake by several tissues of an i.p. injected dose of I^{131} (2.26 μc/fish). The aquarium water (36 liters) of the control fish contained 21.9% of the total of the injected doses while that of the NaSCN treated fish contained 69.7% after 24 hours. This greater loss may be due to reduced uptake as well as increased excretion. The blood $\frac{T}{M}$ values indicate that there was still a fairly large diffusion gradient between the blood and the water (NaSCN $\frac{T}{M}$ 66.36 vs control $\frac{T}{M}$ 1,027.97) at the end of 24 hours. The route of loss was not determined but the lower gill values in the NaSCN-treated fish may indicate an increased loss via this route.

Iodine Levels in the Tissues of Rainbow Trout Having an Adequate Level of Iodide Available to Them

The amount of iodine in fish is dependent upon the availability of iodine in the food and in the water surrounding the fish. The study of Robertson and Chaney (1953) indicates a large difference existed between the iodine content

TABLE 5

INFLUENCE OF NaSCN ON 24 HOUR UPTAKE OF AN INJECTED DOSE OF I^{131} IN RAINBOW TROUT

% Injected Dose per Gram Tissue

CONTROL

Fish no.	Body weight gm	Blood	Gill	Lower Jaw
1	156.7	2.74	0.56	7.82
2	110.7	2.60	0.67	11.42
3	89.1	5.10	0.85	16.25
4	159.1	2.36	0.50	8.48
5	132.7	2.70	0.54	12.02
$\bar{X}$	129.7	3.10	0.62	11.20
SE		± .51	± .06	± 1.50
	20 mg NaSCN per Fish			
1	98.1	0.72	0.26	0.37
2	163.3	1.14	0.47	0.50
3	149.6	0.68	0.31	0.31
4	172.6	0.46	0.22	0.34
5	154.4	0.19	0.06	0.05
$\bar{X}$	147.6	0.64	0.26	0.31
SE		± 0.16	± 0.07	± 0.07

TABLE 6

COMPARISON OF THE IODINE CONTENT OF THYROID AND MUSCLE IN RAINBOW TROUT

Type of trout and Condition	n	Water level of iodine µg/l	Thyroid (µg/100 gm fish) $\bar{X}$	Thyroid (µg/100 gm fish) Range	Muscle (µg/100 gm muscle) $\bar{X}$	Muscle (µg/100 gm muscle) Range
Michigan, wild* spawning run	12	1 or less	0.46	0.10 - 0.74	**0.9	0.3 - 2.4
Michigan, hatchery exposed to 10 µg/l I^{127} for 25-29 days	9	10	15.9	10.3 - 25.2	***15	10 - 27
California* steelhead 0-150 miles upstream spawning run	8	50	16.3	11.5 - 26.9	**16	10 - 22

*Robertson and Chaney (1953).

**Calculated from data of Robertson and Chaney (1953).

***Calculated value from iodine equilibrium study.

TABLE 7

BLOOD IODINE VALUES IN "NORMAL" TROUT VS. TROUT IN IODINE "EQUILIBRIUM"

"Normal"*		Equilibrium study	
PBI µg%	Inorganic µg%	PBI*** µg%	Inorganic** µg%
3.5	10	45.1	470
10.1	5	53.8	301
8.0	2	61.6	276
6.2	0	65.0	325
4.7	0	72.2	216
7.9	3	70.6	451
6.7	0	67.8	233
5.2	0	37.5	335
5.7	0	47.0	184
3.5	0		

*Determinations by Albert L. Chaney Chemical Laboratory, Glendale, California.

**Calculated values.

***Determined by alkaline fusion method (see Appendix E).

TABLE 8

ESTIMATED IODINE STORAGE CAPACITY OF
RAINBOW TROUT TISSUES

Tissue	µg/100 gm $\bar{X} \pm SE$
Plasma, Inorganic	392 ± 50
Plasma, P.B.	58 ± 4
Heart	63 ± 9
Gill	57 ± 5
Lower Jaw	4,641 ±552
Liver	118 ± 19
Spleen	52 ± 10
Caeca	48 ± 5
Head Kidney	108 ± 14
Kidney	117 ± 19
Lower Gut	61 ± 8
Stomach	52 ± 6
Muscle	15 ± 2
Eye	43 ± 2

TABLE 9

BODY WEIGHT-ORGAN WEIGHT RATIOS
IN RAINBOW TROUT

$$\frac{\text{Organ Weight}}{\text{Body Weight}} \times 100$$

	$\bar{X} \pm SE$
Body Weight	169.2 ± 14.1
Heart	0.14 ± .01
Gill	1.59 ± .05
Lower Jaw	0.27 ± .02
Liver	0.95 ± .04
Gall Bladder	0.03 ± .01
Spleen	0.14 ± .01
Caeca	1.34 ± .07
Head Kidney	0.14 ± .01
Kidney	0.64 ± .04
Stomach	1.62 ± .09
Mid-gut	0.47 ± .03
Lower-gut	0.52 ± .03
Eye	0.54 ± .02
Lens	0.039 ± .001

TABLE 10

ESTIMATED MAXIMUM IODINE CONTENT OF A THEORETICAL 100 GRAM RAINBOW TROUT

Tissue	% body weight	av. µg I / 100 gm	µg I	% of total I
Plasma Iodide	1.80	392	7.06	19.91
PBI	0.04	58	0.02	0.06
Heart	0.14	63	0.09	0.25
Gill	1.59	57	0.91	2.57
Lower Jaw	0.27	4,641	12.53	35.34
Liver	0.95	118	1.12	3.16
Spleen	0.14	52	0.07	0.20
Caeca	1.34	48	0.64	1.80
Head kidney	0.14	108	0.15	0.42
Kidney	0.64	117	0.75	2.12
Lower Gut	0.52	61	0.32	0.90
Stomach	1.62	52	0.84	2.37
Muscle	70.00*	15	10.50	29.61
Eyes	1.08	43	0.46	1.30

*From Robertson and Chaney (1953).

of sea-run rainbow trout and Michigan wild trout. They ascribed this to the difference in availability of iodine in the environment. It was of interest to find out whether or not rainbow trout given sufficient iodide in the water, could take up and maintain levels equal to those of trout in an iodine-rich environment. The role of diet was ruled out by fasting the fish.

The level of I^{127} used in the water was 10 μg/l which is about 3-100 times the reported levels of iodine in Michigan waters (Eldridge, 1924) and about one-fifth the iodine level of sea water.

The radioactivity of the tissues listed in Table A-1 was determined as well as the iodine content of the lower jaws and plasma protein precipitates. Using these data the radioactivity ($\frac{\mu c}{gm}$) of the tissues was determined. The iodine measurement of the PBI and lower jaw allowed the calculation of the specific activity of iodine (μc/μg) in these tissues. Assuming the trout were in iodine equilibrium, the iodine content of the remaining tissues was calculated using radioactivity of the tissue in the terms of μc/gm divided by the sp. act. of the PBI (μc/μg I). The specific activity (μc/μg I) of the PBI of each fish was used for the tissues of the fish. The blood PBI was used instead of thyroidal iodine

because it was thought that this iodine would exchange much more readily with blood inorganic iodide and more closely resemble the other body tissues in this respect. As stated earlier Hickman and other workers have shown that PBI is made up of protein-bound thyroxine as well as a much larger amount of non-thyroxine iodine. The actual amount of thyroxine (BEI) could not be determined because the blood samples were too small to do both PBI and BEI determinations. If one assumes that the sea-run trout sampled by Robertson and Chaney (1953) were in iodine equilibrium, the iodine values from those fish should coincide with the values obtained in the present study. Table 6 shows that the thyroidal iodine values do indeed coincide.

Table 8 contains the estimated content of iodine in the tissues of the rainbow trout studied. Note that the calculated iodine content of the muscle is the same as that computed from Robertson and Chaney's (1953) data (see Table 6). Using the data from Tables 8 and 9, the maximum iodine content of the tissues of theoretical 100 gram trout have been calculated (see Table 10).

Assuming the trout in this study were in iodine equilibrium, it is of interest to note that the tissue $\frac{T}{M}$ values, Table A-5, are about the same as the 24 hour $\frac{T}{M}$ values for

trout in tap water with the exception of the lower jaw. This suggests that the values for relative distribution at the end of 24 hours is indicative of the iodine distribution pattern at equilibrium.

Excretion of I^{131} by Rainbow Trout

Net uptake of iodide results when the rate of uptake exceeds the rate of loss. Almost nothing is known of the routes of iodide loss or their relative importance in fishes. Marshall's work suggests that the gill is the most important route of iodine loss in an aglomerular fish. No data are available on glomerular fish such as the trout.

The rate of loss of iodide from the whole fish was studied using the apparatus shown in fig. 2. Trout used in this study were exposed to I^{131} in distilled water for uptake. Distilled water was then used as the circulating medium because (1) its composition could be controlled, (2) it would provide a maximum diffusion gradient for the iodide and (3) by probably creating a diuresis would give the maximum urinary rate of loss. This measured rate of loss then represents the maximum rate at which trout would lose iodide. The data from this experiment are listed in Table 11. Fish No. 1 was over anesthetized with MS-222 and used as a control measuring loss

TABLE 11

WHOLE BODY EXCRETION OF I^{131} FOLLOWING 24 HOUR EXPOSURE TO I^{131}

Fish no.	a*	bx	rxy	T $\frac{1}{2}$ (hours)
1**	3.49412	-.00833	.99	37.5
2	4.29648	-.01286	.99	24.06
3	4.69671	-.00733	.99	41.25
4	3.98372	-.01832	.99	16.0
5	3.90703	-.01797	.99	18.3

*Log cpm = a + bx

**Over anesthetized

TABLE 12

BLOOD DISTRIBUTION OF I^{131} AND $\frac{T}{M}$ VALUES FROM TROUT AT THE END OF WHOLE BODY EXCRETION STUDY

Fish no.	% total I^{131}			$\frac{T}{M}$ values	
	RBC	PBI	Ionic	Blood* / Medium	Lower Jaw / Blood
1	-	-	-	-	-
2	5.31	0.38	94.31	20.36	2.57
3	1.21	1.50	97.19	37.24	2.82
4	0.70	2.13	97.17	12.58	5.69
5	1.03	1.23	97.74	13.15	5.63

*Exposure medium contained from 1.6 - 2.1 μc of I^{131} per two liters of medium.

TABLE 13

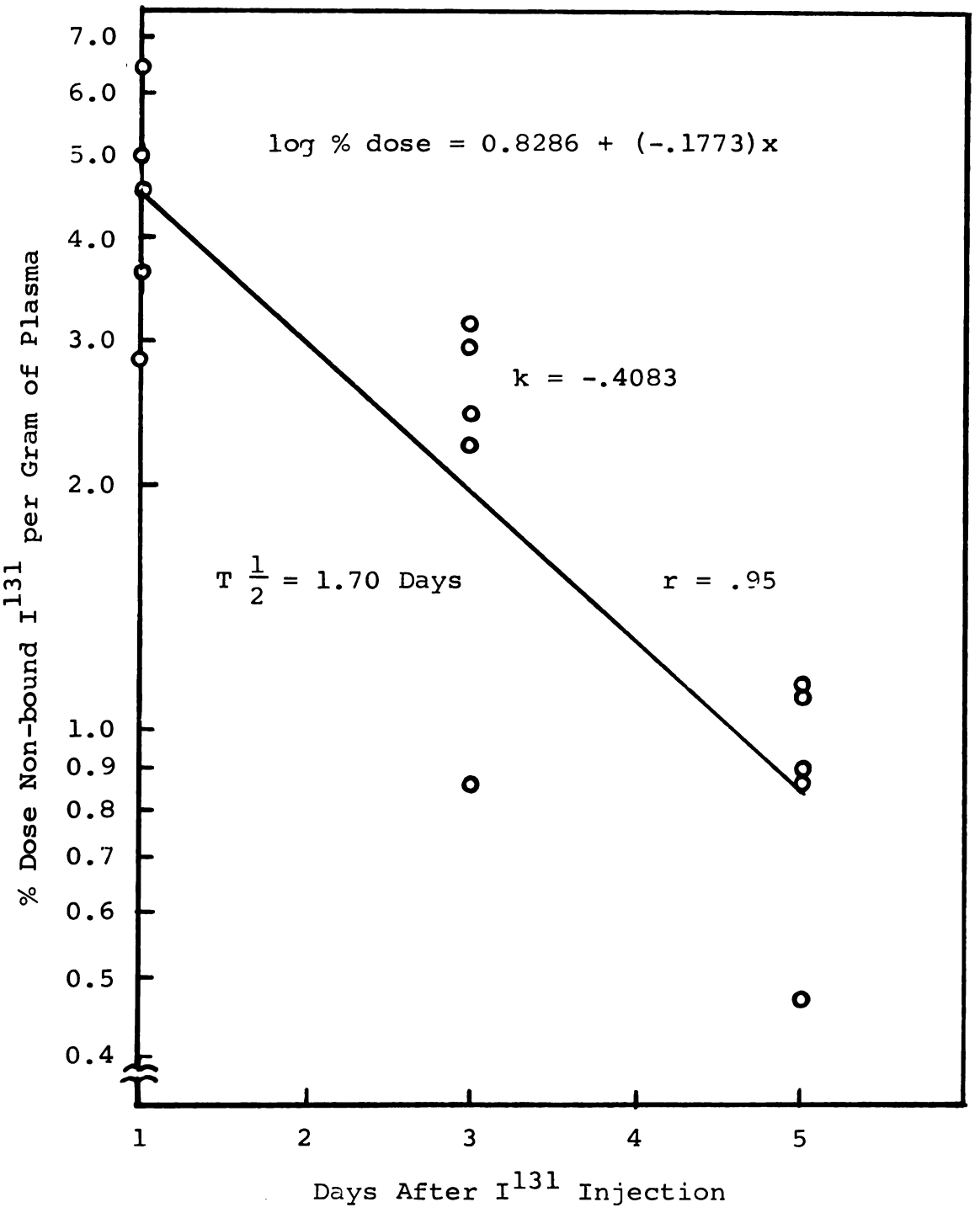
TURNOVER OF I^{131} IN THE BLOOD OF I^{131} INJECTED RAINBOW TROUT

	24 hour $\bar{X}$ *	72 hour $\bar{X}$ *	120 hour $\bar{X}$ *
% dose per gram plasma	4.53	2.35	0.91
% dose PBI per gram plasma precipitated	.037	.016	.026
% dose as non-bound per gram plasma	4.49	2.33	0.88
% I^{131} protein bound	0.83	0.70	2.90
% dose per gram lower jaw	7.87	18.94	31.79
$\frac{\text{Non-bound plasma } I^{131}}{I^{131} \text{ in water}}$	4,681.40	2,761.80	837.76
Lower Jaw $\frac{T}{M}$	1.82	7.89	36.36

*n = 5

Figure 3

Half-life of Non-bound I^{131} in Rainbow Trout Blood


log % dose = 0.8286 + (-.1773)x
k = -.4083
T 1/2 = 1.70 Days
r = .95
% Dose Non-bound I131 per Gram of Plasma
Days After I131 Injection
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
1
2
3
4
5

by diffusion only. Data show that three out of four "normal" fish lost iodine at a rate greater than that by diffusion loss alone (fish no. 1). The blood $\frac{T}{M}$ values (Table 12) suggest, however, that the fish has a rather efficient mechanism for retaining iodide even under conditions calculated to bring about the maximum rate of loss.

Since the blood $\frac{T}{M}$ values remained high following maximum whole body excretion, it was decided that a study be made of the biological half-life of I^{131} in the blood. Fifteen trout were injected with 3.87 μc I^{131} each and placed into three aquaria (5 in each). Samples of five fish were taken at 24, 72 and 120 hours. Data from this experiment are presented in Table 13 and fig. 3.

The apparatus shown in fig. 1 was constructed so that the ions added to water passing over the gill area would not be available for reabsorption and the iodide loss via gill area could be separated from that lost via the kidney. The gut loss was not measured. Data on fish exposed to distilled water and tap water are presented in Tables 14 and 15. The urine flow of the trout (ml/Kg/24 hr.) in both studies is high compared to the normal values for rainbow trout given by Krogh (1939) 60-106 ml/kg/day, Holmes (1961) 75-90 ml/Kg/day and Fromm (1963) 101 $\pm$ SE 8 ml/Kg/day. This could be

TABLE 14

EXCRETION OF I^{131} BY RAINBOW TROUT FOLLOWING EXPOSURE TO I^{131} IN DISTILLED WATER*

Fish	1	2	3	4	5
Urine					
Urine Volumes					
Collection period hr	2.0	2.0	1.25	1.5	2.0
Urine volume ml	1.4	1.9	1.3	1.5	1.9
ml/Kg/hr	5.4	9.3	6.7	8.8	7.5
ml/Kg/24 hr	129.6	232.2	160.8	211.2	190
Clearance					
Osmotic					
Urine mOs/Kg	61	40	34	29	48
Plasma mOs/Kg	-	296	279	235	292
Clearance ml/hr	-	.13	.12	.12	.16
I^{131}					
Urine/plasma ratio	.30	.07	.27	.34	.10
Clearance ml/hr	.22	.06	.17	.25	.10
Urine/gill ratio	3.92	2.74	6.29	3.05	3.61

*Distilled water was also the circulating medium used during the excretion study.

TABLE 15

EXCRETION OF I^{131} BY RAINBOW TROUT FOLLOWING I.P. INJECTION OF I^{131}*

Fish	1	2	3	4
Urine				
Collection period	2 hr	2 hr	2 hr	2 hr
Urine volume ml	1.5	1.3	1.2	1.2
ml/Kg/hr	7.4	6.3	6.4	8.0
ml/Kg/24 hr	176.4	151.2	153.6	192.0
Clearance				
Osmotic				
Urine mOs/Kg	51	38	81	36
Plasma mOs/Kg	302	282	288	299
Clearance ml/hr	.13	.09	.17	.07
I^{131}				
Urine/plasma ratio	.08	.09	.10	.07
Clearance ml/hr	.04	.06	.06	.04
Urine/gill ratio	4.0	4.5	-	2.66

*After injection the trout were placed into four liters of tap water for a 24 hour uptake. The medium circulated over the gill area during the excretion study was also aged tap water.

due to the anesthetic, a "handling diuresis," or in the case of the fish exposed to circulating distilled water, an increased uptake of water. Fish tested in distilled water show a higher $\frac{U}{P}$ ratio and clearance than do those fish tested in tap water. This reduced kidney reabsorption of iodide may be due to the diuretic state of the animals.

The urine to gill ratios indicate that the urinary loss is about four times that of the gill diffusion loss. Under normal conditions where the net movement of iodide may be inward across the gills, the urine would be the major route of excretion.

DISCUSSION

Fresh-water fish may obtain salt from food consumed or by direct uptake via the gills. In starving fish ionic balance or stability is maintained by active uptake of ions by the gills. If most salt necessary for the maintenance of homeostasis is supplied via the gut, it is most probable that the fine control of ionic homeostatic levels is carried out by the gill uptake mechanism. Regulation of the gill mechanism may be assumed to be under the control of hormonal substances present in the blood. The stimuli responsible for altering the circulating levels of these postulated hormonal substances are unknown. It would appear likely, however, that these stimuli arise from changes in the relative ionic composition or osmotic concentration of the blood or environmental water.

The very nature of the uptake mechanism may impose a differential rate on the uptake of various ions available in the habitat, and needless to say, the ionic composition of the habitat water will affect their availability to the fish. The ionic composition may influence ion uptake by affecting the gill epithelium directly. The effect of Ca^{++} on I^{131} uptake by trout is one example. This effect of Ca^{++} in the

medium appears to be similar to that observed by Phillips et al. (1955, 1958) who reported that the uptake of certain ions was related inversely to the level of Ca^{++} in the medium. Calcium in the habitat may be used by fish as a source for body Ca^{++} and it can alter the permeability of cell membranes. Increased permeability due to low Ca^{++} may lead to an increased water influx across the gill which would in turn increase the urine volume and loss of ions. The decrease in ion levels plus the lowered osmotic concentration could trigger a mechanism which increases the rate of ion uptake by the gill.

A high level of Ca^{++} at the gill surface by decreasing permeability probably inhibits movement of ions across the epithelium, a phenomenon observed repeatedly with other biological membranes. By reversing the Ca^{++} gradient (outward to inward) the rate of movement of Ca^{++} into the trout may increase thereby eliminating certain routes of entry for other ions through the gill epithelium.

Anion uptake has been extensively studied using Cl^{-}. Krogh (1937b) was able to show that a number of fresh-water fish could take Cl^{-} or Br^{-} directly from the surrounding medium if they had previously been "washed out" by exposure to distilled water. He was unable to show the uptake of I^{-}

or NO_3^-. Meyer (1948) using goldfish partially covered with a rubber bag found that fish initially lost Cl^- via the gills after transfer from holding tanks to battery jars. Following this initial "shock" phase, they took up Cl^-. By injecting distilled water, he was able to show that dilution of body fluids retarded Cl^- excretion while the uptake remained the same. Salt loading reduced uptake while the excretion remained unaffected. Wikgren (1953) confirmed the observations of Meyer (1948) that fish need not be "washed out" in distilled water before active uptake is initiated. He did not, however, find the so-called "shock" effect observed by Meyer (1948). The author also noted that when Petromyzon were placed into distilled water, the Cl^- level increased rapidly but then dropped back to a low level which was maintained throughout the experiment; however, the conductivity of the medium continued to increase. This indicates a fine degree of control over the Cl^- handling mechanisms in the face of a large diffusion gradient. Jorgensen and Rosenkilde (1956) have shown that Cl^- can be taken up directly from tap water by goldfish. In these fish the excretion or retention of 150 to 238 μEq Cl^- was followed by retention or excretion of one ml of water. They suggest that the osmoregulatory mechanisms in the goldfish are

probably sensitive to variations as small as ± 3% in the osmotic pressure of the body fluids when such change occurs in 24 hours or less.

There are no data which suggest a possible competitive effect between the various halides in relation to the ion uptake mechanism of the gill. Krogh (1937b) stated that the fish gill cannot distinguish Br^- from Cl^-. Data presented were on the uptake of I^{131} by trout from solutions containing various levels of Cl^- show no competitive effect of Cl^- on I^- uptake. A hundred fold increase in Cl^- (tap water to 0.6% NaCl) did not significantly change the uptake of I^{131}. Doubling the Cl^- content 0.6% NaCl to 1.27% NaCl) reduced the I^{131} uptake but it should be noted that the Cl^- diffusion gradient reversed with the two solutions. Whether the reduction in I^- uptake was purely a Cl^- effect is doubtful.

The effect of metabolic inhibitors on gill uptake has received little attention. Meyer (1952) has shown that a mercuric ion at a concentration of 10^{-5} M completely inhibits Na^+ uptake and increases Na^+ gill loss 10 fold in goldfish. Sexton and Russell (1955) suggested this inhibition is due to a reduction in gill succinic dehydrogenase activity. Data reported herein clearly demonstrate that NaSCN can inhibit iodide uptake by the gills of rainbow trout. A

graded response, in terms of blood $\frac{T}{M}$ values, can be seen in relation to the three levels of NaSCN tested. It should be noted that the thyroidal $\frac{T}{M}$ values also showed possible effects of NaSCN indicating it passed through the gill into the blood stream. Injected doses of NaSCN were also effective in reducing gill uptake.

The injection of thiouracil, a compound known to block the iodination of tyrosine showed that organic binding is probably not necessary for the movement of iodide through the gill. Carrier mechanisms have been postulated for cations but few have been mentioned in relation to anions.

The role of the diet in supplying iodide and other salts to fresh-water fish has not been evaluated. There are virtually no data in the literature on the iodine content of fresh-water plants or animals. Just what role diet plays in supplying iodide to fresh-water fish remains to be elucidated.

Blood iodine levels are governed by the amount of iodine taken into the body, the exchange of iodine between the blood and the tissues and the amount excreted. The blood iodine picture in fishes is quite different from that of mammals. In mammals most iodine in the blood is protein-bound and in the form of iodothyronines with little inorganic

iodine. In fishes the converse is true especially if there is adequate iodine in the environment. Leloup (1949), in studying the blood iodine of marine fish, found that the BEI ranged from 2-56% of the total iodine and PBI 7-89% of the total. The BEI comprised 13-74% of the PBI iodine. Similar blood iodine values have been reported for the salmon and shad (Fontaine and Leloup, 1950). Robertson and Chaney (1953) found that about 50% of the total blood iodine was PBI in California steelhead trout. Hickman (1962) reported that in the starry flounder the PBI and inorganic iodide conform to seasonal variations in the environmental level of iodine. The BEI remained constant except for the month of July and represented only a small portion of the total blood iodine. He has also shown a direct correlation between the inorganic iodide levels and the PBI levels whereas the level of BEI remained constant and hence exhibits no correlation with PBI or inorganic iodide levels.

The present study supports the fact that most of the iodine in the blood of fish is in the inorganic form. From 94-99% of the I^{131} in the blood of the trout following a 24 uptake period (Table 3) is in the non-bound form. Data presented in Table 13 show a similar distribution pattern in the blood of i.p. injected I^{131}. Tong et al. (1961) have

shown that 88.4% of plasma iodine is iodide in the hagfish four days after injection of 400 μc of I^{131}.

The amount of blood iodide found in or attached to the red blood cells of fish varies from species to species. Leloup and Fontaine (1960) have expressed the distribution of iodide between the plasma and the RBC's as H/P ratios (the ratio of I^{131} per gram of RBC's to the I^{131} per gram of plasma). Data on mammals and birds show a range of H/P ratios of 0.40 to 0.60. The H/P ratios for teleosts may be similar (eel, carp, conger, sea perch) or lower (Salmonidae, shad, mullet). The minimal value found by Leloup and Fontaine (1960) was 0.098 in salmon. The present study (using data from the uptake studies) indicates that the average H/P ratios for rainbow trout is 0.05 (range 0.01 - 0.16). This is about 1/4 the value reported for rainbow trout by Leloup and Fontaine (1960). Their values are from an *in vitro* study.

The amount of non-hormonal iodine bound to plasma proteins in fish blood may exceed 50% of the PBI. Fontaine and Leloup (1960) have shown that I^{131} will combine with salmon and trout plasma proteins *in vitro*. The present study suggests that *in vitro* binding is similar to that which occurs *in vivo* during 24 hours following injection.

Data from the uptake studies (Table 3) also suggests that some binding takes place as it is improbable that any radioactive T_4 is released into the circulation in so short a time. Berg et al. (1959) reported in studies on intra-thyroidal iodine (I^{131}) metabolism in fish that labeled thyroxine is often slow to appear and is present in only trace amounts or only in certain seasons. Leloup and Fontaine (1960) report that chromatographic studies on thyroid hyrolyzates show that inorganic iodine often exists in significant amounts and may persist for a long time. Tong et al. (1961) report that labeled thyroxine was first detectable in hagfish plasma 6 days after I^{131} injection. Fontaine and Fontaine (1962) report that in non-migratory rainbow trout the pituitary thyrotropic stock is nil or very low.

The distribution of iodine (I^{131}) in the tissues of the trout as related to the blood ($\frac{T}{M}$) is similar to that of mammals (Wallace and Brodie, 1937) except for muscle. In the data presented for rainbow trout the thyroid is the only tissue which concentrates iodine over the blood level. An iodide pump is known to exist in the stomach of tadpoles (Lipner and Hazen, 1962) and in higher vertebrates (Brown-Grant, 1961). No data were obtained on the stomach contents

of trout to confirm the presence of an iodide pump. As previously mentioned, the ovary is also capable of concentrating iodine.

Data on the iodine content of various fish are few. Information as to the environmental level of iodine as well as other pertinent data are often not included in the reports of these values. Robertson and Chaney (1953) reviewed the literature and have contributed data on the iodine content of the blood, muscle, thyroid, eggs, testes and liver of wild Michigan rainbow trout and California steelhead trout. Leloup and Fontaine (1960) have published values for plasma iodine for cylostomes, selachians and teleosts. Hickman (1962) gives values for thyroidal and serum iodine in the starry flounder, rainbow trout and whitefish. In the present study the levels of plasma iodine in hatchery trout maintained as previously described were measured. Lower jaw and plasma PBI iodine levels were also determined in hatchery trout exposed to water containing 10 μg per liter of I^{127}. These data and those of Robertson and Chaney (1953) indicate that there is a large storage capacity for iodine. The tissues of hatchery trout exposed to 10 μg I^-/l contained some 16 times more muscle iodide and 30 times more thyroidal iodine than the same tissues from wild Michigan trout.

The observation (Robertson and Chaney, 1953) that the ovary sequesters large amounts of iodine into the developing eggs should be of interest to trout culturists in a low environmental iodine area such as the Great Lakes. Addition of iodine to the diet during the time when the fish are sexually ripening would probably give rise to a healthier brood stock because the iodine demand of the thyroid and ovary could be adequately met. Survival of the developing embryos and fry might also be enhanced by having adequate iodine stores.

Contamination of waters in the Great Lakes region with sufficient radioactive iodine could have detrimental effects both on aquatic animals and man. Since fish can take up iodine from the water and concentrate it in the developing eggs, sufficiently high levels of radioactive iodine could bring about genetic damage in addition to possible damage to the developing embryonic tissue, especially thyroid.

The human population could be affected by drinking contaminated water or consuming radioactivity contained in sport and/or commercial fish. As stated previously trout muscle has a large capacity for storage of iodine and may contain as much as 30% of the total body iodine.

Iodide may be lost from the body of a trout: (1) via

diffusion across the gills, (2) diffusion from the general body surface, (3) the feces, (4) urine and (5) laying of eggs by the female.

Whole body loss was studied using a flow-by system to prevent reabsorption of the iodide from the surrounding medium. The $T\frac{1}{2}$ values for whole body loss indicate that the amount of I^{131} being lost per hour is reduced by one-half in an average of 24.9 hours.

The whole body loss in terms of percent dose of I^{131} lost into the medium surrounding the fish following an i.p. injection has been studied by a few workers. Leloup (1952) studied two species of marine teleosts and found that Muge lost 24.7% in 24 hours and 45.3% in 72 hours; Congre lost 7% in 24 hours. Chavin (1956) reported that the fresh-water goldfish lose 65% of an injected dose of I^{131} into the aquarium (tap water) in 24 hours. Leloup and Fontaine (1960) have reviewed the literature and compiled a table of data on the rate of excretion of I^{131} by fresh-water teleosts. Their data indicate that rainbow trout kept at $20^{o}C$ lose 33.5% of an injected dose of I^{131} in 24 hours. In the course of the present study one experiment in which five unanesthetized trout were injected with I^{131} (see Table 5) showed that the aquarium water contained 21.9% of the total

injected I^{131} 24 hours after injection. The second experiment involved a series of 10 trout which were anesthetized with MS-222, injected with I^{131} (average 1.31 μc per fish, see appendix B) and placed into plastic containers holding four liters of tap water at 14°C. After 24 hours, the tap water contained an average of 41.0% of the injected dose (range 25.5 - 62.4%). The action of MS-222 on ion regulation in fish is not known. A recent note by Collins and Hulsey (1963) indicates that 0.5 percent NaCl solution with a concentration of 1 gram of MS-222 to two gallons of solution provides a greater survival rate for transport of threadfin shad than a solution of MS-222 without the salt.

It is of interest to note that compounds known to inhibit the normal iodine metabolism of the thyroid also effect the loss of I^{131} from the bodies of fishes. Leloup (1952) has shown that marine teleost Muge kept in sea water containing thiourea (1 g/liter) from 8-21 days lost 55.8 - 58.7% of injected doses of I^{131} in 24 hours whereas untreated controls lost 24.7% in 24 hours. The Congre injected daily with thiouracil (30 mg/kilogram) for 12 days lost 61.4% of injected I^{131} in 24 hours versus 7% in the control. In the present study five rainbow trout which were injected with 20 mg NaSCN/fish concomitant with an injected dose of

I^{131} lost 69.7% of the total dose in 24 hours (control = 21.9%). With the blocking of the thyroidal uptake, it might be expected that the blood level of I^{131} would rise but suprisingly it did not. Leloup (1952) found that in Muge treated with thiourea the blood level of I^{131} (% dose per gram of blood) actually was lower than that of the control: 8 hours post-injection control 3.21%; thiourea 1.68%; 72 hours post-injection control 2.38%; thiourea .74 - .94%. In the present study similar results were obtained for rainbow trout treated with NaSCN; the % dose per gram of blood for controls averaged 3.1% versus 0.64% for the NaSCN treated trout. The increased rate of loss of I^{131} as reflected by the blood levels may have occurred at the renal or gill level. Bricker and Hlad (1955) have shown that SCN^- does not appreciably alter the I^{131}/inulin clearance ratios in humans. It did, however, markedly increase the plasma I^{131} levels in hyperthyroid patients and caused a moderate rise in euthyroid patients.

The data in Table 5 indicate that there is a 50% decrease in the gill I^{131} content in the NaSCN treated as compared to the controls. Whether this means a greater rate of outflux via the gills or a reduced uptake with a "normal" rate of outflux is not known.

An examination of the literature shows that almost nothing is known about the normal rate of turnover of I^{131} in the blood of fresh-water fish. Hickman (1962) studied fresh-water starry flounder and found that the $T\frac{1}{2}$ for blood I^{131} was 202.6 hours and 279.4 hours for two flounder as calculated from data obtained 30 and 70 hours after injection. For rainbow trout blood (fig. 3) the $T\frac{1}{2}$ for the non-bound I^{131} is 1.7 days.

As stated previously Marshall's work on the excretion of iodide by the toadfish is the only published data on actual measurements of the routes and relative rates of excretion of iodine by fish. No data on iodide excretion are available on fresh-water fish having glomerular kidneys. The present study is the first to shed some light on this unknown area. With the same reasoning which has been used with mammalian data, the U/P ratios of I^{131} indicate that iodide is filtered and mostly reabsorbed. Reabsorption of iodide is known to take place primarily in the proximal segment of the tubules in the kidneys of dog and man. The rainbow trout kidney has a proximal segment but no distal segment. Bricker and Hlad (1955), Giebisch *et al.* (1956) and Williamson *et al.* (1962) have shown that the reabsorption of iodide in mammals is a passive diffusion process.

Due to the fact that only one parameter, the U/P ratio of I^{131}, was measured in the trout, no definite conclusion can be reached as to whether or not the reabsorption is an active or passive process.

Renal clearance values of I^{131} for the trout have been expressed in ml per hour rather than the usual ml per minute. In man the normal renal clearance of iodide is 30 to 40 ml of plasma per minute when the glomerular filtration rate is normal. For trout in tap water the average renal clearance of I^{131} is 0.05 ml per hour, measured 24 hours after injection of I^{131}. The average I^{131} clearance for trout exposed to distilled water is three times that of trout in tap water. The work of Giebisch et al. (1956) shows that in the dog renal I^{131} clearance may increase five-fold during a mannitol induced diuresis. Trout exposed to distilled water exhibit a higher rate of urine flow than those exposed in tap water (181.0 $\pm$ SE 16.9 vs. 168.3 $\pm$ SE 9.7 ml/Kg/day). It is, however, improbable that this difference in urine flow could account for the three-fold difference in the clearance values between the two groups of trout.

In trout as well as in mammals the Cl^- clearance is less than the iodide clearance (Cl^- 6.8 ml/Kg/day as determined by Fromm (1963) vs. I^- 12.6 ml/Kg/day in the tap water trout).

Inulin clearance in rainbow trout at 10° is 169 $\pm$ 11.8 ml/Kg/day as measured by Holmes and McBean (1963).

The outflux of iodide from the gills of fresh-water fish was measured simultaneously with the urinary loss. These measurements indicate that 3.8 times as much iodide is lost via the kidneys as by the gills per unit of time. Whether this ratio is constant or changes depending on iodide levels in the blood is not known. The fact that there is an outflux across the gills as well as a kidney loss helps to explain why fresh-water fish in a goitre area have little or no ionic iodine in the blood. But as has been previously shown, if adequate iodide is present in the habitat, fasting trout will take up and retain large amounts of inorganic iodide free in the blood.

SUMMARY AND CONCLUSIONS

1. Rainbow trout can accumulate I^{131} directly from the medium which surrounds them. This uptake is not affected per se by the osmotic gradient between the environment and the fish but is inversely related to the calcium level of the medium.

2. The gill uptake of I^{131} is inhibited by NaSCN. Injected thiouracil does not appear to have any effect on uptake via this pathway.

3. The relative distribution of iodine in trout tissues (tissue vs. blood concentration) is similar to that which occurs in mammals with the exception of muscle which is lower than in mammals.

4. In rainbow trout having adequate iodine available to them, more than 80% of the blood iodine is in the ionic form in contrast to the mammals where 90% or more is protein-bound. The protein-bound iodine of the blood is made up of hormonal iodine from the thyroid and by direct binding of iodide to plasma proteins which can take place both *in vivo* and *in vitro*.

5. The biological half-life of non-bound I^{131} in the blood of rainbow trout at $14^{\circ}C$ is 1.7 day.

6. The retention of injected I^{131} is significantly reduced by NaSCN given intraperitoneally. Reduced gill levels of I^{131} suggest a possible increased loss via this route as the result of NaSCN treatment.

7. Kidney loss of I^{131} was found to be about four times that of the gill when both were measured simultaneously.

8. Kidney clearance of I^{131} has been measured. As in mammals, I^- clearance is greater than that of Cl^-.

9. The urine/plasma ratios for I^{131} indicate that I^{131} is filtered and mostly reabsorbed by the kidney.

10. Comparison of the iodine content of wild trout and trout in iodine "equilibrium" reveals that there is a large iodine storage capacity in trout tissues.

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APPENDIX A

Tissue $\frac{T}{M}$ Values For 24 Hour Studies and 24-29 Day Study

TABLE A-1

UPTAKE OF I^{131} FROM TAP WATER BY RAINBOW TROUT

24 Hour $\frac{T}{M} = \frac{\text{cpm/unit tissue}}{\text{cpm/unit medium}}$ Values

Each value is the average of 10 observations

Tissue	$\bar{X} \pm$ S.E.
Blood*	$1.59 \pm .28$
Heart	$.06 \pm .03$
Gill	$.25 \pm .02$
Lower Jaw	$2.03 \pm .43$
Liver	$.45 \pm .05$
Spleen	$.14 \pm .06$
Caeca	$.20 \pm .05$
Head Kidney	$.18 \pm .07$
Kidney	$.29 \pm .03$
Intestine	$.17 \pm .04$
Stomach	$.18 \pm .02$
Muscle	$.02 \pm .01$
Eye	$.14 \pm .02$

*Average level of activity in exposure medium = 1.57 μc/liter

TABLE A-2

UPTAKE OF I^{131} FROM DISTILLED HOH BY RAINBOW TROUT

24 Hour $\frac{T}{M} = \frac{\text{cpm/unit tissue}}{\text{cpm/unit medium}}$ Values

Each value is the average of 10 observations

Tissue	$\bar{X} \pm$ S.E.
Blood*	6.34 ± 1.12
Heart	.32 ± .03
Gill	.23 ± .03
Lower Jaw	1.49 ± .19
Liver	.36 ± .07
Spleen	.17 ± .02
Caeca	.13 ± .01
Head Kidney	.35 ± .06
Kidney	.30 ± .04
Intestine	.15 ± .03
Stomach	.20 ± .02
Muscle	.06 ± .01
Eye	.19 ± .08

*Average level of activity in exposure medium = 2.38 μc/liter

TABLE A-3

UPTAKE OF I^{131} FROM .6% NaCl BY RAINBOW TROUT

25 Hour $\frac{T}{M} = \frac{\text{cpm/unit tissue}}{\text{cpm/unit medium}}$ Values

Each value is the average of 10 observations

Tissue	$\bar{X} \pm$ S.E.
Blood*	1.40 ± .31
Heart	.20 ± .05
Gill	.56 ± .08
Lower Jaw	2.75 ± .41
Liver	1.07 ± .10
Spleen	.12 ± .04
Caeca	.23 ± .04
Head Kidney	.28 ± .05
Kidney	.37 ± .06
Intestine	.19 ± .03
Stomach	.27 ± .02
Muscle	.04 ± .01
Eye	.21 ± .02

*Average level of activity in exposure medium = 1.20 μc/liter

TABLE A-4

UPTAKE OF I^{131} FROM 66% RINGER'S BY RAINBOW TROUT

24 Hour $\frac{T}{M}$ = $\frac{\text{cpm/unit tissue}}{\text{cpm/unit medium}}$ Values

Each value is the average of 10 observations

Tissue	$\bar{X} \pm$ S. E.
Blood*	.50 ± .06
Heart	0
Gill	.43 ± .06
Lower Jaw	1.30 ± .06
Liver	.40 ± .07
Spleen	.03 ± .03
Caeca	.18 ± .02
Head Kidney	.03 ± .03
Kidney	.23 ± .06
Intestine	.09 ± .03
Stomach	.19 ± .01
Muscle	.02 ± .02
Eye	.14 ± .01

*Average level of activity in the exposure medium = 1.63 μc/liter

TABLE A-5

IODINE EQUILIBRIUM STUDY

25-29 Day $\frac{T}{M}$ Values

Tissue*	$\bar{X} \pm$ S.E.
Heart	0.16 ± .01
Gill	0.16 ± .01
Lower Jaw	18.39 ± 2.49
Liver	0.32 ± .06
Spleen	0.13 ± .02
Caeca	0.13 ± .01
Head Kidney	0.27 ± .01
Kidney	0.29 ± .02
Lower Gut	0.16 ± .01
Stomach	0.13 ± .01
Muscle	0.04 ± .01
Eye	0.11 ± .01

* n = 9

APPENDIX B

Uptake of Injected Doses of I^{131} with Fish Injected Under MS-222 Anesthesia

TABLE B-1

TURNOVER OF I^{131} IN RAINBOW TROUT
FOLLOWING AN I.P. INJECTION
UNDER MS-222 ANESTHESIA

Fish no.	Body weight grams	1% of Injected Dose of I^{131}			Injected dose μc
		Blood per gram	Lower Jaw per gram	Tap water 4 liters	
1	76.2	4.45	6.90	43.09	1.2
2	107.3	2.65	8.43	41.60	1.0
3*	102.0	11.42	17.42	43.10	0.9
4	89.2	9.57	34.25	39.40	1.7
5*	102.8	7.39	14.04	42.10	1.5
6	91.7	3.34	10.75	53.23	1.4
7*	93.4	4.36	7.61	62.41	1.4
8	106.1	6.73	6.03	26.97	1.3
9*	75.0	10.44	28.67	32.65	1.0
10	103.0	8.59	18.94	25.52	1.7
$\bar{X}$	94.67	6.90	15.29	41.0	1.31
$\pm$ SE	3.72	0.98	3.06	3.54	.09

*Samples of blood and lower jaw taken following two hour excretion study.

APPENDIX C

Uptake of I^{131} by Goldfish and Rainbow Trout

Uptake of I^{131} by Goldfish and Rainbow Trout

Twenty-five goldfish (Carassius) purchased from a local store were placed into a 40 liter aquarium of aged tap water and acclimated to a temperature of 14°C for a two week period. During this time they were fed trout pellets. At the end of the acclimation period, the goldfish were placed into an aquarium containing 30 liters of aged tap water to which had been added 68.8 μc of carrier free I^{131}. Twenty-five trout were also placed into another aquarium set up the same as that for the goldfish. During the course of the experiment the fish were not fed nor the water changed.

A sample of five fish was taken from each aquarium at 24, 48, 72, 96, and 120 hours. Each fish was damped dry and weighed to the nearest 0.1 of a gram. The goldfish were wet ashed in 30 ml of concentrated nitric acid. Due to the size variation in the trout, they were wet ashed in various volumes (30 to 100 ml) of concentrated nitric acid.

Ten ml aliquots of the aquarium water and fish digests were counted as described in the materials and methods section. All counts were corrected back to zero time, the beginning of the experiment.

It was thought necessary to determine the availability of the added I^{131}. This was accomplished by running aquarium

water through 2 cc of ion exchange resin IRA 400. The count of the aquarium water before and after passing through the resin was used to estimate the percent I^{131} in the ionic form.

TABLE C-1

UPTAKE OF I^{131} BY GOLDFISH AND RAINBOW TROUT FROM TAP WATER AT 14°C

Exposure time	Fish	No. of fish	Body wt. $\bar{X}$ and range	cpm/gm $\bar{X}$ and range	% ionic I^{131} in water
24 hours*	Goldfish	5	1.7 gm 0.8-2.7	4,308 3,507-5,535	100
	Rainbow trout	5	13.4 gm 6.8-18.8	563 226-807	100
48 hours	Goldfish	5	1.5 gm 0.9-2.4	2,692 2,325-3,090	96
	Rainbow trout	5	17.4 gm 7.8-23.7	495 367-638	96
72 hours	Goldfish	5	2.9 gm 1.4-4.0	4,357 1,119-12,664	100
	Rainbow trout	5	13.0 gm 4.3-25.2	604 475-837	96
96 hours	Goldfish	5	2.4 gm 1.2-3.5	5,038 2,816-7,172	96
	Rainbow trout	5	18.4 gm 15.5-22.2	516 491-562	87
120 hours	Goldfish	5	1.4 gm 1.0-2.4	6,511 1,503-18,242	100
	Rainbow trout	5	9.9 gm 8.2-12.2	535 459-597	89

*Level of radioactivity in water containing the goldfish was 467 cpm/ml, rainbow trout water 462 cpm/ml.

APPENDIX D

Turnover of i.p. Injected Radioactive Thyroxine in Rainbow Trout

Turnover of i.p. Injected Radioactive Thyroxine in Rainbow Trout

Nine unanesthetized rainbow trout ranging from 59.5 grams to 106.4 grams were injected i.p. with 0.21 μg (7.5 μc) of radioactive thyroxine and each placed into a plastic container holding two liters of aged tap water at 14°C. Every 24 hours a sample of two fish was taken. At that time a 10 ml aliquot of the tap water was taken from each container and the remaining fish were placed into two liters of uncontaminated aged tap water.

The fish to be sampled were anesthetized with MS-222 and a blood sample taken. The lower jaw was also removed, trimmed and weighed. The blood was centrifuged and the RBC's, plasma supernatant, plasma precipitates and lower jaw prepared for counting.

Figure D-1

Turnover of Radioactivity Associated with the Protein-bound Fraction of the Blood and the RBC's of I^{131}-Thyroxine Injected Rainbow Trout

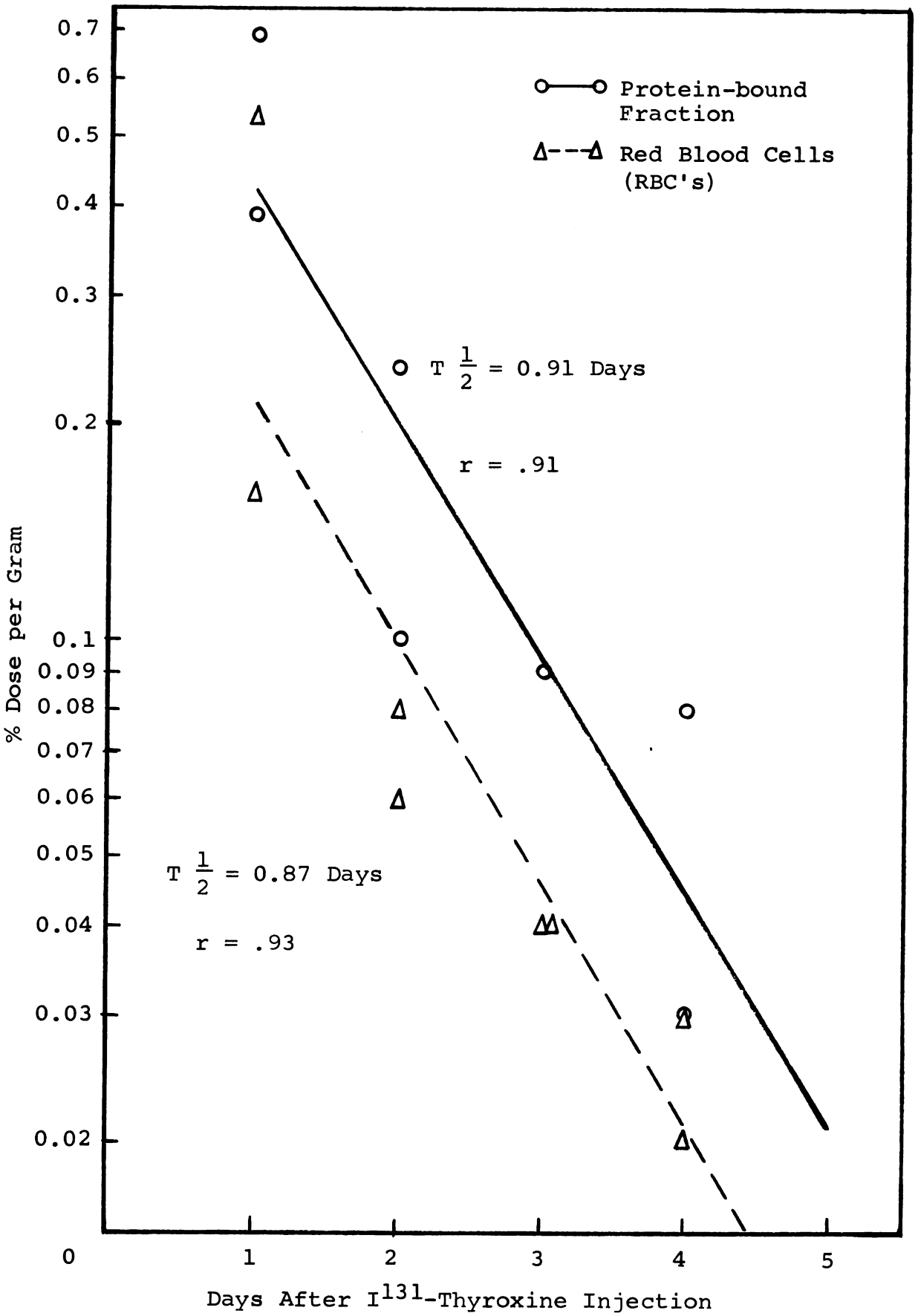
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0
1
2
3
4
5
% Dose per Gram
Days After I131-Thyroxine Injection
Protein-bound Fraction
Red Blood Cells (RBC's)
T 1/2 = 0.91 Days
r = .91
T 1/2 = 0.87 Days
r = .93

Figure D-2

Turnover of Non-bound I^{131} in the Blood of I^{131}-Thyroxine Injected Rainbow Trout

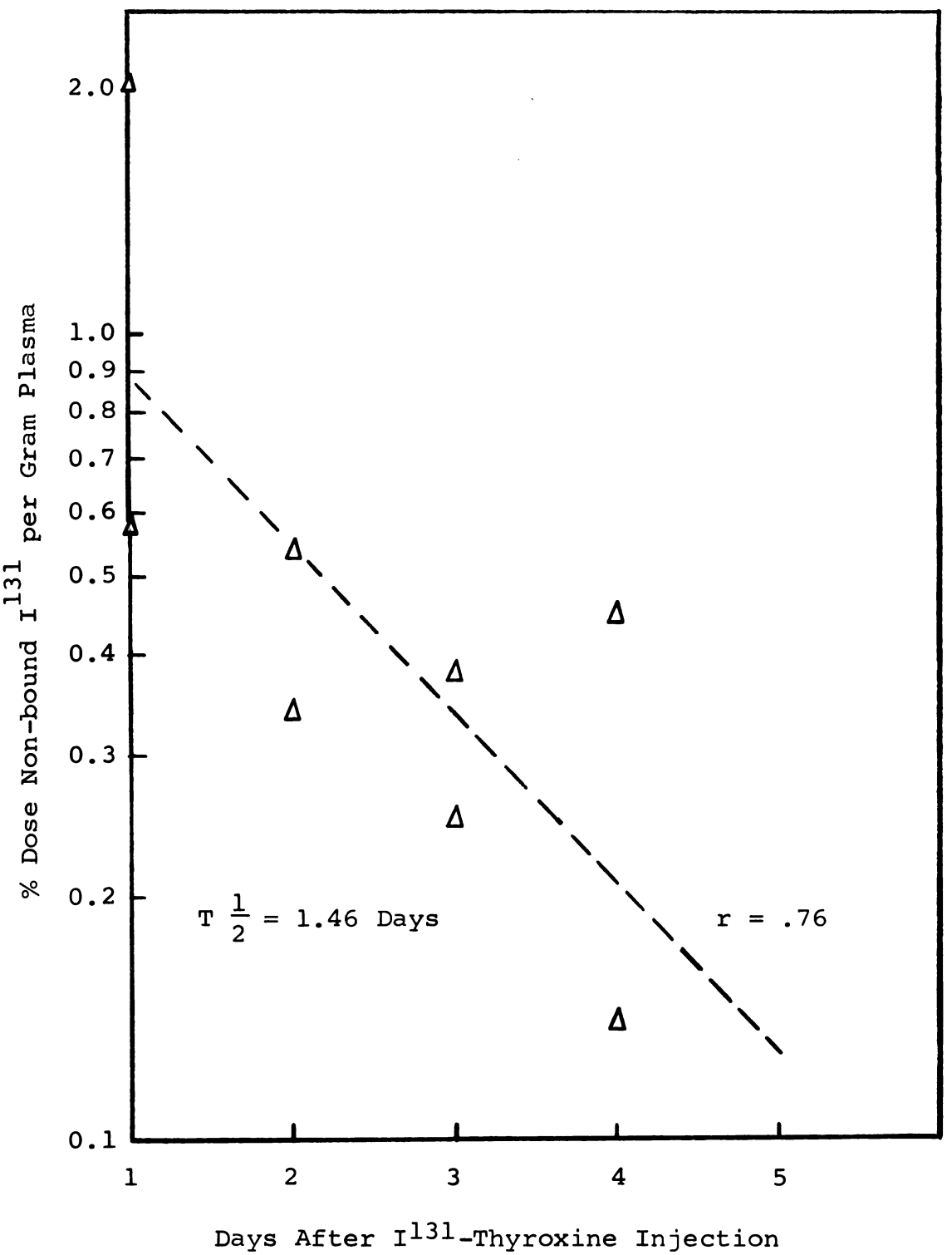
2.0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
% Dose Non-bound I131 per Gram Plasma
T 1/2 = 1.46 Days
r = .76
1
2
3
4
5
Days After I131-Thyroxine Injection

TABLE D-1

TURNOVER OF AN I.P. INJECTED DOSE OF RADIOACTIVE THYROXINE IN RAINBOW TROUT*

	24 hours	48 hours	72 hours	96 hours	120 hours
% dose per	.16	.06	.04	.02	.01
gram RBC	.53	.08	.04	.03	-
% dose per gram	2.04	.34	.38	.45	.62
plasma ppt. supernatant	0.58	.54	.25	.14	-
% dose per gm	.39	.10	.09	.03	.10
plasma protein bound	.69	.23	.09	.08	-
% dose per gram	7.61	1.51	2.53	4.85	8.16
lower jaw	1.76	2.35	2.55	2.38	-

*All observations listed are for individual fish.

TABLE D-2

DISTRIBUTION OF RADIOACTIVITY IN THE BLOOD FOLLOWING AN I.P. INJECTION OF I^{131}-THYROXINE

Time after injection	% of total activity in blood		
	RBC	Protein-bound	Non-bound
24 hours	4.01	15.53	80.46
	12.19	39.93	47.90
48 hours	5.72	21.09	73.19
	5.49	27.19	67.32
72 hours	4.68	18.85	76.47
	5.37	25.60	69.03
96 hours	0.96	6.86	92.18
	5.58	35.14	59.28
120 hours	0.69	13.99	85.32

TABLE D-3

WHOLE BODY EXCRETION FOLLOWING INJECTION OF I^{131}-THYROXINE

Time	No. of fish	$\bar{X}$ % dose excreted per 24 hours	Accumulative $\bar{X}$ % dose excreted
24 hours	9	19.17	19.17
48 hours	7	15.28	35.22
72 hours	5	13.22	47.28
96 hours	3	10.58	61.60
120 hours	1	4.45	84.04

APPENDIX E

Method for the Determination of Plasma Protein-bound Iodine and Total Iodine Content of the Lower Jaw (Thyroid)

Method for the Determination of Plasma Protein-bound Iodine and Total Iodine Content of the Lower Jaw (Thyroid)

Preparation PBI

To 1 ml of plasma add 1 cc of zinc sulfate (10%), 1 cc of sodium hydroxide (0.5 N) and 7 cc of glass distilled water, mix and let stand for 10 minutes. The mixture is then centrifuged and the supernatant saved or discarded as deemed necessary. The precipitate is then resuspended in 10 cc of glass distilled water and the procedure repeated twice. The precipitate is then transferred into a nickel crucible containing 1 cc of 4 N sodium carbonate.

Lower Jaw

The lower jaw was cut from the anesthetized trout, trimmed and weighed to the nearest mg on a Roller-Smith torsion balance. Upon being weighed the lower jaw was placed in a nickel crucible containing 2 cc of 4 N sodium carbonate and 1 cc of glass distilled water.

Common Procedure

The nickel crucibles were then placed into a drying oven at 90-95^{o}C to dry over night. The dried samples were ashed for about two hours at 600 $\pm$ 10^{o}C in an electric muffle

furnace and then allowed to cool. The ash was dissolved in 2 cc of 2 N hydrochloric acid plus 2 cc of 7 N sulfuric acid. The PBI samples were made up to a total volume of 14 cc and the lower jaw samples to 25 cc with glass distilled water. Contents were thoroughly mixed and transferred to a pyrex centrifuge tube. The insoluble carbonaceous material was allowed to settle and when necessary removed by centrifugation.

Two 5 ml aliquots (PBI) and two 0.1 ml aliquots (lower jaw), for duplicate analyses, were pipetted from the centrifuge tubes into round bottomed optically calibrated colorimetric tubes. These tubes contained 0.5 ml of arsenious acid and were made up to volume 5.5 ml with glass distilled water. The tubes were then mixed well and placed into a water bath accurately regulated at 27 $\pm$ 0.2^{o}C for 15 minutes incubation.

0.5 ml of ceric ammonium sulfate was added successively to each tube at 60 second intervals using a "blow-out" pipette and the tubes were thoroughly mixed and returned to the water bath. Exactly 15 minutes after addition of ceric ammonium sulfate the % transmittance was read in a Coleman Model 11 Spectrophotometer using a No. PC-4 blue filter and a wave length of 430 millimicrons. The initial setting of the instrument (10% T) was made up against distilled water.

A reagent blank was prepared exactly as described above except that the sample was omitted.

The reading of the reagent blank was subtracted from the reading of the iodine standard or reading of unknown samples. The concentration of iodine was read directly from a standard curve plotted on arithmetic graph paper. The total iodine was then calculated using the necessary corrections for dilutions.

All determinations were performed in duplicate. A control standard of known concentration of iodine (Hycel's Standard Iodine Reagent) and a reagent blank were included with each series of samples.

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APPENDIX F

Procedure for Paper Chromatography

Procedure for Paper Chromatography

Paper

Paper strips (Whatman 3 MM) one inch by 12 7/8 inches were cut. The origin was marked 2.5 inches from one end. The origin end was tapered, starting 1/2 inch below the origin, to 1/4 inch.

Technique for Applying Solutions

The paper strips were placed between clean glass plates with the origin extending about one inch from the plates. The solutions were applied at the origin with lambda pipettes. The paper was then placed under a heat lamp to dry. The dried strips were afixed to pins and placed on a suspension rack. The rack was then placed into a steam hood and subject to steaming for 15-25 minutes. After steaming the rack was placed into a developing jar containing collidine-H_2O-ammonia. The strips were developed in this system for 18 hours, after which they were removed and the solvent fronts marked. After drying the strips were placed on X-ray film for a 48 hour exposure.

Developing Reagent

Collidine	125 ml
H_2O	44 ml
NH_4OH	beaker of concentrated NH_4OH is placed in chamber

APPENDIX G

Ringer's Solutions for Fish

Ringer's Solutions for Fish

Schiffman's

58.5	grams	NaCl
3.8	grams	KCl
4.8	grams	$CaCl_2$
10	liters	distilled water

Stokes'

7.37	grams	NaCl
.31	grams	KCl
.37	grams	$MgSO_4 - 7H_2O$
.14	grams	$CaCl_2$
1	liter	distilled water

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APPENDIX H

References Pertaining to Iodine in Fish

References Pertaining to Iodine in Fish

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